19th International Zebrafish Conference

July 9-13, 2025 | Madison, WI, USA

Wednesday, July 9, 2025

Shannon Hall

Shannon Hall | 3:45-5:00pm

(Bio)physics of body building: gastrulation, elongation, segmentation, ...

Presenting Author: L. Mahadevan, FRS - Harvard University

Co-Author(s):

I will describe how simple geometrical ideas and physical principles associated with active growth and flow are beginning to help illuminate multicellular tissue morphogenesis in laying out the body plan, using three common motifs, with variations across organisms: gastrulation, elongation, and segmentation. Combining experiments and theory allows us to link molecular genetics to morphogenesis in a developing organism and construct evolutionary phase diagrams across organisms, sharpening the Darwinian question of how "endless forms most beautiful and most wonderful have been, and are being, evolved."

Plenary Session I:

Quantitative Biology, Early Development, Morphogenesis and Patterning

Shannon Hall | 5:00-6:30pm

Session Chairs: Katherine Rogers - *National Institutes of Health (NICHD)* & Jing Chen

Huluwa signaling initiates the regulatory hierarchy of organizer formation in zebrafish

Presenting Author: Anming Meng, PhD - Tsinghua University

Co-Author(s): Yunlong Li – graduate student, School of Life Sciences, Tsinghua University

Abstract: Huluwa is a recently identified novel gene in the zebrafish. Deficiency of maternal huluwa leads to loss of the dorsal organizer at the onset of gastrulation and missing of head and most trunk tissues in zebrafish and frog (Yan et al., 2018). Huluwa protein consists of a short extracellular domain, a single transmembrane domain and a longer intracellular domain without a predictable structure. Our previous study propose that Huluwa induces organizer formation by promoting Axin degradation and thus stabilizing beta-catenin during embryonic development. Careful examination of previous data revealed that overexpression of constitutively active beta-catenin in zebrafish huluwa mutants restored the body axis only in a small proportion of mutants and that only about 1/3 of zebrafish ichabod mutants lacking beta-catenin 2 transcripts showed severely ventralized phenotypes (Kelly et al., 2000) resembling huluwa mutants. These facts suggest that beta-catenin signaling is only one of signals downstream of Huluwa signaling. Our latest data disclose that Huluwa is a GTP-binding protein and activates several pathways that collectively participate in induction of the dorsal organizer during development.

Integrator Complex Subunit 6 Represses Thousands of Genes During Zygotic Genome Activation and Ventrolaterally Restricts the Dorsal Organizer

Presenting Author: William Jones - University of Pennsylvania

Co-Author(s): Mary Mullins, PhD – University of Pennsylvania

The vertebrate body axis is established by the dorsal organizer, a signaling center that promotes dorsal fates and is established shortly after the activation of zygotic transcription. A unique zebrafish loss-of-function mutant, called ints6p18ahub, provides new insights into how the organizer is repressed to balance dorsoventral fates. In this maternal-effect mutant, the progeny of homozygous ints6p18ahub mutant females (M-ints6p18ahub embryos) display an expanded organizer and multiple body axes. The mutated gene, integrator complex subunit 6 (ints6), encodes a subunit of the Integrator complex, which has not previously been implicated in embryonic patterning. Integrator, which binds RNA polymerase II, was first discovered as a small nuclear RNA maturation factor important for mRNA splicing. However, it can also regulate gene expression via its endonuclease and phosphatase modules. I have found that ints6p18ahub is a hypomorphic, temperature-sensitive (ts) allele, and that maternal-effect nonsense alleles of ints6 cause an earlier mid-blastula arrest phenotype, indicating a critical molecular function shortly after zygotic genome activation. To test the timing of Ints6 function, I performed temperature-shifts at different stages using the ints6p18ahub ts allele. I found that Ints6 functions to repress the organizer during the mid-blastula period, shortly after zygotic transcription begins. Next, I tested the spatial function of Ints6. Using regional expression of wild-type Ints6 in M-ints6p18ahub embryos, I found that Ints6 functions ventrolaterally, not dorsally, to repress the organizer. Finally, I performed RNA-seq in ints6 maternal mutants and found thousands of differentially expressed genes (DEGs), including genes that regulate dorsoventral patterning. Surprisingly, over 99% of DEGs were upregulated in mutant embryos during the mid-blastula period, supporting the hypothesis that Ints6 broadly represses early zygotic transcription. We further found that many genes repressed by Ints6 are typically marked with repressive chromatin marks. Overall, Ints6 broadly represses early zygotic transcription, repressing dorsal fates during the mid-blastula period.

Decoding BMP signaling during patterning of the dorsal neural tube

Presenting Author: Hannah Greenfeld, PhD - UCSF

Co-Author(s): Daniel Wagner – UCSF

Abstract: The spinal cord is a highly organized tissue comprised of diverse neurons which are critical for receiving and processing sensory information. The diversity of neural subtypes is generated by opposing gradients of morphogen signaling, Sonic Hedgehog and Bone Morphogenetic Protein (BMP). BMP signaling is required for specification of multiple dorsal neurons, however multiple mechanisms have been proposed for how BMP patterns the dorsal neural tube. To investigate the mechanism of BMP signaling patterning the dorsal neural tube in vivo, we performed single-cell RNA sequencing on zebrafish embryos treated with a BMP signaling inhibitor during neural tube formation. Contrary to the classic morphogen hypothesis, we found that BMP signaling is only required for specification and maintenance of one dorsal interneuron subtype, while all other dorsal neural progenitors remain specified. Our results challenge the existing view that BMP acts as a morphogen within the neural tube to pattern multiple neural progenitors and raises the possibility that neural progenitors are pre-

patterned prior to neural tube formation. We tested if BMP signaling is autonomously required for dorsal neural progenitor specification during gastrulation. We generated mosaic embryos with inhibited BMP signaling in a subset of cells and found that perturbing the BMP signaling gradient during gastrulation, and not during formation of the neural tube, resulted in altered dorsal interneuron patterning. We have generated a single-cell atlas of zebrafish larval development which allows us to predict when gene expression trajectories of dorsal neural progenitors emerge and identify putative genetic drivers that regulate neural specification. Together, our data challenges the morphogen model of dorsal neural tube patterning and shows distinct roles of BMP signaling during spinal cord development.

Probing the duality of temperature and osmotic strength on developmental tempo using deep learning

Presenting Author: Patrick Müller - University of Konstanz

Co-Author(s): Onur Önder – University of Konstanz; Daniel Čapek – University of Konstanz; Grigory Arutyunov – University of Konstanz; Tim Brunnhuber – University of Konstanz; Lisa Dimmler – University of Konstanz; Matvey Safroshkin – University of Konstanz

Abstract: The tempo of embryonic development is highly sensitive to environmental conditions. Recent influential work has proposed a duality of temperature and osmotic strength on cellular function, and suggested that solvent thermodynamics driven by changes in water availability regulate protein activity and macromolecular interactions. However, this hypothesis has not been tested in the context of complex developing multicellular systems, and the relative contributions of temperature and osmotic strength on the regulation of developmental tempo remain unclear. To address this question, we developed zMorphoNet, a deep learning-based tool for detailed morphometric analyses across developmental stages. zMorphoNet combines semantic and instance segmentation for accurate tissue identification and was trained and validated using manually annotated zebrafish datasets. Our tool enables detailed morphometric analyses across developmental stages and precisely delineates embryonic structures, including the yolk, yolk extension, cells, embryonic body, and eyes. By applying zMorphoNet to embryos exposed to varying environmental conditions, we quantified tissue-specific growth rates and morphological changes with unprecedented precision. We found that temperature consistently dominated the regulation of developmental tempo in zebrafish embryos, while modulating osmotic strength had more subtle effects on tissue boundary integrity and morphogenetic processes. These findings suggest that temperature-driven solvent thermodynamics play a primary role in regulating the biochemical activity essential for developmental progression. By combining deep learning with experimental manipulations, our study underscores the utility of zMorphoNet for quantifying tissue dynamics and developmental tempo in complex biological systems.

Pressure gradients define how the notochord responds to mechanical perturbations

Presenting Author: Parsa Zareiesfandabadi - Duke University

Co-Author(s): Michel Bagnat – Duke University; Sonke Johnsen – Duke University; James Norman – Duke University; Sean Sun – JHU; Yufei Wu – JHU

Abstract: The notochord, a defining feature of chordates, is a pressurized rod that provides essential structural support during vertebrate development and spine formation. In invertebrate chordates such

as ascidians, the notochord comprises an impermeable epithelial layer surrounding a continuous fluid-filled lumen. In contrast, vertebrate notochords compartmentalize fluid within individual vacuolated cells. Previous studies in zebrafish demonstrated that vacuolated cells are critical for embryonic axis elongation and proper spine morphogenesis. Notably, disruption of vacuoles leads to localized deformation of the notochord due to compressive forces from vertebral formation, resulting in localized spine malformations. These observations led us to hypothesize that the compartmentalization of the vertebrate notochord into vacuolated cells underlies its ability to absorb mechanical stresses locally. Using quantitative microscopy, we characterized the mechanical properties of the zebrafish notochord and found that vacuolated cell-cell boundaries exhibit significant curvature that changes dynamically throughout development, indicating non-uniform pressure gradients at the tissue scale. To investigate how these gradients influence notochord responses to mechanical perturbations, we punctured individual cell-cell boundaries using two-photon laser ablation and measured the magnitude and extent of perturbation propagation. Remarkably, responses were highly localized, confined to cells adjacent to the puncture, and exhibited asymmetry dependent on initial pressure gradients. Finally, we developed a simple mathematical model based on osmotic processes coupled with mechanical interactions that recapitulated our experimental observations. Our findings suggest that osmotic gradients between vacuolated cells and interstitial fluid generate tissue-wide pressure gradients that enable mechanical compartmentalization and local stress insulation within the vertebrate notochord.

Critical Role of Spatio-Temporally Regulated Maternal RNAs in Zebrafish Embryogenesis

Presenting Author: Gopal Kushawah - Stowers Institute for Medical Research

Co-Author(s): Ariel Bazzini – Stowers Institute for Medical Research

Abstract: The maternal-to-zygotic transition (MZT) reprograms embryonic gene expression by shifting regulatory control from maternal to zygotic transcripts, primarily through maternal mRNA degradation. While the temporal dynamics of maternal mRNA decay are well-characterized, spatial mechanisms remain underexplored. Using CRISPR-Cas9 and CRISPR-Cas13d systems, we functionally dissected maternal and zygotic mRNA contributions and overcame challenges of studying embryonic lethal genes. We identified differentially distributed maternal mRNAs in specific cells and evidenced the critical role of five maternal mRNAs, cth1, arl4d, abi1b, foxa, and lhx1a, in early embryogenesis. Further, we focused on the functionally uncharacterized cth1 gene in zebrafish. We demonstrate its essential role in gametogenesis and early development. Cth1 mutants exhibit severe multiorgan pathogenesis and infertility, suggesting its critical biological function. Given that embryonic lethality restricts functional embryonic studies, we leveraged CRISPR-Cas13d to bypass these limitations and dissect cth1 function during early embryogenesis. Our massively parallel reporter assays and RNA-seq time-course analyses under knockdown conditions reveal Cth1 as a spatiotemporal RNA decay factor. It regulates mRNA stability and its potential targets transcript levels through 3'UTR-mediated recognition, orchestrating early developmental gene expression. Furthermore, the Cth1 3'UTR itself drives spatiotemporal localization and stability, emphasizing its role in post-transcriptional regulation. Our findings provide critical insights into spatiotemporal RNA decay mechanisms and establish dual CRISPR-Cas strategies as powerful tools for studying essential embryonic genes.

Thursday, July 10, 2025

Plenary Session II:

Disease Models — Shannon Hall | 8:30-10:00am

Session Chairs: Summer Thyme - *UMass Chan Medical School* & Yonghua Sun - *Institute of Hydrobiology, CAS*

Fishing for Cures: Zebrafish as a Pioneering In Vivo Model to Help Solve Mitochondrial Medicine Odysseys

Presenting Author: Ankit Sabharwal, PhD - Dell Medical School, The University of Texas at Austin

Co-Author(s): Victoria Diaz – Department of Pediatrics, Dell Medical School, The University of Texas at Austin, USA; Jun Morisue – Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; Mireya Mota – Department of Pediatrics, Dell Medical School, The Un

Abstract: Mitochondrial DNA (mtDNA) pathogenic variants underlie diverse mitochondrial disorders, impacting multiple organ systems and posing significant clinical challenges. Historically, the absence of precise animal models has limited mechanistic understanding and therapeutic development. Leveraging recent advances in mitochondria-targeted base editing technologies, we have successfully established zebrafish (Danio rerio) models using via FusX TALE Base Editors (FusXTBE) to recapitulate human mtDNA disorders, providing potential for unprecedented opportunities for mechanistic insights and therapeutic exploration. First, we explored germline transmission dynamics and systemic consequences of near-complete (>80% heteroplasmy) mt-tl1 mutations in zebrafish. Embryos harboring these edits exhibited impaired mitochondrial bioenergetics and altered transcriptomic signatures involving respiration and cholesterol synthesis pathways. Intriguingly, differential segregation patterns across generations correlated with distinct transcriptomic profiles and hearing impairments analogous to clinical mitochondrial disorders, highlighting the utility of this model for studying inheritance and systemic manifestations of mtDNA mutations. Second, we introduced a novel premature stop codon mutation in mt-nd5 m.13311C>T mutation using DddA-derived cytosine base editors (DdCBEs), achieving stable heteroplasmy levels between 40-70%. Phenotypically, these mutants demonstrated significantly reduced swimming activity, diminished Complex I enzyme activity, elevated lactate levels, increased oxidative stress markers, and altered mitochondrial metabolic fluxes indicative of Complex I deficiency. This model accurately mirrors biochemical and behavioral phenotypes observed in primary mitochondrial diseases, facilitating translational studies aimed at therapeutic intervention. Finally, we generated zebrafish harboring the prevalent LHON-associated m.11778G>A mutation. These mutants displayed stable retinal heteroplasmy exceeding 90%, similar to other tissues such as liver and heart across successive generations. Current studies focus on retinal ganglion cell-specific degeneration characteristic of LHON, bioenergetic profiling, and cellular responses under stress conditions to elucidate tissue-specific vulnerability mechanisms and potential moonlighting roles beyond bioenergetics. Collectively, these zebrafish avatars offer potential to be used as platforms to dissect mitochondrial pathophysiology, significantly advancing mechanistic understanding and targeted therapeutic strategies.

It's all about the context: deciphering permissive conditions and constraints in somatic mosaic disorders

Presenting Author: Nicola Blum - Boston Children's Hospital, Harvard Medical School

Co-Author(s): Matthew Harris – Harvard Medical School, Boston Children's Hospital

Abstract: Somatic mutations are both inevitable and ubiquitous across tissues. The consequences of these mutations, however, are highly context dependent, influenced by a complex interplay of timing, genetic, cellular and microenvironmental factors. We model mosaic disorders in zebrafish to unveil key determinants in phenotypic outcome of somatic mutations and open up novel areas for treatment and early interception. Joseph Merrick, famously known as the "The Elephant Man", is believed to have suffered from Proteus syndrome – a somatic mosaic disorder characterized by progressive focal bone overgrowth with no cure or specific treatment. Patients with Proteus syndrome carry clones with a somatic activating mutation in AKT1 scattered throughout their body. Recent studies have shown that mutant clones can also be detected in unaffected bones suggesting that the mutation itself is not sufficient to drive progressive overgrowth. We have developed a zebrafish model for Proteus syndrome to unravel the factors that fuel or repress the overgrowth potential of AKT1 mutant bone clones. Of note, our model is the first animal model that recapitulates bone phenotypes seen in patients. Our experiments reveal differential growth dynamics of clones. While all clones cause an initial burst in excess bone formation, only a small subset of clones result in rapidly progressive bone overgrowth as seen in patients. Surprisingly, some clones show uncontrolled growth resembling osteosarcoma. Comparisons between these subset of clones hint to distinct microenvironmental factors in fueling growth of the bone lesion and transformation into an osteosarcoma-like state. We have begun to identify these signals and testing whether these attributes can be leveraged to design novel treatments. Our work provides a conceptual framework to understand differences in phenotypic outcome of somatic activating AKT1 mutations and, more broadly, demonstrates how zebrafish can be used to identify modifying factors in somatic mosaic disorders.

NEW INSIGHT ABOUT AUTOSOMAL DOMINANT HYPER-IGE SYNDROMES: THE INTERPLAY BETWEEN P727STAT3 AND VITAMIN D AS PROMISING THERAPEUTIC TARGET.

Presenting Author: Annachiara Tesoriere - Università di Padova

Co-Author(s): Francesco Sernesi – Department of Biology – University of Padova, Italy; Rachele Ghirardo – Department of Biology – University of Padova, Italy; Matteo Gasparotto – Hector Institute for Translational Brain Research – Central Institute of Mental Health,

Abstract: Autosomal Dominant Hyper-IgE Syndromes (AD-HIES) are rare immunodeficiency disorders primarily caused by mutations in the STAT3 gene. These conditions severely impact tissue regeneration, immune system and skeletal development. Most cases involve de novo mutations, making prenatal diagnosis nearly impossible. No specific therapy has been developed yet, and the syndrome is associated with significant morbidity and mortality. Understanding the molecular mechanisms behind the disease is crucial for identifying new therapeutic strategies. As observed in AD-HIES patients, our recently developed stat3 knockout (stat3-/-) zebrafish model exhibits severe defects in tissue regeneration, immune response and bone tissue development. Stat3 mutants show a significant reduction in tail regeneration at both larval and adult stages, linked to impaired immune response at the injury site. Additionally, they present pronounced bone defects that closely mirror the craniofacial alterations seen in AD-HIES patients. STAT3 functions depend on several post-translational modifications, such as serine 727 (S727) phosphorylation. We performed site-directed mutagenesis to replace serine 751 (the zebrafish homolog of mammalian S727) with alanine obtaining the new stat3S727A mutant line. Surprisingly, this mutation also leads to severe bone defects, alterations in wound healing and immune

response, highlighting the importance of this modification in the disease's pathogenesis and providing new insight into the molecular mechanisms. Vitamin D plays a critical role in bone health, immune response, and wound repair. Stat3 mutants show reduced expression of genes involved in vitamin D maturation, suggesting that the stat3 impairment could decrease vitamin D levels. Administering already mature vitamin D on stat3 mutants, we managed to partially rescue wound healing and immune response defects, indicating vitamin D as a promising therapeutic target for AD-HIES.

Zebrafish erf mutation reveals evolutionarily conserved mechanisms underlying craniosynostosis

Presenting Author: Shannon Fisher - Chobanian and Avedisian School of Medicine, Boston University

Co-Author(s): Anita He – Pharmacology, Physiology, & Biophysics – Chobanian and Avedisian School of Medicine, Boston University

Abstract: During early development, the bones of the cranial vault are held together by connective tissue at sutures. Sutures serve a mechanical purpose, providing flexibility to the skull, but also are the sites of new bone deposition during rapid growth of the brain. One of the most common craniofacial birth defects in humans is craniosynostosis (CS), where one or more of the sutures is replaced with bone. Heterozygous mutations in the transcription factor gene ERF cause severe CS, often affecting multiple sutures. ERF encodes a repressor in the ETS2 family of transcription factors. We have shown physical interactions of Erf with enhancers for the RUNX2 and BMPER genes, both associated with genetic risk for CS, and hypothesize that reduction in Erf leads to increased expression of both genes. We have created zebrafish mutants for erf, which similarly display severe multi-sutural CS. This remarkable conservation of function suggests that loss of Erf leads to upregulation of common genes in both humans and zebrafish, which underlies development of CS in both species. We are using erf mutants to screen both for regulatory elements that are more accessible and active, and for genes that are more highly transcribed, to identify additional targets of Erf that contribute to the CS phenotype. We are also using live confocal imaging to follow the development of CS in erf mutants. Preliminary results show that delineation of the edges of the skull bones, normally observed as they approach during skull growth, fails to occur in the erf mutants. This suggests that a defect in patterning of the edges of the bones may underlie the failure of the bones to remain separate at the sutures.

Functional Characterization of Autism with Disproportionate Megalencephaly Candidate Genes in Larval Zebrafish

Presenting Author: Nicholas Haghani - University of California Davis, Genome Center

Co-Author(s): Emily Xu – University of California Davis, Genome Center; Megan Dennis – University of California Davis, Genome Center

Abstract: Among individuals with autism spectrum disorder (ASD), some of the worst prognoses come from comorbidity with accelerated brain growth, known as disproportionate megalencephaly (DM). ASD-DM is associated with regressive autism, slower gains in IQ, greater difficulties with expressive language, and more severe cognitive defects. Recently, through whole genome sequencing of ASD-DM patients, we identified likely gene-disrupting mutations in 138 candidate genes, many of which have unknown functions or relation to autism. While brain anatomical differences can be readily modeled in mice and cortical organoids, functional characterization of multiple genes in parallel remains a major challenge. Due to their small size, robust reproduction, external fertilization, and rapid development,

zebrafish are ideally suited for higher-throughput functional studies of conserved neurodevelopmental genes. Generating mosaic knockout embryos for an initial 12 candidate zebrafish orthologs, we assayed behavior and morphometric features at 5 days post-fertilization (dpf). We observed significant differences (p-value < 0.05) in distance between the eyes relative to body length for eight crispant mutants (nckap1, pink1, chd8, kmt2e, arid1b, huwe1, ythdc1, and ptena) and distance moved without stimuli over 1 hour for six crispant mutants (nckap1, chd8, wdfy3, ptena, ythdf2, and ythdc1) versus controls. Work is ongoing to characterize crispants for an additional eight genes at 5dpf and all twenty at 3 dpf alongside generation of single-cell (sc) RNA-sequencing libraries of isolated larval heads for all twenty conditions at 3 dpf. While we and others have shown mutagenesis of ASD-associated genes reproduces larval head size phenotypes, notably no sc RNA-sequencing approach has been applied at scale. Data generated from this work will serve to expand the list of known genes for ASD diagnosis and highlight those resulting in similar cellular and transcriptional outcomes for a comprehensive insight into ASD etiology.

Establishing zebrafish as a model to study Down syndrome and a platform for drug development for neural disorders

Presenting Author: Summer Thyme, PhD - UMass Chan Medical School

Co-Author(s): Mary Shay Capps – UMass Chan Medical School; Anna Moyer – UMass Chan Medical School; Alexia Barcus – UMass Chan Medical School; Ari Ginsparg – UMass Chan Medical School; Jessica Chrabasz – UMass Chan Medical School; Claire Conklin – University of Alabama

Abstract: Down syndrome occurs in approximately 1 in every 700 live births and is the most common genetic cause of intellectual disability worldwide. There are currently no FDA-approved drug therapies targeting intellectual disability, which is a major factor limiting quality of life for people with Down syndrome. In addition to deficits in learning and memory, individuals with Down syndrome possess reduced cerebellar volume, changes in the proportion of brain cell types, craniofacial abnormalities, and Alzheimer's disease. We are establishing a new research program that aims to use zebrafish, a small vertebrate model system, to investigate how overexpression of chromosome 21 orthologs affects brain development. We generated two independent lines with ubiquitous overexpression of more than 20 individual chromosome 21 orthologs and performed whole-brain activity and structure mapping of larval zebrafish. Overexpression of the transcription factor erg causes reduced cerebellar volume and upregulation of genes marking astrocyte-like cells. Given that cerebellar hypoplasia and an increased number of astrocytes are prominent phenotypes in people with Down syndrome, we aimed to understand how erg overexpression causes these phenotypes in zebrafish. To determine the cell types required for cerebellar hypoplasia, we are now overexpressing erg under the control of cell-type-specific promoters. We also used paired single nucleus ATAC-seq and RNA-seq of the adult telencephalon to identify additional regulatory elements for the generation of new cell type-specific drivers. In parallel to this project, our lab has developed a new pipeline for computational screening of virtual compound libraries, and we will use this approach once our studies of Erg nominate ideal downstream targets. In summary, we have generated a zebrafish model that phenocopies two important Down syndrome-associated phenotypes, which paves the way for additional hypothesis-driven research, small molecule screens, and rational drug discovery.

Concurrent Session I

Neurodevelopment — Shannon Hall | 10:30am-12:00pm

Session Chairs: Bushra Raj - *University of Pennsylvania* & Eric Horstick - *West Virginia University*

Multiple genes encoded within the 22q11.2 neurodevelopmental risk locus interact within juxtaventricular glial cells to regulate sensorimotor behavior

Presenting Author: Philip Campbell, MD, PhD - University of Pennsylvania

Co-Author(s):

Abstract: Deletion of a 3Mb region on chromosome 22 (22qDS) encoding 45 protein coding genes predisposes humans to multiple neurodevelopmental disorders and is one of the greatest genetic risk factors for schizophrenia, though the cellular and molecular mechanisms underlying these associations remain incompletely understood. Through an unbiased zebrafish behavioral screen of loss of function animals of conserved genes within the 22qDS deleted region, we identified that loss of prodha, a mitochondrially localized enzyme involved in L-Proline metabolism, leads to marked sensorimotor behavioral phenotypes. Further, we find that prodha is enriched in juxtaventricular glial cells and that it is specifically required acutely, rather than developmentally, within these cells to regulate sensorimotor behaviors. RNA sequencing of prodha-deficient juxtaventricular glial cells also reveals an upregulation of genes involved in mitochondrial translation and microRNA biogenesis. Notably, two other 22qDS genes (mrpl40, a mitochondrial ribosomal component, and dgcr8, a key regulator of microRNA processing) play direct roles in these pathways and double mutant analysis suggests that mrpl40 and dgcr8 can partially compensate for prodha loss. Together, these findings reveal a convergent role for multiple 22qDS genes within juxtaventricular glia and suggest that glial dysfunction may contribute to brain and behavioral phenotypes in 22qDS.

Circuit mechanisms of gravity-guided locomotion and balance deficits

Presenting Author: Yunlu Zhu, PhD - NYU Grossman School of Medicine

Co-Author(s): Hannah Gelnaw – NYU Grossman School of Medicine; Franziska Auer – NYU Grossman School of Medicine; Kyla Hamling – NYU Grossman School of Medicine; Paige Leary – NYU Grossman School of Medicine; Qing Bai – University of Pittsburgh School of Medicine; Shin

Abstract: The sensation of gravity anchors our perception of the environment and is fundamental to balance control during locomotion. Balance impairments manifest as postural instability and gait variability. However, the neural circuits that transform gravity into commands for balance control remain unclear. We first defined sensorimotor circuits underlying gravity-guided balanced behaviors in free-swimming larval zebrafish. Using a loss-of-function approach, we delineated two vestibular circuits consisting of evolutionarily conserved brainstem architecture: a hindbrain-spinal-cord circuit underlying postural stability and a hindbrain-midbrain-spinal-cord circuit that maintains swim consistency. Next, we examined the same circuits in dysfunction by modeling tau-mediated balance deficits that define a sporadic tauopathy. We observed that the severity of circuit-specific deficits in postural stability and swim consistency correlated with the human tau load in corresponding balance neurons, even in the absence of cell death. Surprisingly, using 2-photon imaging, we observed network-level compensation of balance neuron function following tau accumulation. Our results parametrically linked tau load in specific neurons and circuits to behavioral deficits. These findings reveal the neural basis of balance

control and bridge the gap between cell types, pathological characteristics, and clinical presentations of tauopathy.

Radial astroglia cooperate with microglia to clear neuronal cell bodies during zebrafish optic tectum development

Presenting Author: Heather Barber, MS - University of Virginia

Co-Author(s): Robin Brown – University of Virginia; Zachary Cutler – University of Virginia; Sarah Kucenas – University of Virginia; Cassidy Robbins – University of Virginia

Abstract: During development, networks between neurons must be established, refined, and maintained, with unnecessary neurons requiring timely removal. The contributions of astroglia to these developmental processes are only just being explored. The zebrafish optic tectum (OT) is a common model for investigating neural circuitry. Using in vivo, time-lapse imaging of Tg(slc1a3b:myrGFP-P2A-H2AmCherry) transgenic larvae, we observe OT radial astroglia extending projections from their basal processes between the glial limitans and ventricular zone. These small processes form large spherical structures, measuring approximately 5 micrometers in diameter and lasting up to 12 hours before dissipating. The formation of these structures coincides with optic nerve invasion into the OT and a wave of neuronal apoptosis. To our knowledge, this phenomenon has not been previously described. We thus utilized the extensive genetic, pharmacological, and imaging techniques available in zebrafish to further investigate. Our work reveals that these projections surround the cell bodies of dying neurons concurrent with an increase in neuronal apoptosis. Due to their physical characteristics and engulfment of other cell bodies, we tentatively dub these structures “scyllate heads”, in reference to the multi-headed Odyssean figure Scylla. Scyllate heads rarely acidify or internalize into the astroglial soma, suggesting they are not phagosomes. We observe microglia interacting with scyllate heads by invading them and removing their contents, which the microglia then efficiently degrade. When we deplete microglia via drug treatment, scyllate head duration doubles, indicating that interactions with microglia are important for their timely dissipation. Due to their containment of dying neurons, non-degradative nature, and association with microglia, we hypothesize that scyllate heads sequester developmental debris for later phagocytosis by microglia. These studies provide new insight into the diverse roles of astroglia and the way that non-professional phagocytes cooperate with professional phagocytes to effectively clear large numbers of dying cells during development.

Dynamics of the neuronal cytoskeleton in zebrafish

Presenting Author: Pascale Bomont, PhD - INMG-PGNM

Co-Author(s): Leticia Arias – INMG-PGNM; Farah Kotaich – INMG-PGNM; Stéphanie Portet – University of Manitoba Canada

Abstract: Neurofilaments (NFs) are neuronal Intermediate Filaments, a large family that forms the cytoskeleton of the cell together with the actin filaments and microtubules. Structural scaffold of the cell, NFs exhibit essential functions in neurons and are key actors in neurodegeneration. Indeed, NFs are not only a genetic cause of several neuropathies in human, their abnormal aggregation is an early common pathological hallmark of neurodegenerative diseases. While the proof-of-concept of removing NFs as a therapeutic avenue has been obtained in disease mouse models, it could not be translated to human due to the lack of appropriate biological systems and our poor knowledge on NF biology. Our

laboratory exploits the advantages of the zebrafish species to scrutinize in vivo the dynamics of this cytoskeleton network, with the aims to monitor the behavior of NFs in a physiological context and to uncover the yet unknown mechanisms triggering neurodegeneration in disease. Combining novel NF zebrafish lines with state-of-the-art imaging methodologies, we demonstrate that NFs exhibit anterograde and retrograde transport in axons, with a balance that evolves during development. We provide mathematic modeling for the quantification in space and time of NF flux and for the deciphering of the behavior of individual filaments. Moreover, we reproduced selected NF-disease mutants to dissect in zebrafish and other systems the mechanisms underlying aggregation and neuronal dysfunctions in disease. This project will shed light into the dynamics and signaling of NFs, a cytoskeleton network mostly seen as static, that can be targeted to develop effective therapies for human diseases.

Serotonin acutely regulates acoustic behavior selection in zebrafish through multiple HTR2 receptor subtypes

Presenting Author: Roshan Jain, PhD - Haverford College

Co-Author(s): Nicholas Roland – Cornell University; Matthew Curran – University of Pennsylvania Perelman School of Medicine; Rebecca Osbaldeston – Haverford College; Kevin Villafañe – Haverford College; Jacob Krawitz – Haverford College

Abstract: Neuromodulators such as serotonin (5-HT) regulate many aspects of behavior including mood, social interactions, sleep, and decision-making to allow animals to flexibly respond to their changing environment. We focused on simple acoustically-evoked behavior selection in zebrafish to model and elucidate the effects of serotonin on decision-making and behavioral flexibility. Following sudden acoustic stimuli, zebrafish larvae select between two stereotyped escape behaviors: an explosive short-latency response or a kinematically distinct and less vigorous long-latency response, biasing their response selection based on the perceived threat, environmental context, and recent stimulus history. This behavioral flexibility is modulated by serotonin, and using an array of pharmacological antagonists and agonists of various serotonin receptors, we show that serotonin receptor subtypes 5-HT1A, 5-HT2, and 5-HT4 acutely bias zebrafish escape behavior selection in response to acoustic stimuli. To pinpoint the specific serotonin receptors responsible for biasing this behavior selection, we designed CRISPR/Cas9 gene knockout tools to target candidate serotonin receptor subtype genes. Through combined pharmacology and genetics, we demonstrate that 5-HT2B and 5-HT2CL2 receptor activity promotes the selection of long-latency escape behaviors over short-latency responses. Surprisingly, we also find that the paralogous 5-HT2CL1 receptor biases behavior selection in the opposite direction, promoting short-latency escape behavior. Together, these findings reveal multiple receptor mechanisms through which serotonin bi-directionally modulates simple and ethologically relevant vertebrate decision-making following acoustic threat.

The heart-brain balancing act: The function and development of motor and sensory circuits for cardiac feedback control

Presenting Author: Luis Hernandez-Nunez, PhD - Harvard University / Stanford University

Co-Author(s): Joana Avrami – Harvard University; Sky Shi – Stanford University

Abstract: Neural control of cardiac function is essential for survival, yet the functional diversity of the sensory and motor circuits of the heart remains poorly understood. Here we take a multidisciplinary approach, combining systems neuroscience techniques, genetics, and control theory to study the role of cardiac sensory and motor circuits in larval zebrafish. While larval zebrafish's optic and genetic accessibility has made it a widely used organism for studying how the brain processes environmental cues to modulate behavior, it had not yet been used to study organ control or the autonomic nervous system (ANS) from a systems neuroscience perspective. Thus, we use calcium imaging, optogenetics, pharmacology, and electron microscopy to map the developmental time course of anatomical and functional innervation of the heart. We identify the emergence of parasympathetic and sympathetic control of the heart, as well as the anatomically defined neural populations needed for heart modulation. We also show the onset of cardiac sensing and identify a new type of interoceptor. Our study provides a timeline of developmental landmarks of the autonomic circuits for heart feedback control and sets the stage for future mechanistic studies of neurocardiac circuits.

Infection & Immunity — Play Circle Theater | 10:30am-12:00pm

Session Chairs: Sofia de Oliveira – Albert Einstein College of Medicine & Penny Lam – Medical College of Wisconsin

Neutrophil Dynamics in Polytraumatic Injury: Insights from a Zebrafish Model

Presenting Author: Sofia de Oliveira - Albert Einstein College of Medicine

Co-Author(s): Cassia Michael – Graduate Student, Development and Molecular Biology, Albert Einstein College of Medicine; Joaquin Canton Sandoval – Postdoc, Development and Molecular Biology, Albert Einstein College of Medicine

Abstract: Unintentional injuries are the leading cause of death for individuals under 50, with polytraumatic injuries posing significant challenges. Despite medical advancements in the field, mortality and complications remain high, particularly in individuals with metabolic conditions such as diabetes and cardiovascular disease. Polytraumatic injury involves concurrent damage to multiple organs, where immune responses play a crucial role in outcomes. Neutrophils, the first responders to injury, rapidly react to inflammatory signals and regulate tissue regeneration. Our lab has demonstrated that neutrophils orchestrate the inflammatory response to injury and that metabolic dysfunction disrupts their function, leading to delayed and dysregulated responses associated with impaired regeneration. While neutrophil behavior in single injuries is well studied , their response to polytrauma remains poorly understood, particularly on a systemic level. Using transgenic zebrafish larvae as an in vivo model, we developed innovative approaches to study neutrophil behavior across multiple organs, including the liver, eye, spinal cord, and skin. Our findings show that neutrophils prioritize recruitment to complex organs such as the liver and spinal cord over simpler skin epithelial injuries. Their migration patterns and behavior also differ between polytrauma and single-injury contexts, with a specific behavior signature emerging in polytrauma. However, diet-induced systemic inflammation disrupts this prioritization, with neutrophils displaying lower capacity to respond to injury in complex organs and favoring recruitment to less complex injuries like skin epithelium. Additionally, when zebrafish larvae are exposed to lipopolysaccharide (LPS) 24 hours post polytrauma, simulating sepsis, diet-induced inflammation dramatically worsens survival following polytrauma, reducing survival rates from 93% to 45%. Our work reveals that neutrophil recruitment in polytrauma follows distinct behavior signatures and is significantly impaired by the presence of diet-induced systemic inflammation. Furthermore,

zebrafish provide a valuable model for studying immune responses across multiple organs, offering insights into systemic inflammation and healing in polytraumatic injuries.

The molecular basis of neutrophil reverse migration

Presenting Author: Yiran Hou, PhD - University of Wisconsin-Madison

Co-Author(s): Julie Rindy – University of Wisconsin-Madison; Shreya Vattem – University of Wisconsin-Madison; Anna Huttenlocher – University of Wisconsin-Madison

Abstract: Neutrophils are first responders to infections and tissue injury. While the initial presence of neutrophils provides the immune response required to fight infections, their extended stay at wounds could lead to chronic inflammation, hindering tissue repair. Reverse migration, as one of the major mechanisms for neutrophil clearance from wound site, is an attractive target for achieving local inflammation resolution without causing systemic immunosuppression. Although past works have identified individual factors and pathways involved in reverse migration, a systemic understanding of its molecular basis is still missing. To achieve unbiased characterization of neutrophil reverse migration, we aimed to profile neutrophils in sterile wounding conditions in zebrafish, the model organism where the phenomenon of neutrophil reverse migration was first discovered. Using the transgenic fish line Tg(mpx:Dendra2) with neutrophils carrying a photoconvertible fluorescent reporter, we labeled neutrophils arriving at the wound site in early recruitment stage by photoconversion and separated them from the rest of when local inflammation resolves through fluorescence-activated cell sorting (FACS). By comparing the single cell transcriptomes between the sorted photoconverted and other neutrophils, we found enrichment of padi2+ population in the reverse migrated group, suggesting biased instead of equal involvement of neutrophil subpopulations. In addition, we identified key molecular signatures up or down-regulated in the reverse migrated population. Preliminary genetic manipulation on kdelr2b, one of the top candidates, revealed its necessity in regulating wound site inflammation. This suggests that kdelr2b may act in the same pathway as its putative ER rentation target myeloid-derived growth factor (mydgf) in limiting neutrophil inflammation. Further functional testing on other top candidates showing myeloid-specific expression pattern in the Zebrahub atlas are ongoing, potentially revealing the molecular network involved in regulating neutrophil reverse migration. Our targeted molecular profiling provides new perspectives in understanding neutrophil reverse migration.

Visualizing Chemoattractant Gradients in Zebrafish Using GEM-Sensors: A Novel Family of Genetically Encoded Biosensors

Presenting Author: Balazs Enyedi - Semmelweis University

Co-Author(s):

Abstract: Chemoattractant gradients play a central role in directing cellular migration through the activation of specific G protein-coupled receptors (GPCRs). While the signaling pathways controlling chemotaxis are well characterized, direct visualization of chemoattractant and chemokine distribution in live tissues remains a major technical hurdle. Previously, we developed GEM-LTB4, a genetically encoded fluorescent biosensor for leukotriene B4 (LTB4), a key lipid mediator of chemotaxis and neutrophil swarming. This biosensor enabled the detection of LTB4 release from neutrophils ex vivo, however, in vivo measurements have remained challenging due to sensor sensitivity and dynamic range limitations. Here, we present a high-throughput platform for engineering and evolving GPCR-based

fluorescent biosensors. By inserting conformation-sensitive green and red fluorescent protein variants (cpEGFP or cpmApple) into the third intracellular loop of various GPCRs, with randomized linker lengths and amino acid compositions, we generate large libraries of sensor variants. Screening these libraries to identify optimal sensor variants, followed by iterative cycles of directed evolution, has yielded sensors showing 200-1000% fluorescence signal increase upon ligand binding. Using this approach, we have successfully converted multiple GPCRs into biosensors, generating a suite of sensors capable of detecting extracellular gradients of key chemoattractants and chemokines, including fMLF, IL-8, and 5-KETE, in both green and red fluorescence channels. Additionally, we have developed GEM-LTB4-2.0, an improved LTB4 biosensor with a fivefold increase in signal response upon ligand binding compared to the original GEM-LTB4, significantly enhancing sensitivity for in vivo applications. These genetically encoded GEM-sensors enable real-time visualization of chemoattractant dynamics in live zebrafish. We have also developed a toolset for their tissue-specific expression, allowing the measurement of chemoattractant production near neutrophils, macrophages, epithelial, and endothelial cells in zebrafish using the QF2-QUAS system. This technology provides a powerful platform for investigating the regulation of cell migration and inflammatory processes in vivo.

Decoding innate immune PRRs in zebrafish

Presenting Author: Emily Rosowski, PhD - Clemson University

Co-Author(s):

Abstract: Innate immune cells use pattern recognition receptors (PRRs) to recognize microbe-associated molecular patterns (MAMPs) to protect against infection. How different PRRs recognize MAMPs, and how PRR-MAMP interactions are integrated into a complete immune response is not fully understood. In non-mammalian models like zebrafish, this problem is compounded by a lack in understanding of PRR homology across species. To investigate these questions, we use a larval zebrafish-Aspergillus fumigatus infection model. A. fumigatus is a fungal pathogen that infects organisms in the spore form, in which its MAMPs are masked, then grows through germination into hyphae and its MAMPs become exposed, making it an excellent model to study the role of different PRRs throughout a multi-day infection. In human cells, A. fumigatus is sensed by the Toll-like receptor (TLR) Tlr2, the Nod-like receptor (NLR) Nod1, and the C-type lectin receptor (CLR) Dectin-1. We find that in larval zebrafish, mutation of tlr2 leads to a significant decrease in macrophage recruitment to A. fumigatus and a significant decrease in spore killing, suggesting that Tlr2 is used to initially sense and respond to fungal spores. Zebrafish also possess a predicted copy of nod1, however we have identified an alternative transcript of this gene that does not contain the domains generally required for function of an NLR. Expression of this transcript with a fluorescent tag in macrophages demonstrates that this protein can associate with A. fumigatus spores inside of macrophages, but its function is still unknown. Zebrafish homologs for Dectin-1 have long been unidentified, and we have confirmed that two putative homologs, Clec4c and Sclra/Cldc1, mediate sensing of fungal MAMPs by macrophages and neutrophils in the larval model. Further understanding of PRR function in larval zebrafish will improve this model of human disease and illuminate how different PRRs work together to fight infection.

A whole animal drug screen reveals a molecule stimulating sustained immune responses

Presenting Author: Hannah Young - University of Utah

Co-Author(s): Nels Elde – University of Utah

Abstract: Drug screens play a crucial role in identifying broadly acting modulators of immune responses to viruses. However, screens are typically performed in cultured cells, which lack most of the complexity of whole organisms, such as mucosal barriers and immune effectors. Culture-based screens are therefore limited in the ability to model organism-level effects. We developed a high-throughput, organism-level drug screening method using a transgenic zebrafish line that expresses GFP in response to activation of the type 1 interferon response—a key component of the innate immune defense against viruses. We used these fish to screen the Prestwick Chemical Library and identified small molecules that activate the interferon response throughout the animal. We identified the quaternary ammonium cation dequalinium (DEQ) to be a potent interferon activator. DEQ has not been previously identified in culture-based screens, yet it induces a systemic interferon response in larval zebrafish, which we verified by quantitative PCR and confocal microscopy. Zebrafish larvae treated with DEQ express high levels of interferon stimulated genes even 3 days post treatment, suggesting they experience a sustained immune response. These results starkly contrast what we observe with traditional interferon agonists that typically induce a transcriptional response that decreases after 8-12 hours. Additionally, treating larvae with DEQ prior to infection with a natural pathogen, zebrafish picornavirus 1, significantly reduced the amount of virus relative to untreated controls. We hypothesize DEQ may be activating the interferon response through its mitotoxic activity, as we find disrupted mitochondrial function in the fish following DEQ treatment. These results highlight the importance of organism-based drug screens and indicate that DEQ may be a potential antiviral therapeutic.

Integrating archive-wide virus discovery with whole immune system profiling

Presenting Author: Keir Balla - Chan Zuckerberg Biohub SF

Co-Author(s): Yttria Aniseia – Chan Zuckerberg Biohub SF; Eric Waltari – Chan Zuckerberg Biohub SF; Deepika Sundarraman – Chan Zuckerberg Biohub SF; Jordao Bragantini – Chan Zuckerberg Biohub SF; Jennifer Doherty – Chan Zuckerberg Biohub SF; Jared Nigg – Chan Zuckerbe

Abstract: Viruses are nearly ubiquitous but often unobserved elements of biological systems. RNA sequencing (RNA-seq) has emerged as an unbiased means for detecting viruses and is also a common tool for investigating gene expression in any organism. We have developed a generalizable pipeline that takes raw sequencing data as input and generates host gene expression data coupled with virus discovery and quantification as output. We applied this pipeline to uniformly process all publicly available RNA-seq data generated with zebrafish and discovered hundreds of thousands of virus sequences along with signatures of antiviral gene expression. These discoveries enable multiple opportunities for leveraging the strengths of zebrafish to investigate immune system organization and function in vertebrates at an unprecedented scale. We generated transgenic zebrafish to label all immune cells and have developed image acquisition and analysis tools that enable dynamic segmentation and tracking of every cell in the immune system. Sequencing revealed that our imaging approach captured every kind of immune cell that has been described so far in zebrafish. Analysis of cell movements revealed distinct classes of immune behaviors. We are currently determining the extent to which cell type and state can be decoded from morpho-kinetic parameters alone, and are applying these measurements to understand how virus infection is resolved at the organismal scale.

Cell Biology — Wisconsin Historical Society | 10:30am-12:00pm

Session Chairs: Alexa Burger - *University of Colorado Anschutz Medical Campus* & Chuck Kaufmann

Mechanosensing-driven cell competition ensures robust morphogen gradient formation.

Presenting Author: Tohru Ishitani - RIMD(Biken), Osaka University

Co-Author(s):

Abstract: Morphogen gradients instruct cells to pattern tissues. Although the mechanisms by which morphogens transduce chemical signals have been extensively studied, the roles and regulation of the physical communication between morphogen-receiver cells remain unclear. By zebrafish in vivo imaging, genetic modification, and transcriptome analyses, we reveal that Mechanical communication between morphogen-receiver cells ensures robust morphogen gradient formation. The Wnt/β-catenin–morphogen gradient, which patterns the embryonic anterior-posterior (AP) axis, generates intercellular tension gradients along the AP axis by controlling membrane cadherin levels in zebrafish embryos. This “mechano-gradient” is used for the cell competition–driven correction of noisy morphogen gradients. Naturally and artificially generated unfit cells, producing noisy Wnt/β-catenin gradients, induce local deformation of the mechano-gradients that activate mechanosensitive calcium channel PIEZO1 in the neighboring fit cells, which then secrete Annexin A1 to kill unfit cells. Thus, our study shows the mechano-gradient and the morphogen gradient correction mediated by the mechano-gradient, which is a previously unidentified mechano-chemical intercellular communication mechanism during embryogenesis. This discovery has prompted a re-evaluation of the conventional concept of morphogen systems and provides new insights into intercellular communication mechanisms regulating development, regeneration, and homeostasis. At this meeting, we will also introduce our latest findings, including the involvement of this mechano-sensing system in the elimination of de novo mutant cells and the prevention of congenital diseases.

PCM1 Coordinates Centrosome Asymmetry and Polarized Endosome Dynamics to Regulate Neural Progenitor Cell Fate

Presenting Author: Xiang Zhao, PhD - Chan Zuckerberg Biohub San Francisco

Co-Author(s):

Abstract: Vertebrate radial glia progenitors (RGPs), the principal neural stem cells, balance self-renewal and differentiation through asymmetric cell division (ACD), during which unequal inheritance of centrosomes is observed. Mechanistically, how centrosome asymmetry leads to distinct daughter cell fate remains unclear. Here we find that Pericentriolar material 1 (Pcm1), asymmetrically distributed at the centrosomes, regulates polarized endosome dynamics and RGP fate. In vivo time-lapse imaging and nanoscale-resolution expansion microscopy of zebrafish embryonic RGPs detect Pcm1 on Notch ligand-containing endosomes, interacting with the polarity regulator Par-3 and dynein motor, either directly or indirectly. Loss of pcm1 disrupts endosome dynamics, with clonal analysis uncovering increased neuronal production at the expense of progenitors. Pcm1 facilitates an exchange of Rab5b (early) for Rab11a (recycling) endosome markers and promotes the formation of Par-3 and dynein macromolecular complexes on recycling endosomes. Finally, in human-induced pluripotent stem cell-derived mitotic neural progenitors of brain organoids, PCM1 shows asymmetry and co-localization with CEP83, a

centriolar component necessary for primary cilia formation. PCM1 also co-localizes with PARD3 and RAB11A mitotic human neural progenitors. Our data reveal a new mechanism by which centrosome asymmetry and polarized endosome dynamics are coordinated by Pcm1 in regulating ACD and progenitor fate.

The core planar cell polarity complex regulates pronephric collective cell migration

Presenting Author: Sarah Paramore, PhD - University of Chicago

Co-Author(s): Sally Horne-Badovinac – University of Chicago; Victoria Prince – University of Chicago

Abstract: During the second day of development, the epithelial cells of the zebrafish pronephros begin a days-long collective migration towards the anterior of the embryo, driving morphogenesis of the pronephric duct. It was previously shown by the Drummond lab that 1) this migration is powered by the extension and retraction of migratory protrusions at the basal cell surface and 2) lumenal fluid flow is the directional cue for migration orientation. These data pose an intriguing question: if fluid flow sensed at the apical surface dictates the orientation of migratory protrusions at the basal surface, how is this polarity information relayed through the cell? Here, we investigate the role of the core planar cell polarity (PCP) complex in localizing migration machinery. The core PCP complex consists of three transmembrane proteins: Vangl, Celsr, and Frizzled. These proteins form intercellular junctions that localize asymmetrically across the plane of the tissue, allowing relay of polarity information. PCP is required for the asymmetric localization of cilia within the pronephros, but its possible role in migration is unexplored. Using immunofluorescence analysis and mosaic expression of the PCP component Vangl2, we have found that Vangl2 is asymmetrically localized within the pronephros by 24 hpf, indicating that PCP is expressed in the right time and place to regulate migration. Moreover, we show that acute degradation of Vangl2 at 2 dpf immediately halts migration, demonstrating a requirement for the core PCP complex. Our current work is focused on investigating the interplay between pronephric fluid flow and the polarization of PCP proteins. To this end, we have recently optimized high-resolution live-imaging of pronephric migration, which we are using in combination with an endogenously-tagged Vangl2 transgenic line to observe – in real time – how Vangl2 polarization changes when we manipulate fluid flow using both surgical and genetic methods.

Developmental Regulation of Epithelial Polarization by pre-mRNA Splicing

Presenting Author: Andressa Pacheco Czaikovski - Duke University

Co-Author(s): Daniel Levic – Assistant Research Professor of Cell Biology, Cell Biology, Duke University; James Norman – Lab Research Analyst II, Cell Biology, Duke University; Claudia Carugati – Student - Undergraduate, Duke University; Manuel Irimia – Group Leader,

Abstract: In the developing zebrafish intestine, epithelial cells mature over a period of several days before they become fully polarized. This polarization is critical for intestinal physiology as it mediates fluid and macromolecule transport across the apical or basolateral plasma membrane. Previous research identified sorting signals and adaptors essential for basolateral transport of membrane proteins. However, mechanisms regulating sorting of apical membrane proteins are more complex and have remained poorly understood. We previously demonstrated that acidification of the trans-Golgi network (TGN) is crucial for apical sorting. Here, using a forward genetic screen, we found that minor-type (U12) intron splicing regulates TGN acidification and apical sorting via the expression of select ion channels.

While mutations in minor intron splicing machinery exhibit broad changes in U12 intron retention, loss of individual ion channels is sufficient to perturb apical sorting. We also conducted transcriptomic analyses from purified intestinal cells to investigate the relationship between minor intron splicing and epithelial maturation during gut development. Our results suggest that minor intron splicing is a regulatory module to control expression of TGN-localized ion channels that promote acidification and aid in apical sorting, which we propose is mediated by cargo clustering. Finally, we developed high precision, quantitative imaging assays to monitor protein oligomerization in the TGN in vivo. Overall, our study provides a mechanistic view of how epithelial cells polarize their apical surface and how this process is regulated to allow the intestine to conform to physiological demands over time.

BMP receptor trafficking and sub-functionalization in signal transduction during embryonic patterning in the zebrafish

Presenting Author: Jeet Patel, PhD - University of Pennsylvania

Co-Author(s): Benjamin Tajer – University of Pennsylvania; Mary Mullins – University of Pennsylvania

Abstract: Bone morphogenetic protein (BMP) signaling is a major driver of developmental processes, including bone development, organ system formation, and patterning of the dorsoventral (DV) axis, neural tube, and limbs. The role of BMP signaling in DV patterning is conserved from insects through humans, one interesting feature of which is signaling via BMP heterodimers. In zebrafish, Bmp2-Bmp7 heterodimers signal through a heteromeric BMP receptor complex of Type I Acvr1l and Bmpr1, along with two Type II Acvr2. We recently showed that, while both Type I receptors are required for DV patterning, only Acvr1l phosphorylates Smad1/5, while Bmpr1a kinase activity is dispensable. While prior paradigms postulated that both Type I receptors activate downstream Smads, this sub-functionalization for a single kinase when two are available has also been observed in other contexts. Specialized receptor requirements present a range of possible mechanisms by which heteromeric receptor complexes could be leveraged to regulate signaling. Using live-imaging approaches, I found Acvr1l traffics intracellularly via endocytosis, while Bmpr1a localizes primarily to the membrane and is rarely trafficked. As Acvr1l is the required kinase, trafficking may facilitate signal transduction. Indeed, Acvr1l and Acvr2 are frequently found in the same endosomes. Receptor co-trafficking is consistent with a model in which Acvr2 phosphorylates Acvr1l to activate Smad5. Overexpression of both Acvr1l and Acvr2 increases Acvr1l endosome density and embryonic ventralization, a phenotype indicative of increased BMP signaling, not observed with either receptor alone. Interestingly, more Acvr1l endosomes are observed with constitutively active Acvr1l, which obligately phosphorylates Smad5 to ventralize the embryo, suggesting a link between receptor trafficking and signaling. Using mutants for extracellular BMP regulators, BMP receptors, and Smad5, I will further interrogate the requirements for endocytosis of Acvr1l in signal transduction and embryonic patterning, defining cell biological mechanisms of BMP regulation in vertebrate development.

Phosphotyrosine-Independent Interactions Between the Cell Adhesion Molecule Jam2a and Adaptor Proteins Crk/Crkl During Zebrafish Myoblast Fusion

Presenting Author: Zhou Luo - UT Southwestern Medical Center

Co-Author(s): Zhi-Rong Ruan – UT Southwestern Medical Center; Danqing Tong – UT Southwestern Medical Center; Yingying Hu – UT Southwestern Medical Center; Xueya Cao – UT Southwestern Medical Center; Elizabeth Chen – UT Southwestern Medical Center

Abstract: Myoblast fusion is an indispensable process in skeletal muscle development and regeneration. Previous study revealed that zebrafish myoblast fusion is mediated by F-actin-propelled invasive protrusions at the fusogenic synapse, and the cell adhesion molecule, Jam2a, is the major organizer of the invasive structure. However, how Jam2a engages with the actin cytoskeletal regulators to generate invasive protrusions is unknown. Here, we show that the SH2 and SH3 domain-containing adaptor proteins, Crk and Crkl, are enriched within the F-actin structure at the fusogenic synapse and function redundantly to promote actin foci formation and myoblast fusion. Using a split-GFP assay, we show that Crk and Crkl interact with the intracellular domain of Jam2a, as well as downstream regulators of the actin polymerization machinery, including Wasp, Wip and Dynamin. Strikingly, fusing Crk to the C-terminus of a truncated Jam2a that lacks its cytodomain (Jam2a□C-Crk) effectively rescues the fusion defect of jam2a mutant, demonstrating that the major function of the Jam2a cytodomain is to recruit Crk, which in turn brings downstream actin regulators to the fusogenic synapse to promote actin polymerization and filament bundling. Interestingly, we found that the SH2 domain of Crk does not interact with the two tyrosine residuals in the Jam2a cytodomain, nor are these tyrosines required for Jam2a's function, suggesting that Crk interacts with Jam2a via a phosphotyrosine-independent mechanism. Indeed, we mapped the Crk-binding motif in the Jam2a cytodomain and identified a novel four amino acid motif that is required for Crk interaction. Taken together, our study demonstrates that Crk/Crkl are the intermediary adaptor proteins that link Jam2a to the downstream actin machinery via a novel mechanism to promote invasive protrusion formation and myoblast fusion.

Plenary Session III:

Organ Formation & Function — Shannon Hall | 1:00-2:30pm

Session Chairs: Daniela Panáková - *Max Delbrück Center for Molecular Medicine* & Thomas Juan - *Uppsala University*

Live Imaging of Compensatory Lymphangiogenesis in Zebrafish Larvae During Edema Resolution

Presenting Author: Hyun Min Jung, PhD - University of Illinois at Chicago

Co-Author(s): Olamide Olayinka – Pharmacology and Regenerative Medicine – University of Illinois at Chicago; Hanna Ryu – Pharmacology and Regenerative Medicine – University of Illinois at Chicago; Xiaowei Wang – Pharmacology and Regenerative Medicine – University of I

Abstract: Edema, defined by the accumulation of interstitial fluid, is a hallmark of numerous pathological conditions and poses significant therapeutic challenges. Efficient lymphangiogenesis plays a critical role in edema clearance, and delayed or insufficient lymphatic responses severely impair the healing process. Despite its importance, real-time tracking of lymphangiogenesis during edema resolution in animal models remains underexplored. In this study, we employed an osmotic imbalance strategy to induce edema and investigate the dynamic remodeling of lymphatic vessels during recovery. Using intravital imaging of transgenic zebrafish larvae, we observed significant lymphatic vessel remodeling during edema resolution. Notably, we identified an increase in lymphatic endothelial progenitor cells and a sustained expansion of primary lymphatics, highlighting the active processes of lymphangiogenesis

during recovery. Furthermore, employing the innovative RiboTag system allowed us to profile the translatome of lymphatic and venous endothelial cells, revealing the upregulation of key pro-lymphangiogenic VEGF signaling pathways during edema resolution. Inhibition of compensatory lymphangiogenesis hindered edema fluid clearance, underscoring the crucial role of lymphatic activation in this process. Our findings establish a robust in vivo model for live imaging of compensatory lymphangiogenesis and offer novel insights into the molecular mechanisms driving lymphatic activation during edema resolution.

Reverse genetics at single-cell resolution reveals lineage-specific programs in shared tissues

Presenting Author: Lauren Saunders - Heidelberg University

Co-Author(s): Arish Shah – Postdoc, Centre for Organismal Studies, Heidelberg University; Sanjay Srivatsan – Assistant Professor, Basic Sciences Division, Fred Hutchinson Cancer Center; David Kimelman – Professor Emeritus, Genome Sciences, University of Washington; Co

Abstract: Single cell transcriptomics facilitates characterization of cell fate decisions embryo-wide and, when coupled with perturbations, enables molecular phenotyping to dissect genetic programs during development. Our new experimental and analytical approach for high-resolution phenotyping of individually barcoded zebrafish embryos captures multi-scale responses to dozens of perturbations over developmental time. Using this approach, we profiled 3.2M cells from 1800 barcoded individuals, during normal development and in response to 23 genetic perturbations over time. The high degree of replication enables sensitive detection of perturbation-dependent cell type composition changes. Time-series profiling of classic notochord mutants revealed a cryptic population of brachyury-independent cells with a striking transcriptional resemblance to notochord sheath cells. Given the contribution from multiple embryonic sources — neural crest and mesoderm – to cranial development, this unexpected similarity highlights parallel paths to cartilage development in which transcriptional signatures reflect embryonic origins rather than tissue function. Convergent differentiation, in which multiple lineages produce similar cell types, is increasingly detected during vertebrate development. This offers a unique vantage to understand the conserved and divergent genetic modules underlying shared cell fate decisions. To better understand mechanisms of this process, we are investigating genetic perturbations in which either mesodermal or neural crest-derived cranial cartilage is affected. For example, cranial neural crest cells are uniquely sensitive to global ribosome biogenesis disruption. By integrating our high-resolution phenotyping approach with fate mapping and cross-species studies, our new lab is exploring the plasticity of genetic networks and cellular behaviors underlying morphological diversity in vertebrates.

Expanded knock-in targeting to visualize cellular niches and stimulate pancreatic beta-cell maturation

Presenting Author: Olov Andersson - Uppsala University

Co-Author(s): Agnese Kocere – Uppsala University; Lipeng Ren – Uppsala University; Jiarui Mi – Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University

Abstract: We have previously reported a method to generate 3’ non-destructive knock-in zebrafish lines using CRISPR-Cas9. However, such method using short homologous arms relies heavily on the availability of gRNA targets spanning over the STOP codon region. Here, we introduced a new strategy, which is based on the same PCR amplification strategy to yield dsDNA with 5' end protection as the knock-in

template but utilized gRNA targeting sites upstream of the STOP codon in the last exon. This method easily results in a knock-in at the expense of a short truncation of the C-terminus of the endogenous gene product. We successfully used this method to generate zebrafish lines with dual cell labeling and lineage tracing functions at the hand2 and nkx6.2 loci. In addition, using these strains, we identified that the hand2+ cells are heterogeneous in the liver and can form an important niche factor for hepatic ducts. In the pancreas, we found that nkx6.2 labels the functional proportion of beta-cells indicating maturity, and following beta-cell ablation the regeneration of beta-cells appears in different cellular states. As the functional proportion of beta-cells appears in the posterior part of the pancreatic islet we use this property to screen for secreted peptides/proteins that can accelerate maturation of beta-cells in the anterior part of the islet, a feature that may be a useful for identifying future treatments for diabetes. In summary, our research pipeline further expands the utility of 3' end knock-in in zebrafish, and the generated zebrafish lines are powerful tools in addressing key questions in developmental biology and tissue regeneration.

Enteroendocrine cell signaling wires the gut-brain vagal axis

Presenting Author: Lihua Ye - The Ohio State University

Co-Author(s): Melissa Brewer – The Ohio State University; Christina Lillesaar – University Hospital of Würzburg; Alexander Runyon – The Ohio State University

Abstract: The vagal sensory neurons innervate visceral organs, including the digestive tract, and transmit the visceral information to the brain. How the vagal innervation coordinates with visceral organ development is an intriguing question that remains largely unknown. Using new zebrafish genetic models to trace the development of the vagal sensory neurons and the intestinal vagal network over time, our data revealed that zebrafish vagal sensory neurons start to innervate the intestine at 2 days post fertilization. Vagal nerve fibers branch out toward the newly formed enteroendocrine cells (EECs) in the intestine epithelium. We found that zebrafish EECs form synaptic connections with vagal sensory nerve fibers like mammals. Importantly, our data showed that the intestinal vagal network development is coupled with the formation of EECs. Genetically ablating EECs impairs vagal intestinal innervation and alters the vagal central projection pattern. Vagal axon tracing revealed that vagal peripheral innervation and central projection mirror each other, and the intestine-innervating vagal sensory neurons project to the posterior branch in the hindbrain. Ablating EECs induces apoptosis in a subset of vagal sensory neurons and reduces the posterior vagal central branch width. Furthermore, ablating EECs completely alters the brain's response to nutrient ingestion and diminishes nutrient-induced hindbrain and hypothalamus neuron activation. Finally, we discovered that subsets of EECs uniquely express Brain-Derived Neurotrophic Factor (Bdnf). Loss of Bdnf impairs the intestinal vagal network. Selectively overexpressing Bdnf in EECs promotes vagal intestinal innervation and enhances the vagal central projection. Together, our study revealed for the first time that EECs guide vagal neuron sensory development and intestinal vagal network formation. We uncovered the novel role of EEC-derived Bdnf signaling in promoting vagal innervation and gut-brain signaling transmission. Selectively manipulating EEC Bdnf signaling is expected to provide novel approaches to modulate intestinal vagal innervation patterns and change gut-brain signaling transmission.

Nr2f1a-Notch signaling interactions balance chamber-specific endocardial identity necessary for cardiac development

Presenting Author: Bitan Saha, PhD - Cincinnati Children's Hospital Medical Center

Co-Author(s): Krishna Krothapalli – Cincinnati Chidren's Hospital Medical Center; Amulya Adavalli – Cincinnati Chidren's Hospital Medical Center; Joshua Waxman – Cincinnati Chidren's Hospital Medical Center

Hypoplastic Left Heart Syndrome (HLHS) is a congenital heart defect characterized by underdeveloped ventricular chambers and can be caused by impaired endocardial development and dysregulation of cardiac jelly, the extracellular matrix (ECM) between myocardium and endocardium. NR2F2, an orphan nuclear receptor predominantly associated with the differentiation of atrial cardiomyocytes, has surprisingly been associated with HLHS. However, how loss of NR2F factors may underlie HLHS is not understood. Using a knock-in transgenic reporter for endogenous expression of zebrafish nr2f1a, the functional homologue of human NR2F2, we find that nr2f1a is uniquely expressed in the atrial endocardium starting at 48 hours post fertilization, just prior to the initiation of trabeculation. We hypothesized that Nr2f1a and Notch signaling, which is active in the ventricular endocardium during the same developmental window, may mutually antagonize each other to pattern the endocardium into atrial and ventricular domains. We found that pharmacological inhibition of Notch signaling led to an expansion of nr2f1a expression into the ventricular endocardium. Conversely, nr2f1a mutants had expanded Notch activity in the endocardium as well as increased trabeculation. Previous work has shown that cardiac jelly is differentially regulated between the atrium and the ventricle. Our single cell RNA-seq data revealed that ECM remodeling factors are excluded from the atrial endocardium during chamber development and one such factor, hyal2b, may potentially mediate chamber-specific cardiac jelly degradation. Furthermore, we observed that ECM deposition correlated with nr2f1a expression within the chambers, as increased deposition of cardiac jelly was associated with Notch inhibition and ventricular endocardial nr2f1a expression, while cardiac jelly was virtually absent in nr2f1a mutant hearts with ectopic Notch signaling. Altogether, our findings highlight a novel mutually-antagonistic interaction between Notch signaling and Nr2f1a within the ventricular and atrial endocardium that dictates trabecular and chamber morphology, and may provide insights into the etiology of HLHS.

Cardiac contractions repress venous fate in the endocardium.

Presenting Author: Thomas Juan - Uppsala University

Co-Author(s): Shivam Govind Jha – Uppsala University; Stefan Günther – Max Planck Institute for Heart and Lung Research; Didier Stainier – Max Planck Institute for Heart and Lung Research

Abstract: The cardiovascular system responds to hemodynamic forces generated by blood flow during development and under pathological conditions. Globally, cardiovascular diseases are the leading cause of mortality, and blood flow defects strongly contribute to their pathogenicity. In particular, the development and maintenance of the cardiac valves are under the control of blood flow. Here, we find that the entire endocardium is also mechanosensitive prior to cardiac valve development, and that loss of cardiac contraction/blood flow leads to endocardium-to-venous transdifferentiation. Using single-cell transcriptomic analysis and live imaging, we show that perturbations in cardiac contraction/blood flow induce the loss of endocardial markers as well as the ectopic expression of venous markers in endocardial cells. Cardiac microsurgery experiments reveal that cardiac contractions, but not blood flow, are essential to suppress venous fate in endocardial cells. Mechanically, we show that cardiac valve mechanosensors are not responsible for endocardial mechanosensation. Furthermore, using loss and

gain-of-function strategies, we identify Notch signaling as a key regulator of venous fate repression in the endocardium. Taken together, these data suggest that cardiac contractions regulate cardiac development and function by repressing venous fate in the endocardium, thereby advancing our understanding of the consequences of heart failure.

Christiane Nüsslein-Volhard Award Lecture — Shannon Hall | 2:30-3:15pm

Seeking Mechanisms of Brain Development: A Career Path Guided by Local Cues
Corinne Houart - *King's College London*

Workshop Session I

Integration of Computational Modeling and Quantitative Biology — Shannon Hall | 4:00-5:30pm

Integration of Computational Modeling and Quantitative Biology

Presenting Author: Bakary Samasa - University of Pennsylvania

Co-Author(s):

Zebrafish have emerged as a central model organism for dissecting complex biological mechanisms. This workshop, â€œIntegration of Computational Modeling and Quantitative Biology,â€• offers a unique platform to explore methodologies in multi-scale modeling, image data processing, and robust AI-driven analytical techniques. We begin with a biology-centered overview of zebrafish modeling approaches, offering foundational insights into how computational models inform experimental studies on embryonic development. We follow with advanced computational workflows of multi-scale modeling approaches from single cell to multi-cellular. Further, we will discuss how machine learning and AI approaches enhance image data processing and quantification, and also accelerate model optimization and predictions. The workshop will shine a spotlight on diverse zebrafish research projects, illustrating integrative collaborations between biologists and engineers. These projects include computational models for BMP patterning in early dorsal-ventral body-axis formation, mechanistic models for understanding epiboly, notochord mechanics, and Ca2+ signaling models in zebrafish larval fin wound healing. By bridging experimental and computational disciplines, this forum seeks to empower attendees with cutting-edge tools and insights in zebrafish research.

Skeletal Development — Play Circle Theater | 4:00-5:30pm

Moderators: Shannon Fisher - *Chobanian and Avedisian School of Medicine, Boston University*; Matthew Harris - *Boston Children's Hospital*; Thomas Schilling - *University of California, Irvine*

Zebrafish Sustainability Network — Wisconsin Historical Society | 4:00-5:30pm

Moderators: Ashley Bruce - *University of Toronto;* Manjari Trivedi - *Harvard Medical School*

Friday, July 11, 2025

Plenary Session IV:

Neurobiology — Shannon Hall | 8:30-10:00am

Session Chairs: Cagney Coomer & Konstantinos Ampatzis

Cell Adhesion Molecules Required for Electrical Synapse Assembly

Presenting Author: William Crow - University of Oregon

Co-Author(s): Adam Miller – Associate Professor, Biology, University of Oregon

Abstract: During early development of the nervous system, fast intercellular communication relies mainly on electrical synapses which are later supplemented or replaced by chemical synapses as the nervous system matures. Despite their ubiquity in early neural development, little is known about how neurons assemble the gap junction (GJ) channels required for electrical communication. The model of electrical synapse assembly is based on chemical synapses, where cell adhesion molecules (CAMs) direct target selection and junction formation, yet the molecules regulating neural GJs remain unknown. Using a proximity labeling approach, we identified ~250 proteins associated with the neural GJ forming protein Connexin 34 (Cx34) in developing and adult zebrafish. This electrical synapse proteome included a group of immunoglobulin superfamily (IgSF) CAMs previously linked to regulation of GJs in epithelia and muscles. We found that three of these CAMs (Clmpa, Clmpb, and Jam3a) colocalize with Cx34 and the cytoplasmic synaptic scaffold ZO1 at developing electrical synapses. Electrical synapses in the hindbrain and spinal cord contained distinct combinations of these CAMs suggesting that electrical synapses may have unique molecular organization dependent on circuit function. Loss of function experiments revealed Clmpa/b were both required for synaptic Cx34 and ZO1 hindbrain localization, while only Clmpa was required in the spinal cord. By contrast, loss of Jam3a disrupted synaptic Cx34 and ZO1 organization and overall electrical synapse morphology in both the hindbrain and spinal cord. These experiments identify the first CAMs required for building electrical synapses and support a model in which synaptic protein localization and morphological construction are molecularly separable during electrical synapse assembly. Further, ~10 IgSF CAMs were identified in the Connexin-associated proteome, suggesting that this family of proteins may broadly regulate various aspects of electrical synapse structure and function within the developing and adult nervous system.

Searching for rhythmic cell populations – a time-series single-cell RNA-sequencing analysis of zebrafish adult brains

Presenting Author: Han Wang - Center for Circadian Clocks, Soochow University, Suzhou 215123, Jiangsu, China

Co-Author(s): Taole Liu – Soochow University

Abstract: The circadian clock orchestrates rhythmic functions and activities in the nervous system of the brain. However, the circadian fingerprints in various brain regions and cell types, and their functional roles remain unclear. We have conducted time-series single-cell RNA-sequencing and bulk RNA-sequencing analyses of the zebrafish adult brain to identify brain cell types and characterized their circadian patterns and cellular functions. 11 brain regions and 18 major cell types are revealed to display distinct patterns of circadian gene expression. Compared to approximately 10% rhythmic genes identified by the time-series bulk RNA-sequencing analysis, approximately 67% genes are revealed to be rhythmic by the time-series scRNA-sequencing analysis. Further, heterogeneous circadian gene expression patterns are observed between brain regions such as the habenula, pineal gland and

hypothalamus. Together, these findings shed light on uncharted circadian roles of a huge portion of uncharacterized rhythmic cell populations in the zebrafish brain.

Psilocybin-induced subcortical plasticity promotes stress resilience

Presenting Author: Takashi Kawashima - Weizmann Institute of Science

Co-Author(s):

Abstract: Stress resilience determines animals' vulnerability to external disturbances and is a crucial factor in the treatment of mood-related disorders. Psilocybin, a psychedelic agonist for excitatory serotonin receptors, may exert its mood-improving action by enhancing stress resilience through neuroplasticity mechanisms in the brain. We recently discovered that acute psilocybin pretreatment enables stress resilience in larval zebrafish, which have evolutionarily conserved subcortical structures. The analysis of brain-wide neural dynamics and systematic serotonin receptor mapping indicated focal suppression of stress response in the HTR2A+ neurons in the habenula. Importantly, habenular neurons' sensorimotor responses remained intact, indicating that psilocybin induces specific neuroplasticity in stress response pathways in the brain. Downstream of the habenula, we found that psilocybin alters spatiotemporal patterns of brain-wide serotonin release. These results suggest the presence of subcortical networks that can be reinforced to enhance stress resilience.

Mapping the neural basis for individual differences in the exploratory behavior of adult zebrafish.

Presenting Author: Neha Rajput - Wayne State University

Co-Author(s): Kailyn Fields – Wayne State University; Barbara Fontana – Wayne State University; Dea Kanani – Wayne State University; Justin Kenney – Wayne State University; Kush Parikh – Wayne State University; Ada Squires – Wayne State University; Matheu Wong – Wayne

Abstract: Individual differences in behavior have been observed across a wide range of taxa, including humans, rodents, and fish, yet we know little about the biological basis of these differences. One significant axis of behavioral variation is risk-taking, where animals displaying a greater willingness to take risks are classified as bold, while those exhibiting less inclination are characterized as shy. To investigate the neural mechanisms underlying these behavioral differences, we employ adult zebrafish as a model. We assess behavioral differences in zebrafish by subjecting them to the novel tank test, quantifying their exploration of the new environment to classify them as bold and shy. To gain a better understanding of the neural basis for bold and shy behavior, we performed whole-brain activity mapping. We used in situ hybridization chain reaction (HCR) to detect the expression of c-fos, an immediate early gene, as a means of labeling active neurons. To visualize brain-wide c-fos expression, we combined tissue clearing technique with light sheet microscopy to generate whole brain images. For automatic detection of c-fos positive cells, we employed CellFinder, a deep learning-based cell identification approach integrated into the BrainGlobe computational environment. The images were then registered to our recently created adult zebrafish brain atlas (AZBA) using advanced normalization tools (ANTs). With this approach, we identified distinct signatures of c-fos activity, associated with bold and shy behavior.

Defining the cellular and molecular dynamics of astrocytes during axon regeneration

Presenting Author: Alexandria Hulegaard, PhD - University of Pennsylvania

Co-Author(s): Michael Granato – Professor, Cell and Developmental Biology, University of Pennsylvania

Insults to the central nervous system including spinal cord injury cause tissue-wide changes involving various cell types to neutralize toxic debris and contain damage. Astrocytes have primarily been studied in the formation of a glial scar around the site of damage, but only a subset of astrocytes forms this barrier. Nonetheless, many astrocytes in the vicinity become reactive changing their morphology and expression profiles. Despite their prominence, the role of reactive astrocytes in injury and regeneration is not well understood. For example, what are the dynamic behaviors of astrocytes in direct response to spinal cord injury and what molecular signals regulate their in vivo responses? Using zebrafish, we interrogate these questions by examining astrocytes in the spinal cord following injury. Using a laser, we transect the Mauthner cell axon in the spinal cord and record its regeneration over the course of a few days. Using live-imaging, we monitor astrocytes with single-cell resolution following axotomy and during acute stages of regeneration. Thus far, we observe that astrocytes near the transection site exhibit dynamic behavior and send processes towards the injury site within 1-2 hours of injury. By combining live imaging with a genetically encoded calcium sensor, we observe that within seconds of axon transection, astrocytes have increased calcium activity radiating away from the injury site. Using CRISPR/Cas9 injected into single-cell embryos, we are conducting a candidate screen to target genes upregulated in astrocytes in various injury models. This work will identify genetic factors that regulate astrocyte dynamics in vivo and elucidate molecular pathways in astrocytes that promote CNS axon regeneration. The goal of this work is to define the cellular and molecular nature of astrocytes in the injured spinal cord and how they influence axon regeneration.

A cell-state switch establishes competence for target-specific regeneration in the zebrafish vagus nerve

Presenting Author: Lindsey Qian - University of Minnesota

Co-Author(s): Adam Isabella – University of Minnesota

Abstract: Peripheral nerve damage caused by injury, disease, or aging is widespread and can often be debilitating. While many factors regulating the regrowth of injured axons have been characterized, how regenerating axons are guided to the proper targets is still poorly understood. This topic is of particular importance, as in mammals misdirected axonal regrowth remains a major barrier to circuit reformation and functional recovery. The zebrafish is a powerful model for addressing these questions, as it is highly regenerative, genetically tractable, and has optically clear embryos and larvae allowing for live imaging. Motor neurons of the conserved vagus nerve extend five primary axon branches from the hindbrain into the pharyngeal arches and viscera in a stereotyped topographic pattern, making it an excellent model for studying axonal decision making. Using a novel larval neuron transplantation approach to examine axon regeneration with single-cell resolution, we found that vagus motor axons regrow robustly and with high topographic accuracy. Notably, we observed that vagus motor axons use distinct molecular guidance programs between developmental (embryo) and regenerative (larval) stages. To understand this “program switching”, we performed heterochronic neuronal transplantation between several embryonic and larval stages and define a competency change in which vagus neurons deactivate their developmental guidance program and activate their regenerative guidance program concomitant with the conclusion of embryonic axon target selection. Finally, we examine potential competency signals that drive this developmental-to-regenerative cell state switch. This work reveals novel insights into the processes underlying the acquisition of regenerative capabilities.

Concurrent Session II

Disease Models — Shannon Hall | 10:30am-12:00pm

Session Chairs: Misha Ahrens & Lihua Ye – *The Ohio State University*

Very efficient recovery of precisely edited alleles harboring multiple base substitutions

Presenting Author: Kazuyuki Hoshijima, Ph.D - University of Utah

Co-Author(s): Kazuyuki Hoshijima – Senior Research Associate, Human Genetics, University of Utah; Michael Jurynec – Associate Professor, Human Genetics, University of Utah; Shengzhou Wang – Graduate Student, School of Biological Sciences, University of Utah; Kendell C

Abstract: Whereas current genome editing methods can produce almost any type of modified allele in zebrafish, the poor efficiency with which precisely edited alleles can be recovered is an obstacle to their widespread use. The ability to easily generate base-substitution alleles would have a transformative effect on research with zebrafish. Given the depth with which phenotypic analyses can be performed, the effects of sequence variants proposed to be associated with human disease, intra-specific, or inter-specific differences could be readily analyzed. Given the precision with which gene expression patterns can be analyzed at single-cell resolution, the quantitative, temporal, and spatial gene expression effects of variants affecting regulatory sequences or proteins could be analyzed at a level that cannot be approached presently in other animals. Finally, the combination of zebrafish carrying human disease alleles and high throughput screening methods should allow identification of disease-modifying drugs.As edited alleles with recessive effects or without clearly predicted phenotypes need to be identified by labor-intensive sequence-based screening methods, we have worked to dramatically improve the efficiency with which edited alleles are generated, improving both the fraction of treated F0 animals as well as the fraction of gametes per F0 germ line that transmit a precisely edited allele, devoid of unwanted accompanying mutations.We find that Cas9-induced nick-stimulated can be used to achieve high efficiency precise editing. We use ssDNA donor molecules as templates to generate alleles in which sequence stretches of >50bp have been modified (longer replacements are being tested). Multiple base changes of any kind can be produced simultaneously. ~1 in 10 F0 animals transmit a precisely edited allele present in 1/8 – 1/2 of the germ line. The method appears to be useful for the introduction of short non-homologous regions, such as might encode an epitope tag or loxP site.

A Novel Zebrafish Model for Diffuse Midline Glioma (DMG)

Presenting Author: Elissar Alhaj Kadour - School of Medicine and Public Health, University of Wisconsin-Madison

Co-Author(s): Owen Tamplin – School of Medicine and Public Health, University of Wisconsin-Madison; Richard White – Ludwig Institute for Cancer Research, University of Oxford

Abstract: Diffuse midline glioma (DMG), previously defined as diffuse intrinsic pontine glioma (DIPG), is a highly aggressive brain tumor that appears in children between 4-9 years of age and is always lethal within 1-2 years after diagnosis. These cancers are very rare, with only a few hundred children being diagnosed in the US each year. For decades, there has been no significant improvement in treatment options for DIPG/DMG, making it a high priority for further investigation. The development of new DIPG/DMG animal models is critical for advancing our understanding of this deadly brain cancer. Our

objective is to develop a DIPG/DMG model to reveal the earliest stages of tumor development and dissect the mechanisms of tumor initiation, thereby creating a platform to test therapeutics for early intervention. We over-expressed DIPG/DMG oncogenes in zebrafish oligodendrocyte precursors cells (OPCs) using the myelin basic protein (mbp) promoter that turns on at 4 days post fertilization (dpf). Together with mbp:Cas9 and U6-driven gRNAs to generate spontaneous tumor suppressor mutations (ptena, ptenb, tp53), we observed aggressive and invasive lesions from 7-8 weeks, and lethality from 10-12 weeks. The mbp:GFP reporter allows direct observation of OPCs in the central nervous system (CNS). We will pinpoint the precise timing of tumor onset when DIPG/DMG cells diverge from normal OPCs between embryonic and juvenile stages. We are using live imaging and single cell RNA sequencing to identify the critical timepoints in tumor initiation and development. We believe this model will allow us to gain unique insights into the molecular and genetic mechanisms of DIPG/DMG and identify potential therapeutic targets.

Intrinsic and TDP-43 loss-induced catabolic stress elicits neuroprotective cellular degradation in ALS-vulnerable motor neurons

Presenting Author: Kazuhide Asakawa, PhD - National Institute of Genetics

Co-Author(s): Takuya Tomita – The University of Tokyo; Shinobu Shioya – National Institute Of Genetics; Hiroshi Handa – Tokyo Medical University; Yasushi Saeki – The University of Tokyo; Koichi Kawakami – National Institute Of Genetics

Abstract: Selective neuronal vulnerability is a defining feature of neurodegenerative disorders, exemplified by motor neuron degeneration in amyotrophic lateral sclerosis (ALS). The nature of motor neurons underlying this selectivity remains unresolved. Here, by monitoring autophagy at single-cell resolution across the translucent zebrafish spinal cord, we identify motor neurons as the cell population with the highest autophagic flux. Large spinal motor neurons (SMNs), which are most susceptible to ALS, exhibit higher flux than smaller SMNs and ALS-resistant ocular motor neurons. Notably, large SMNs accelerates both autophagy and proteasome-mediated degradation, which are further augmented by TDP-43 loss. Additionally, acceleration of unfolded protein response pathways in large SMNs indicates an innate tendency to accumulate misfolded proteins. The enhanced cellular degradation in large SMNs is neuroprotective as its inhibition halts axon outgrowth. These findings propose that cell size-associated degradation load underlies selective neuronal vulnerability in ALS, highlighting the alleviation of catabolic stress as a target of therapy and prevention.

CK189 is linked to neurodevelopmental disorder in a zebrafish knockout model

Presenting Author: Dilan Wellalage Don - Department of Biology, Chungnam National University, South Korea

Co-Author(s): Seda Susgun, PhD – Department of Genetics, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Turkiye; Cheol-Hee Kim, Professor – Department of Biology – Chungnam National University, South Korea

Abstract: Neurodevelopmental disorders (NDDs) are a group of conditions encompass developmental delay, intellectual disability, autism spectrum disorder, epilepsy, and attention-deficit/hyperactivity disorder. Advances in next-generation sequencing has discovered pathogenic variants in genes particularly participating protein synthesis pathways. Here, we present a novel candidate gene involved

in RNA modification and its association with NDDs. A consanguineous family with NDD affected children were recruited in this study. Comprehensive genetic analyses revealed biallelic loss of function in CK189 RNA modification gene. In order to study CK189 function in vivo, we generated a knockout (KO) zebrafish model using CRISPR-Cas9 technique. The adult KO zebrafish displayed impaired behavioral patterns in social behavior, startle response and anxiety-related behavior, followed by dysregulated stress-evoked c-fos expression in the pallium, reflecting possible deficits in neuronal plasticity and stress resilience. This work serves as a foundation for future research into genes associated with RNA modifications and their role in NDDs. Further studies will contribute in better understanding genotype-phenotype correlation.

Gene-ethanol interactions and epithelial morphogenesis: The shape of things to come

Presenting Author: C. Lovely, PhD - University of Louisville School of Medicine

Co-Author(s): John Klem – university of louisville school of medicine; Tae-Hwi Schwantes-An – Indiana University School of Medicine; Michael Suttie – University of Oxford; Raedèn Gray – University of Louisville School of Medicine; Hieu Vo – University of Louisville Sc

Abstract: Fetal Alcohol Spectrum Disorders (FASD) describe a continuum of ethanol-induced developmental defects, including malformations to the facial skeleton. While ethanol-sensitive genetic mutations contribute to facial malformations, the impacted cellular mechanisms remain unknown. Formation of the facial skeleton requires complex signaling interactions between the neural crest, from which the facial skeleton is derived, and the facial epithelial, in particular the endoderm. Proper endoderm morphogenesis is critical to setting up these tissue interactions. Bone Morphogenetic Protein (Bmp) signaling is a key regulator of endoderm morphogenesis providing a possible ethanol-sensitive mechanism. Here, we have established an in vivo FASD model, where mutations in the Bmp pathway sensitize embryos to ethanol-induced facial defects by disrupting endoderm morphogenesis. Ethanol-treated Bmp mutants display significant changes in endoderm shape and size, disrupting subsequent tissue interactions driving facial development. Using quantitative morphometric readouts, we document that changes in endoderm shape mirror shape changes to the facial skeleton. Strikingly, ethanol does not impact Bmp signaling or its downstream targets directly but does increase apoptosis in migratory neural crest in Bmp mutants. This suggests that Bmp-dependent signals from the endoderm are ethanol sensitive leading to neural crest defects. We go on to show that our data are predictive for Bmp-ethanol associations in jaw development in humans, with variants in BMPR1B being associated with ethanol-related differences in jaw volume ($p=4.0x10-4$). Overall, our results show that zebrafish analyses can model gene-ethanol associations in humans, strongly phenocopying both the malformation and the variation inherent in human data. Thus, the zebrafish model remains a powerful, efficient model to simultaneously generate a deeper mechanistic understanding of gene-ethanol interactions on the complex tissue interactions and provide a conceptual framework for ethanol-induced structural birth defects. Ultimately, this work will connect ethanol exposure with concrete cellular events that could be sensitive beyond the facial skeleton.

A single nucleus RNA-seq atlas of the larval zebrafish response to a high-lipid meal

Presenting Author: Catherine Brown - Johns Hopkins University

Co-Author(s): Michelle Biederman – Biology – Johns Hopkins University; Steven Farber – Biology – Johns Hopkins University; Meredith Wilson – Biology – Johns Hopkins University

Abstract: Single cell (sc) and single nucleus (sn) RNA sequencing (RNA-seq) have been applied to fundamental problems of development by elucidating the transcriptional trajectories of cell types as they differentiate. However, one can also apply these technologies to describing the cellular response of an animal to a normal metabolic stimulus like eating a high-lipid meal (HLM). The advantage of snRNA-seq over scRNA-seq is that it only captures newly transcribed RNAs, so it can better report on small and/or rapid transcriptional changes. Here we describe the first vertebrate snRNA-seq atlas of whole 6 dpf larval zebrafish immediately following a HLM. To achieve the cellular and temporal granularity of interest, we developed a feeding and rapid screening protocol and performed snRNA-seq at 15 min, 30 min, 1 h, 2 h and 4 h of feeding. This study yielded ~183K nuclei for transcriptional analysis (10X Genomics). To our surprise, we found 650 unique differentially expressed genes (DEG) in 74 cell types within 15 minutes of a HLM. For example, multiple digestive cell types downregulate endopeptidases, while fos is increased in the pharyngeal epithelium among other places. Although anterior enterocytes of the intestine, the cells that uptake dietary lipid, exhibit some rapid transcriptional responses, it takes an hour for these cells to significantly upregulate critical lipid transport proteins. Many other cell types also have striking transcriptional changes over the course of processing the meal. Additionally, we performed snRNA-seq on fish mutant for the transcription factor creb3l3, a known regulator of lipid responsive gene expression, which will allow us to understand the role of creb3l3 in a cell type specific manner. Our wild-type snRNA-seq atlas combined with mutant snRNA-seq datasets will enable us to tease apart the complex cell-type specific transcriptional networks driving the response to a HLM.

Tissue Regeneration — Play Circle Theater | 10:30am-12:00pm

Session Chairs: Zhaoxia Sun - *Yale University School of Medicine &* Matthew Harris - *Boston Children's Hospital*

Hb-egf-mobilized epicardial cells direct morphogenesis and regeneration of compact cardiac muscle

Presenting Author: Fei Sun, PhD - Morgridge Institute for Research

Co-Author(s): Masashi Sada – Morgridge Institute for Research; Lauren Parker – Duke University; Adam Shoffner – Duke University; Kelsey Oonk – Duke University; Mikayla Utnehmer – Morgridge Institute for Research; Yanchao Han – Soochow University; Ravi Karra – Duke Uni

Abstract: Unlike adult mammals, which have limited capacity for cardiac regeneration, adult zebrafish can replace lost or damaged heart tissue and restore organ function. Upon cardiac injury, morphogenesis programs first important in juvenile zebrafish are reactivated to enable heart regeneration. Here, we describe identification of a potent epicardial mitogen and the key epicardial subpopulation that it acts upon. We find that the EGF receptor ligand heparin binding epidermal growth factor (hb-egf) is expressed in the epicardial cells covering the juvenile zebrafish ventricle during cardiac morphogeneis, and then is reactivated in adult hearts in response to cardiac injury. Induced cardiac overexpression of hb-egf stimulates early expansion of epicardial tissue and emergence of cortical muscle in juvenile zebrafish hearts, accompanied by premature formation of coronary vessels. Genetic mutations in hb-egf paralogs disrupt heart regeneration, whereas induced cardiac hb-egf overexpression profoundly augments epicardial and myocardial regenerative responses, creating a grossly enlarged cortical muscle layer filled with epicardial-derived cells but without fibrosis. Ex vivo epicardial culture

and Hb-egf pulsing experiments indicate that Hb-egf production has a primary effect on epicardial cells, promoting their cycling, migration, and invasion of muscle, where they exert pro-myogenic and -angiogenic effects. Our study identifies Hb-egf as a highly potent, instructive mitogen for epicardial subpopulations that guide cortical muscle formation at the juvenile stage and promote muscle regeneration upon cardiac damage.

Neural crest-like cell transdifferentiation underlies a new mode of neuronal regeneration in the zebrafish retina

Presenting Author: Romain Madelaine, PhD - MDIBL

Co-Author(s): Aissette Baanannou – MDIBL; Bidhi Diwedi – MDIBL; Pritha Das – MDIBL; Anindita Neog – MDIBL; Caroline Halluin – MDIBL; Romain Menard – MDIBL; Kevin Emmerich – Johns Hopkins University; Joel Graber – MDIBL; Jeff Mumm – Johns Hopkins University

Abstract: In humans, retinal neurons death, optic nerve injuries and associated neurodegenerative diseases, such as glaucoma or age macular degeneration, often lead to permanent loss of vision. While the regenerative capacity is low in the human nervous system, including retina, the endogenous neuronal regenerative process after injury occurs in some non-mammalian vertebrate species, like the zebrafish. Unlike mammals, zebrafish do not form a glial scar that inhibits axonal and neuronal regeneration after injury. Rather, they harbor neural progenitor and stem cell populations allowing them to regenerate entire parts of the nervous system and restore tissue integrity. In the zebrafish retina, cycling neural progenitor cells of the ciliary marginal zone and quiescent resident neural stem cells (also called Müller glial cells) have been involved in the process neuronal regeneration following different types of injury. We have identified an additional cellular source participating in the regeneration of neurons in the zebrafish retina after ablation of retinal ganglion cells. Before injury, these cells express molecular markers of neural crest cells identity, such as sox10, foxd3 or pdgfrb, while after neuronal ablation they also express proneural factors like ascl1a and olig2. Combining genetic ablation of neurons with photoconversion or Cre/Lox dependent genetic lineage tracing of sox10-expressing cells, and cell proliferation analysis, we demonstrated that these cells can differentiate into post-mitotic retinal neurons in the ganglion cell layer in the absence of cell proliferation. This work reveals an unexpected cellular mechanism of transdifferentiation, dependent on a neural crest-like cell population, participating in the process of neuronal regeneration in the zebrafish retina. The discovery of this plastic cell population could potentially open new strategies to promote the formation of neurons in the mammalian retina.

Molecular mechanisms underlying axon target selection during regeneration in zebrafish vagus nerve

Presenting Author: Rabab Ibrahim - University of Minnesota

Co-Author(s): Adam Isabella – Genetics, Cell Biology and Development – University of Minnesota

Abstract: During regeneration, injured axons must be re-guided to the correct targets, but little is known of how this process is regulated. Zebrafish have a highly regenerative nervous system, making them an ideal model to study regeneration. In the zebrafish vagus nerve, motor neurons project five major branches to the pharyngeal arches and viscera in an anterior-posterior (A-P) topographic pattern. While the mechanisms regulating topographic target selection during vagus development are well understood, how this intricate innervation pattern is reconstructed after injury is unclear. We have established two

techniques – a whole-nerve severing model and a transplantation-based single-neuron injury model – to injure the vagus nerve and track motor axon regrowth with single-cell resolution. Using these models, we found that injured axons robustly regrow to topographically correct branches. This process does not require developmental guidance cues and instead uses an unidentified regeneration-specific guidance mechanism that involves interaction with a component of the preexisting branch. We hypothesize that anterior and posterior vagus neurons possess unique molecular identities that promote differential responses to guidance cues present on cells associated with distinct vagus branches. To understand this mechanism, we performed branch-specific RNAseq of vagus neurons to identify candidate guidance factors that exhibit an A-P expression bias specifically during the regenerative stage. This revealed several candidate genes with putative roles in axon guidance. To elucidate the roles of these candidates in axon targeting, we are combining loss- and gain-of-function with our two in vivo injury models. To elucidate the branch-specific cell-cell interactions that promote axon target selection, we have established a system to co-cultured vagus motor neurons with branch specific cell types. This work will allow us to identify the molecular mechanisms that guide axon target selection in the regenerating zebrafish vagus nerve.

Hypothermia induces cardiac regeneration via ERAD-mediated Nfe2l1 stabilization and proteasome activation

Presenting Author: Tao P. Zhong - East China Normal University

Co-Author(s): Yansong Tang, Bangjun Gao, Mengying Feng, Yiping Zhu, Zhaohui Ouyang, Shan Chen, Dongliang Li, Peilu She, Ke Wei, Tao P Zhong

A strong correlation exists between declining heart regeneration and evolving of endothermy. Body temperature in mammals lowers upon environmental exposure after birth. Whether and how cardiomyocytes (CMs) sense temperature fluctuations to regulate cell proliferation remain unknown. We employed a comprehensive set of cell and animal models to investigate

temperature-dependent CM proliferation and cardiac regeneration. We show that hypothermia directly simulates CM division in isolated NRVCs and hiPSC-CMs, and promotes hyperplastic myocardial growth in adult zebrafish and neonatal mice. On the contrary, elevated temperature inhibits cultured human, rodent and zebrafish CM dedifferentiation and proliferation. Adult zebrafish and neonatal mice that are subjected to cold exposure augment myocardial regeneration following apical injury. Integration of snRNA-Seq and proteomic data identifies upregulation of proteasome genes that facilitates the degradation of sarcomere components upon hypothermia, promoting CM dedifferentiation and proliferation. We uncover ER-associated Nfe2l1 as a key transcription factor that activates proteasomal gene expression to drive CM renewal and myocardial regeneration. Importantly, a crucial ER-associated degradation (ERAD) element serves as a temperature sensor to regulate Nfe2l1 stability for proteasomal gene expression. Either inhibiting ERAD or activating proteasome activity enhances cardiac regeneration in both zebrafish and mouse. Hence, the conserved ERAD/Nfe2l1-proteasome network across vertebrate CMs links temperature sensing to cardiac dedifferentiation and proliferation, providing potential therapeutic targets for heart repair

Live visualization of ECM dynamics during development and regeneration in zebrafish

Presenting Author: Jingwen Shen - Morgridge Institiute for Research

Co-Author(s): Kazunori Ando – Morgridge Institute for Research; Stefano Di Talia – Duke University; Pierre Gillotay – Morgridge Institute for Research; Ranjay Jayadev – Duke University; Jianhong Ou – Morgridge Institute for Research; Kenneth Poss – Morgridge Institute

Abstract: Extracellular matrix (ECM) plays fundamental roles in animal development, regeneration, and disease. The difficulty of tagging endogenous matrix proteins has been a hurdle to understanding ECM composition and dynamics. To visualize vertebrate ECM components, we tagged zebrafish Laminin, gamma 1(Lamc1), Collagen, type I, alpha 2 (Col1a2), and Transforming growth factor, beta-induced (Tgfbi) using C-terminus in-fusion genome editing. Analysis of these knock-in lines revealed distinct expression domains of the different components during stages of development and regeneration in various tissues. FRAP (fluorescent recover after photobleaching) analysis indicated that Lamc1 is stable matrix in fin fold but more dynamic in myosepta of developing zebrafish, and that Col1a2 and tgfbi are stable matrix components in myosepta. Unexpectedly, we found that Col1a2-mScarlet protein accumulates at the amputation plane during tailfin regeneration, where it remains concentrated for several days though distant from the regeneration blastema. This “foundation” region also displayed a distinct transcriptome along with enhanced cell cycling signatures, suggesting active renewal and repair events at the base of the regenerating appendage. Our resource enables live capture of ECM dynamics that can identify new biology of developmental and regenerative events.

Macrophage-derived Extracellular Vesicles Modulate Stem Cell Functions During Muscle Regeneration In Zebrafish

Presenting Author: Quoc Duy Tran - Australian Regenerative Medicine Institue

Co-Author(s): Peter Currie – Australian Regenerative Medicine Institute; Avnika Ruparelia – Melbourne University

Abstract: Background: Muscle regeneration is a highly coordinated process involving interactions between various cell types, including macrophages and muscle stem cells (satellite cells). Following injury, macrophages migrate to the damaged site and regulate satellite cell activation, proliferation, and differentiation into myotubes through direct contact or the release of soluble factors. Extracellular vesicles (EVs), tiny membrane-enclosed particles that transport regulatory molecules such as microRNA and growth factors, are key mediators of intercellular communication. It remains unclear whether macrophages influence satellite cell activities through EVs. A significant challenge in this field is that most EV research has been conducted using fixed tissues, limiting our understanding of the origin, destination, and real-time dynamics of EVs within living organisms. This study aims to investigate the role of macrophage-derived EVs in regulating satellite cell behaviour during muscle regeneration.

Results: To enable real-time observation of macrophage-satellite cell interactions via EVs, a zebrafish macrophage-specific EV reporter line was generated. Live imaging revealed that macrophage-derived EVs bind to and are internalized by satellite cells, subsequently inducing their division. Isolation and analysis of macrophage-dẻived EVs identified Nampt, a pro-regenerative factor. The functional role of EVs in muscle regeneration was assessed using an EV inhibitor compound and a macrophage-specific genetic knockout, which impairs EV production and secretion. Zebrafish larvae lacking macrophage-derived EVs exhibited defective muscle regeneration, with the most pronounced effects observed during the early stages of regeneration. Further analysis showed a significant decrease in satellite cell

proliferation and differentiation, while macrophage migration remained unaffected in the absence of EVs.

Conclusion: The macrophage-derived EV reporter line provides a valuable tool to study EV-mediated communication between macrophages and satellite cells in real time. This study demonstrates that macrophage-derived EVs are essential for promoting satellite cell proliferation and differentiation during muscle regeneration.

Tissue Patterning — Wisconsin Historical Society | 10:30am-12:00pm

Session Chairs: Kelle Siegfried & James Gagnon - *University of Utah*

Distinct tissue kinematics shape the embryonic body plan

Presenting Author: Susan Wopat, PhD - UC Santa Barbara

Co-Author(s): Vishank Jain-Sharma – Physics – UC Santa Barbara; Pieter Derksen – UC Santa Barbara; Gary Han – UC Santa Barbara; Sebastian Streichan – UC Santa Barbara

Throughout vertebrate gastrulation, collective cell movements form the embryonic axes and germ layers. While the biochemical signals guiding embryogenesis are well studied, hurdles related to in toto analysis encumber our understanding of the physics that underlie formation of the body plan. Therefore, we sought to establish a quantitative framework that enables investigation of whole-embryo tissue kinematics. In order to achieve this objective, we first generated in toto movies of zebrafish development from the onset of gastrulation through tailbud outgrowth using Multiview Selective Plane Illumination Microscopy (MuVi SPIM). We pair this with a new, user-friendly, Blender-based tissue cartography interface to transform these rich, three-dimensional (3D) datasets into layered two-dimensional (2D) surface maps of the embryo, substantially reducing data complexity and enabling quantitative tissue specific analyses of cell movements across germ layers. This analysis revealed that the sparsely populated extraembryonic tissues (i.e. the enveloping and yolk syncytial layers) exhibit distinct flow dynamics from those of embryonic body precursors, suggesting that tissue-autonomous movement behaviors are necessary for proper embryogenesis. Furthermore, the global patterns of these instantaneous flow fields remain spatially fixed for extended periods, and only shift with discrete developmental stages, supporting the idea that body architecture arises through a sequence of iterative ‘morphogenetic modules’. This quantitative platform provides a foundation for exploring how ectopic induction or perturbation of biochemical patterning cues modulates global flow patterning and ultimately alters embryogenesis.

Precision in Motion: Cytoneme-Driven Wnt Signalling in Neural Crest Fate Decisions

Presenting Author: Gemma Sutton, PhD - Living Systems Institute, University of Exeter

Co-Author(s): Karen Camargo Sosa – Research Fellow, Department of Life Sciences, University of Bath; Robert Kelsh – Deputy Head of Department, Department of Life Sciences, University of Bath; Steffen Scholpp – Professor of Cell & Developmental Biology, Department of B

Abstract: The Wnt/β-catenin signalling pathway activates gene regulatory networks during development to enable cell fate transitions in precise locations in the embryo. In vertebrates, Wnt/β-catenin signalling has an ongoing role in the development of the neural crest (NC) lineage, a

multipotent cell population with extraordinary migratory capacity. The NC forms a myriad of cell derivatives, including pigment cells, neurons and glia of the peripheral nervous system, cardiomyocytes and skeletogenic cells in craniofacial tissue. During NC fate restriction, Wnt/β-catenin signalling promotes melanocyte specification at the expense of neuronal derivatives. However, it remains unclear how Wnt/β-catenin signalling is spatially and temporally controlled during this process. Specifically, it is not well understood how certain cells within this migratory population activate the pathway while neighbouring cells do not. We employ high-resolution in vivo imaging in zebrafish to track the transport of Wnt signalling components from Wnt-producing cells of the dorsal midbrain to Wnt-receiving NC progenitors. We focus on the dispersal of Wnt3a, the primary candidate ligand regulating NC specification. We demonstrate that Wnt-producing cells in the midbrain-hindbrain boundary (MHB) form dynamic cell protrusions. These cell protrusions can act as signalling filopodia, known as cytonemes, by carrying Wnt3a to neighbouring cells. We are expanding our analysis of Wnt signalling components transported to NC cells, and we now have increasing evidence that the Wnt receptor, Frizzled10, can also be transported along cytonemes in complex with Wnt3a. Furthermore, using Lightsheet microscopy together with a live Wnt/β-catenin signalling reporter, we show that migrating NC cells robustly activate Wnt signalling, suggesting that the MHB acts as a signalling centre to cranial NC. Overall, our findings reveal a novel mechanism for precise, targeted delivery of Wnt ligands to a migratory population of NC cells, providing new insights into how spatially restricted signalling influences cell fate decisions during development.

Agouti and BMP signaling drive a naturally occurring fate conversion of melanophores to leucophores

Presenting Author: Delai Huang, PhD - University of Virginia

Co-Author(s): Emaan Kapadia – University of Virginia; Yipeng Liang – University of Virginia; David Parichy – University of Virginia

Abstract: The often-distinctive pigment patterns of vertebrates are varied in form and function and depend on several types of pigment cells derived from embryonic neural crest or latent stem cells of neural crest origin. These cells and the patterns they produce have been useful for uncovering features of differentiation and morphogenesis that underlie adult phenotypes, and they offer opportunities to discover how patterns and the cell types themselves have diversified. In zebrafish, a body pattern of stripes arises by self-organizing interactions among three types of pigment cells. Yet these fish also exhibit white ornamentation on their fins that depends on the transdifferentiation of black melanophores to white cells, “melanoleucophores.” To identify mechanisms underlying this conversion we used ultrastructural, transcriptomic, mutational, and other approaches. We show that melanophore–melanoleucophore transition depends on regional BMP signals transduced through noncanonical receptors (Rgmb-Neo1a-Lrig2) as well as BMP-dependent signaling by Agouti genes, asip1 and asip2b. These signals lead to expression of transcription factor genes including foxd3 and runx3 that are necessary to induce loss of melanin, curtail new melanin production, and deploy a pathway for accumulating guanine crystals that, together, confer a white phenotype. These analyses uncover an important role for positional information in specifying ornamentation in zebrafish and show how tissue environmental cues and an altered gene regulatory program have allowed terminal addition of a distinct phenotype to a preexisting cell type.

Cell-type specific expression of rRNAs in sex determination and differentiation

Presenting Author: Miranda Wilson - Icahn School of Medicine at Mount Sinai

Co-Author(s): Florence Marlow – Icahn School of Medicine at Mount Sinai

Differences of sexual development encompass various congenital conditions with diverse etiologies arising during embryogenesis and persisting throughout sexual development. We have identified the vertebrate-specific RNA binding protein of multiple splice variants 2 (Rbpms2) as an essential regulator of zebrafish oogenesis. Specifically, Rbpms2 represses translation of testis-promoting RNAs and promotes translation of the Gator2 complex protein, Mios, upstream of mTorc1 signaling and ribosome biogenesis in oocytes. Reduced nucleolar recruitment of RNA polymerase I in rbpms2 mutant (rbpms2DM) oocytes suggests disrupted ribosomal RNA (rRNA) transcription. Zebrafish rRNAs have been reported to be predominately expressed from two loci: chromosome 5 in all cells (somatic rRNA; S rRNA) and chromosome 4 in germline, specifically oocytes (maternal rRNA; M rRNA). Additionally, demethylation of the M rDNA locus is associated with ovarian development while decreased expression biases to testis development. As rDNA accessibility and rRNA transcription limit nucleologenesis, we determined the spatiotemporal expression of the M and S rRNAs in developing wild-type and rbpms2DM gonads. By targeting the nascent M and S rRNAs, we found that M and S rRNA transcription was mutually exclusive in mitotic germ cells of wild-type and rbpms2DM ovaries and M rRNA transcription correlates with meiotic initiation. Only M rRNA was detected in wild-type oocytes while M and S rRNAs were co-transcribed in rbpms2DM oocytes, implicating Rbpms2 in S rRNA repression. Mitotic germ cells of wild-type and rbpms2DM testes transcribed M or S rRNA, though a subset transcribed both. A transcriptional switch from M to S rRNA was observed in maturing spermatocytes before ceasing transcription of either. Our findings demonstrate that, within normal germ cells, rRNA transcription is mutually exclusive. Further, we identified a mitotic to meiotic switch in rRNA transcription and associate S rRNA activation with failed oogenesis, supporting the notion that differential rRNA switching is the elusive sex determination trigger.

Prkra dimer senses double-stranded RNAs to dictate global translation efficiency

Presenting Author: Ming Shao - School of Life Sciences, Shandong University

Co-Author(s): Tong Lu – School of Life Sciences, Shandong University

Abstract: Double-stranded RNAs (dsRNAs), known as conserved pathogen-associated molecular patterns, activate the integrated stress response via interferon-induced protein kinase R (PKR), leading to global translation inhibition. However, the interferon system is inactive in pluripotent cells, leaving the mechanisms of dsRNA sensing and translational control unclear. In this study, we utilized early zebrafish embryos as a model of pluripotent cells and discovered a PKR-independent blockage of translation initiation by dsRNA stimulation. Prkra dimer was identified as the genuine dsRNA sensor. Upon dsRNA binding, the dimerized dsRNA binding domain 3 of Prkra becomes activated to sequester the eIF2 complexes from the translation machinery, inhibiting global protein synthesis. This distinctive embryonic stress response restricts RNA virus replication in zebrafish embryos, is conserved in mouse embryonic stem cells, and compensates PKR function in differentiated cells. Therefore, the Prkra-mediated dsRNA sensing and translation control may serve as a common strategy for cells to adapt environmental stresses.

Plenary Workshop:

Advances and Challenges in Genome Engineering — Shannon Hall | 1:00-2:30pm

Advances and challenges in genome engineering

Presenting Author: Koichi Kawakami - National Institute of Genetics, Laboratory of Molecular and Developmental Biology, Mishima, Japan

Co-Author(s):

The goal of this workshop is to share recent developments and new strategies for targeted manipulation of the zebrafish genome. We will also address issues regarding chromatin accessibility and transgene silencing.

Mary Goll	Surveying the chromatin landscape for optimal transgenesis
Koichi Kawakami	A safe harbor locus for UAS-effector transgenesis
Christian Mosimann	Beyond pIGLET: applications, challenges, and next steps in landing site-based transgenesis
Thomas Juan	Approaches to knock down function of endogenous genes
David Grunwald	Easy, reliable, and efficient generation of precise base substitution mutations
Filippo Del Bene	Optimization of prime editing in zebrafish by inhibition of microhomology-mediated end-joining
Darius Balciunas	Oligonucleotide-mediated HDR for engineering of epitope tagged and floxed alleles
Maura McGrail	Zebrafish Community Cre/lox resource for lineage tracing and cell type-specific gene studies
Marnie Halpern	Generation of transgenic driver lines by targeted CRISPR/Cas9 integration

George Streisinger Award Lecture — Shannon Hall | 2:30-3:15pm

The MBL Zebrafish Course: A Quarter Century Shaing Tricks of the Trade

MBL Course Directors

Workshop Session II:

Challenges Facing Early-Career Zebrafish Researchers — Shannon Hall | 4:00-5:30pm

Moderators: M. Brent Hawkins – *Boston Children's Hospital*; Carl Berggren

A Practical Workshop for Analyzing Whole-Brain Calcium and Voltage Imaging Data — Wisconsin Historical Society | 4:00-5:30pm

Moderator: Takashi Kawashima - *Weizmann Institute of Science*

Designing Sustainable Activities for Outreach and Education — Play Circle Theater | 4:00-5:30pm

Moderator: Jason Meyers - *Colgate University*

Saturday, July 12, 2025

Plenary Session V:

Emerging Technologies **Shannon Hall | 8:30-10:00am**

Session Chairs: Diana Pinheiro - *IST Austria* & Saba Parvez - *University of Utah*

CRISPR prime editing made precise by inhibition of microhomology-mediated end-joining

Presenting Author: Filippo Del Bene - Institut de la Vision

Co-Author(s): Francois Kroll – Institut de la Vision; Malo Serafini – Institut de la Vision; Thanh-mai Dang – Institut de la Vision; Luna de Barbarin – Institut de la Vision; Marion Rosello – Institut de la Vision; Jean-Paul Concordet – MNHN; Carine Giovannangeli – MN

Abstract: Thanks to their fast and external development, zebrafish larvae are a potentially revolutionary model for genetic diseases. However, faithfully introducing pathogenic variants found in patients remains ineffective. The usual strategy relies on homology-directed repair of a double-strand break made by CRISPR-Cas9. The approach has been under development for a decade in zebrafish but still usually yields edit rates < 10% with high frequency of unwanted mutations. We are developing an alternative solution based on prime editing, a new CRISPR method that uses a Cas9 nickase fused to a reverse transcriptase which introduces the edit encoded in a guide RNA extension. Prime editing makes a single-strand break, termed a nick, and therefore rarely produces unwanted mutations in mammalian cells. However, in zebrafish embryos, prime editing yields surprisingly high rates of unwanted mutations (insertions and deletions at the cut site), around 2–6× the rate of edits. Most unwanted deletions are flanked by microhomologies and insertions are often templated from sequences neighbouring the cut—both hallmarks of repair of double-strand breaks via microhomology-mediated end-joining (MMEJ). In vitro, replication forks convert nicks to double-strand breaks. The same mechanism is probably at play here, and would explain the discrepancy with mammalian cells as the cell cycle of early zebrafish embryos is almost 10× faster. Knockout of polq, a helicase/DNA polymerase essential to MMEJ, abolishes almost every unwanted mutation generated during prime editing and drastically increases rates for some edits. We are developing a pharmaceutical approach to enable wider use of this strategy. We screened 26 compounds modulating DNA repair pathways and identified six promising hits, three of which inhibit MMEJ. By removing virtually all unwanted mutations generated during CRISPR prime editing, our work resolves a major obstacle towards routine and rapid phenotyping of pathogenic mutations in zebrafish larvae.

Optimized Cytosine Base Editors Overcome GC/CC Editing Barriers to Enhance Zebrafish Models of Human Genetic Diseases

Presenting Author: Yanmei Liu, Professor - Institute for Brain Research and Rehabilitation (IBRR), South China Normal University Guangzhou, Guangdong, China

Co-Author(s): Shaohui Zheng – South China Normal University; Yang Liu – South China Normal University; Yu Zhang – South China Normal University, Oklahoma Medical Research Foundation; Wei Qin – Oklahoma Medical Research Foundation; Xinxin Xia – South China Normal Unive

Abstract: Base editing technology enables precise single nucleotide mutations in animal models, aiding in the modeling of human genetic diseases—a key advantage for precision medicine. Cytosine base editors (CBEs) can convert C•G pairs to T•A pairs, accounting for nearly half of human pathogenic variations. However, traditional CBEs in zebrafish struggle with editing GC and CC motifs, limiting their utility. To address this issue, our team has developed two optimized CBE systems for zebrafish: zTadA-CBEs and zevoCDA1. These tools overcome sequence preferences, enhancing GC and CC motif editing.

For NGG PAM targets, zTadA-BE4max (window 4-8), generated by replacing the deaminase in the traditional CBE with the mutated adenosine deaminase TadA8e, generally produces fewer indels compared to zevoCDA1-BE4max (window 1-9). zTadA-Bemv (window 9-16), created by adjusting the relative positions of nSpCas9 and TadA, provides a complementary editing window to zTadA-BE4max.

SpRY-CBEs, which are not restricted by PAM sequences, show better performance at NRN PAM sites than at NYN PAM sites. zTadA-SpRY-BE4max (window 4-8) typically exhibits lower efficiency at positions 4 and 8, potentially making it more precise in practice. In contrast, zevoCDA1-198 (window 0-5), which edits at positions -1 and -2 at some loci, shows higher efficiency. zevoCDA1-NL (window 1-7) and zevoCDA1-SpRY-BE4max (window 1-9) have even higher editing efficiencies and can target some NYN PAM sites that zTadA-SpRY-BE4max cannot.

Using these tools, we successfully established several previously challenging disease models, including Hermansky-Pudlak syndrome and Axenfeld-Rieger syndrome, and corrected a missense mutation (T>C) in the fms ts± mutant of the zebrafish FMS receptor gene back to the wild type, restoring macrophage numbers. Overall, these two cytosine editor toolkits significantly enhance zebrafish's capability as a model for human genetic diseases, offering more possibilities for elucidating the pathogenic significance of Variants of Unknown Significance, exploring disease pathogenesis, and developing personalized treatment strategies.

High-efficiency TadA Cytosine Base Editors for Precise Genetic Variant Modeling

Presenting Author: Wei Qin, PhD - OKLAHOMA MEDICAL RESEARCH FOUNDATION

Co-Author(s): Kevin Huang – OMRF; Sheng-Jia Lin – OMRF; Cassidy Petree – OMRF; Gaurav Varshney – OMRF; Pratishtha Varshney – OMRF; Yu Zhang – OMRF

Abstract: Base editors have emerged as powerful tools for introducing precise genomic modifications, but their application in model organisms has been limited by efficiency and targeting constraints. Here, we report the development and characterization of TCBE-Umax, a highly efficient TadA-derived cytosine base editor optimized for zebrafish. Through systematic engineering of the TadA deaminase domain, we achieved significantly improved editing efficiency with minimal sequence context bias. We further expanded the targeting scope by creating TCBE-Umax variants compatible with different PAM requirements and developed precision variants with reduced bystander editing and indel formation. The optimized TCBE-Umax2 enabled efficient biallelic editing, allowing rapid functional assessment of genetic variants in F0 generation zebrafish. As a proof of principle, we demonstrated the utility of this

system by evaluating 15 variants of uncertain significance (VUS) associated with hereditary hearing loss, successfully determining their pathogenicity through phenotypic analysis. The high efficiency and versatility of TCBE-Umax make it a valuable tool for precise genome editing in zebrafish, offering a powerful platform for studying genetic variants and disease mechanisms. This work establishes a framework for implementing improved base editors in model organisms and demonstrates their potential for advancing both basic research and clinical genomics.

Functional Genetic Testing of the Mitochondrial Genome Using Enhanced TALE Base Editors

Presenting Author: Stephen Ekker, PhD - University of Texas at Austin

Co-Author(s): Santiago Castillo – Mayo Clinic; Karl Clark – Mayo Clinic; Bibek Kar – Mayo Clinic; Mireya Mota – University of Texas at Austin; Kavini Nanayakkara – Mayo Clinic; Eiko Ogiso – Children's Hospital of Philadelphia; Ankit Sabharwal – University of Texas at

Abstract: The mitochondrial genome represents one of the most highly conserved known stretches of vertebrate DNA, with all 37 genes found in the same order from zebrafish (Danio rerio) to humans. Recently CRISPR-free, DddA-derived mitochondrial TALE base editors have enabled the precise edits of mitochondrial DNA (mtDNA). Deploying an enhanced version of this tool that can induce near-complete levels of heteroplasmy enabled the molecular tagging, biochemical testing, functional genetic assessments and the establishment of stable, multi-generation novel disease models using zebrafish. To further expand the utility of mtDNA base editors, we deployed a TALE architecture that does not require any defined target sequence. We show this unconstrained scaffold enables enhanced editing activity and improved precision in nearly all loci tested. Further, we identified and then screened a family of active DNA interacting proteins enabling DNA base editing on double-stranded DNA substrates. We used enhanced in silico biochemistry approaches with these diverse proteins to map protein/DNA/cofactor complexes, resolving discrepancies between static structures derived from X-ray crystals and base editing activities observed from biochemical reaction kinetics. This mechanistic understanding will open up the targeting of new pathogenic edits and corresponding biology. Further, this system also enabled the establishment of human cell lines with sufficiently high heteroplasmy levels to induce key biochemical deficits in vitro. Together, we are triangulating between zebrafish in vivo testing with human in vitro models to understand the molecular nature of genes in this fully syntenic circular chromosome.

Auxin inducible protein degradation in zebrafish

Presenting Author: Benjamin Martin, PhD - Stony Brook University

Co-Author(s): Robert Morabito – Stony Brook University; Ryan Swick – Stony Brook University; Samantha Stettnisch – Stony Brook University

Abstract: Conditional protein depletion is a powerful tool for understanding temporal or tissue specific protein function. Current methods for rapid protein depletion in zebrafish are limited. The plant derived auxin inducible degron (AID) system has been widely used in cell culture and several model organisms but has yet to be fully adapted for use in zebrafish. The system utilizes a degron fused to a protein of interest, which is recognized and targeted for degradation by the plant specific F-box protein Tir1 only in the presence of the plant hormone auxin. Here we show that the updated AID2 system works efficiently in zebrafish embryos. We generated Tir1 expressing transgenic lines in which degron tagged proteins

can be rapidly degraded only when auxin is added to the media. We use the AID2 system to examine maintenance and fate decisions of neuromesodermal progenitor cells, which can generate neural or mesodermal tissue. We show using degron tagged Sox2 that Sox2 maintains the undifferentiated neuromesodermal progenitor state, and that mesoderm can only be induced upon depletion of Sox2. We expect that the zebrafish AID2 system will be an effective tool for understanding temporal and tissue specific protein function in various developmental and disease model contexts.

Engineering of epitope tagged proteins: how, which and where?

Presenting Author: Darius Balciunas - Temple University

Co-Author(s): Henri Chung – Iowa State University; Tom Coyne – Temple University; Eileen Dalessandro – Temple University; Iddo Friedberg – Iowa State University; Ari Geller – University of Pennsylvania; Parnal Joshi – Iowa State University; Maura McGrail – Iowa State

Abstract: Lack of sufficient-quality antibodies continues to be a significant challenge for scientists using the zebrafish model system. This limitation is best overcome by expressing an epitope tagged protein, ideally by engineering the epitope tag into the endogenous locus coding for the protein of interest. Our laboratory has been using single stranded oligonucleotide (ssODN) templates to engineer epitopes and other short sequences such as loxP sites tags into the zebrafish genome for about a decade. While we were able to successfully modify every gene of interest, it is worth noting two important limitations of this approach: (i) indels are commonly observed at the 5' junction between the ssODN template and the endogenous sequence, and (ii) length of added exogenous sequence is limited by oligonucleotide synthesis. We have been primarily using the V5 tag, since we and others have found that 1xV5 is sufficient for immunohistochemistry and Western Blotting. In contrast, 1xHA tagged Tbx5a is not effectively recognized by commercially available antibodies, indicating that several repeats of the HA are likely to be needed in other scenarios as well. We have recently switched to the ALFA tag. In addition to ALFA-tagged lines we are generating auxiliary transgenic lines ubiquitously expressing anti-ALFA nanobody fused to GFP for live detection or Degron for protein degradation. Epitopes are traditionally added to the N- or C-terminus of the protein of interest. Our target site selection used to rely on manual identification of regions of relatively low homology. Iddo Friedberg's laboratory has recently developed a software package named EpicTope, which automatically considers additional features, such as solvent accessibility and predicted structure, in addition to evolutionary conservation. EpicTope was used to successfully select function-preserving tag integration sites in Smad5 and Hdac1. It is available on GitHub.

Concurrent Session III

Organogenesis — Shannon Hall | 10:30am-12:00pm

Session Chairs: Kristen Kwan - *University of Utah* & Holger Knaut

Wnt signaling directs epithelial tubule interconnection in the regenerating zebrafish kidney

Presenting Author: Iain Drummond, PhD - MDI Biological Laboratory

Co-Author(s): Bruce Draper – U.C. Davis; Samuel Hughes – MDI Biological Laboratory; Caramai Kamei – MDI Biological Laboratory; Denise Marciano – The University of Texas Southwestern Medical Center; Kyle McCracken – Cincinnati Children's Hospital Medical Center; Leif O

Abstract: Epithelial tubule fusion is fundamental for kidney morphogenesis. Differentiating nephron tubules interconnect with collecting system epithelia to generate a lumenal pathway for fluid excretion. In the adult zebrafish kidney, nephrogenesis occurs as a regenerative response to injury and provides a model to explore cell signaling pathways required for tubule interconnection. We show that canonical Wnt signaling at the junction between two tubules induces a mesenchymal, invasive cell phenotype and is required, along with Src kinase and rac1, to generate basal cell protrusions. The Wnt ligands wnt9b and wnt4 are both required for new nephron formation after injury. Mutation in wnt4 or treatment with the canonical Wnt inhibitor IWR1 blocks formation of basal protrusions in forming nephrons. Mutation in the Wnt receptor frizzled9b reveals a fusion-associated non-canonical Wnt pathway that acts to 1) restrict canonical Wnt gene expression, 2) drive Rho kinase-dependent apical constriction of epithelial cells, and 3) position basal protrusions and generate orthogonal tubule lumenal connections. As a result, frizzled9b mutant nephrons fail to fully interconnect with target distal tubules. Our results indicate that canonical and non-canonical Wnt signaling interact in the same cells to orient and drive tubule interconnection in the regenerating zebrafish kidney.

Hand2 functions upstream of Jam2 signaling to induce Etv2 expression in organ-specific vascular progenitors

Presenting Author: Saulius Sumanas, PhD - University of South Florida

Co-Author(s): Julius Martinkus – University of South Florida; Sanjeeva Metikala – University of South Florida; Diandra Rufin Flora – University of South Florida; Nicole Restrepo – University of South Florida; Zhitao Ming – Iowa State University; Fang Liu – Iowa State

Abstract: The mechanisms involved in the formation of organ specific vasculature have been poorly understood. It has been debatable whether it comes from existing vasculature by angiogenesis, sprouting from the existing vasculature, or by vasculogenesis, the emergence of new vascular progenitors de novo. We have recently identified a population of late-forming endothelial progenitor cells in zebrafish embryos (termed secondary vascular field, SVF), which emerge from the lateral plate mesoderm after 24 hpf stage, when blood circulation has been initiated, and show strong expression of ETS transcription factor Etv2. Here we investigated the molecular mechanisms that govern the emergence of these SVF cells and their contribution to vasculature. We identified that the bHLH transcription factor Hand2 and Junctional Adhesion Molecule Jam2b are expressed in the SVF-forming region and are required for the emergence of SVF cells in zebrafish embryos. Time-lapse imaging and hand2:Cre and jam2b:Cre based lineage tracing showed that SVF cells serve as the major source of intestinal vasculature, including the supraintestinal artery (SIA) and subintestinal vein (SIV), which are subsequently remodeled to provide the blood flow to many internal organs. To analyze the functional role of Jam2b and a related Jam2a gene in vascular development, we generated double maternal-zygotic jam2a; jam2b mutants, which display a greatly reduced number of Etv2-positive SVF cells and show defects in the intestinal vasculature development. Further analysis showed that Hand2 functions in the SVF-forming region upstream of Jam2b and is required to induce Etv2 expression in SVF cells. In summary, our results identify new roles for Jam2 signaling and Hand2 function in the emergence organ-specific vascular progenitors.

Investigating the roles of notochord membrane proteins in vacuole integrity and chordoma initiation.

Presenting Author: James Norman - Duke University Department of Cell Biology

Co-Author(s): Jennifer Bagwell – Senior Research Analyst, Cell Biology, Duke University; Daniel Levic – Assistant Research Professor, Cell Biology, Duke University; Courtney Karner – Principal Investigator, UT Southwestern Medical Center; Michel Bagnat – Principal Inv

Abstract: The notochord is a conserved axial structure that serves as a hydrostatic scaffold for embryonic axis elongation and a template for vertebral column formation. This organ consists of a core of large vacuolated cells, each containing a giant fluid-filled vacuole that is a lysosome related organelle (LRO) and defines the physical properties of the notochord. Here, we used an unbiased biochemical approach to identify notochord vacuole membrane-associated proteins and discovered a set of candidate proteins controlling LRO biogenesis. Perturbation of this pathway causes vacuole defects, such as fragmentation and stalled membrane fusion, that results in a shortened antero-posterior (AP) axis. To investigate the role of this pathway in de novo vacuole formation, we used mechanical cell ablation and found that following vacuolated cell collapse, notochord sheath cells lose their ability to form new vacuoles and replace the damaged cells. Furthermore, experiments in zebrafish and mice revealed that upon injury, impaired vacuole formation leads to the expression of early notochord markers, cell proliferation, and the formation of cell masses reminiscent of chordoma, an aggressive spinal tumor. Our findings highlight the critical roles of vacuole maintenance, de novo vacuole formation, and notochord repair mechanisms in AP axis biology and suggest a mechanism for chordoma initiation.

Functional identification of neurons and pace-maker like cells involved in various types of motility in the zebrafish gut

Presenting Author: Kohei Hatta - Grad Sch of Science, Univ of Hyogo

Co-Author(s): Miku Kato – Univ of Hyogo; Daiji Takamido – Univ of Hyogo; Shin -ichi Okamoto – Univ of Hyogo; Koichi Kawakami – National institute of Genetics; Masataka Nikaido – Univ of Hyogo

Abstract:

The gut of the zebrafish larva serves as a useful in vivo model for investigating the motor function of vertebrate digestive organs, due to its simplicity and transparency. Various types of gut motility have been observed, but little is known about which cells are involved in their generation. In the posterior intestine at 8 days post-fertilization (dpf), two distinct movements can be observed: a strong peristaltic reflex-like movement (PRLM) occurring at intervals of a few minutes, and a periodic regular movement (RM) occurring approximately every 30 seconds.

To identify the cells involved in each type of motility, we expressed the calcium indicator GCaMP3 stochastically in various gut cell types using a heat-shock-inducible transgenic line. We screened for cells activated in association with each type of motility. We found that PRLM is associated with neuronal activity involving multiple neurotransmitter systems, including serotonin and nitric oxide. In contrast, RM is closely associated with rhythmic activity of a non-neuronal cell group, pacemaker-like cells (PMLCs).

In some cases, the morphology of individual PMLCs, including their multiple branching processes, can be visualized at the peak of Ca^{2+} transients. We also observed modulation of PMLC activity in response to the passage of a bolus. Furthermore, we are employing a broader range of transgenic lines that enable

tissue-specific reporter expression, to further elucidate the neuronal and non-neuronal circuits involved in diverse gut motility patterns at single-cell resolution in vivo.

Forward Genetic Screen and new mapping pipeline uncover novel regulator of ApoB lipoprotein biogenesis

Presenting Author: McKenna Feltes, PhD - Johns Hopkins University

Co-Author(s): Aleksey Zimin, PhD – Biomedical Engineering – Johns Hopkins University; Steven Salzberg, PhD – Biomedical Engineering – Johns Hopkins University; Steven Farber, PhD – Biology – Johns Hopkins University

Abstract: Forward genetic screening is a powerful approach that has led to numerous discoveries across biology. Unbiased chemical mutagenesis is cheap and efficient, but identifying the single base pair change causing the phenotype in the large polymorphic zebrafish genome is challenging and time-consuming. To address this limitation, we have designed a state-of-the-art and freely available mapping pipeline called WheresWalker. Recent improvements and ideal mapping parameters for WheresWalker will be discussed in addition to exciting findings from our screen, which identified 27 new mutants with "dark yolk" (DY), a signature of abnormal Apo-B lipoprotein (B-lp) processing. Of the mutant collection, 10 mutants map to known DY loci (mttp, dgat2, apobb.1, mia2) while the 17 others represent ≥13 unique and novel loci. We have used novel mttp, apobb.1, and mia2 mutants to develop and optimize WheresWalker mapping. WheresWalker has been used to map 6 of the novel loci from our screen and has been successfully deployed across the zebrafish community. One of the novel mutants, zion, maps to slc3a2a. SLC3a2 binds with SLC7 family members to transport amino acids, and in human GWAS datasets, many of the SLC7 genes are associated with phenotypes linked to abnormal B-lp metabolism. When each zebrafish slc7 was targeted by F0 CRISPR, only slc7a7 targeted animals developed DY, suggesting slc3a2a/slc7a7 mediates the phenotype. Excitingly, supplementing larval growth media with amino acids rescues B-lp production in slc7a7 mutants, suggesting amino acid balance is key to B-lp synthesis. The mechanism of this regulation is currently under investigation. Interestingly, inhibition of the amino acid sensor mTORC1 also results in DY and reduced B-lp. Insights from the zion mutant are shedding light on how metabolic signals influence B-lp synthesis and will help us to better understand how human disease states, such as cardiovascular disease and diabetes, develop.

Dietary protein absorption in the ileum is regulated by local metabolism of circulating lipoproteins

Presenting Author: John Rawls, PhD - Duke University School of Medicine

Co-Author(s): Jia Wen – Molecular Genetics and Microbiology – Duke University School of Medicine; Laura Childers – Cell Biology – Duke University School of Medicine; Tiffany Liu – Molecular Genetics and Microbiology – Duke University School of Medicine; Sarah Gorbatov

Abstract: The vertebrate intestine consists of distinct functional domains along its anteroposterior length, each with distinct roles in nutrient absorption. The anterior domain is specialized for absorption of dietary fats which are packaged into lipoproteins that are released into circulation. In contrast, the ileal domain has the unique capacity for uptake and intracellular digestion of dietary proteins. This ileal activity is performed by lysosome-rich enterocytes (LREs) which are present in pre-weaning mammals and in zebrafish throughout the lifespan. However, coordination between these distinct intestinal functions was unknown. We discovered that the ileum in zebrafish and pre-weaning mice expresses

Lipoprotein lipase (Lpl), the rate-limiting enzyme for triglyceride hydrolysis from circulating lipoproteins. To test the role of Lpl in the intestine, we mutated the Lpl inhibitor angiopoietin-like 4 (angptl4). After high-fat diet (HFD) challenge, angptl4 mutants exhibited highly abnormal accumulation of lipid droplets in ileal LREs accompanied by reductions in circulating lipid levels. LRE lipid accumulation in angptl4 mutants was abolished by further mutating lpl, indicating that it is due to uncontrolled Lpl activity. Similarly, disrupting lipoprotein synthesis by mutating microsomal triglyceride transfer protein (mttp) prevented lipoprotein export from the anterior intestine and eliminated lipid accumulation in ileal LREs after HFD. Thus ileal LREs produce Lpl, which, when active, liberates lipids from circulating lipoproteins which they take up and store. Using fluorescent protein gavage assays in angptl4 mutants, we also found that lipid storage in LREs after HFD is accompanied by reduced uptake and degradation of dietary protein and downregulation of endocytic and lysosomal genes. This establishes a novel role for lipoprotein metabolism in LRE protein uptake, and a new pathway regulating intestinal nutrient absorption. Through regional Lpl-Angptl4 interactions in the intestine after HFD, LREs accumulate lipoprotein-derived lipids which in turn reduces their uptake and intracellular digestion of dietary proteins.

Toxicology, Environmental Biology and Sustainability **Play Circle Theater | 10:30am-12:00pm**

Session Chairs: C. Ben Lovely - *University of Louisville School of Medicine* & Jessica Plavicki

Valproic Acid Disrupts Redox Homeostasis and Alters Cellular Composition in the Optic Tectum of Larval Zebrafish

Presenting Author: Bailey Calder - Brigham Young University

Co-Author(s): Jaquelyne Howell – Brigham Young University; Michael Zeyer – Brigham Young University; Sierra Dixon – Brigham Young University; Annalie Martin – Brigham Young University; Brandon Davies – Brigham Young University; Jason Hansen – Brigham Young University;

The integration of multi-modal sensory inputs (e.g., visual, auditory) into a cohesive framework is critical for guiding an organism's interactions with its environment. In mammals, this function is partially executed by the superior colliculus (SC), a midbrain structure which utilizes multisensory integration to guide involuntarily behaviors toward or away from relevant environmental stimuli, ultimately influencing social behavior. Accumulating evidence has implicated the SC in neurodevelopmental disorders such as autism spectrum disorder (ASD), highlighting the need to understand its developmental mechanisms. One such mechanism involves the regulation of neuronal differentiation through the redox state of the cell. Proper regulation of redox states is crucial as it leads to protein modifications, which fundamentally alters protein function within the cell and the tissue as a whole. Interestingly, valproic acid (VPA)–an antiepileptic drug–has been shown to both affect the redox state of differentiating neurons in culture and is associated with higher incidence of ASD in children following fetal exposure. To better understand the connection between redox stress, VPA, and regions of multisensory integration like the SC, we treated developing zebrafish with VPA and characterized the development of the optic tectum (OT)–the homologous non-mammalian structure of the SC. We found that VPA treatment altered redox states within the developing OT and altered neuronal specification. Furthermore, single-cell RNA sequencing in treated vs untreated larvae reveals changes in both neuronal and glial composition within the OT. Together, these data provide strong evidence that VPA affects

development of the OT and, by association, might influence human neurodevelopment and the possible molecular changes leading to ASD.

Preconception paternal alcohol exposure leads to physical and behavioral changes commonly associated with fetal alcohol spectrum disorders.

Presenting Author: Yohaan Fernandes - Univeristy of South Dakota

Co-Author(s): Mindy Rampersad, B.ED. – Research Associate, Biology, Univeristy of South Dakota; Spencer Hurst – Undergraduate Research Associate, Biology, University of South Dakota; Kaia Olson – Undergraduate Researcher, Biology, University of South Dakota

Abstract: Prenatal alcohol exposure can lead to a range of physical and/or central nervous system birth defects in offspring jointly called fetal alcohol spectrum disorders (FASDs). Many factors can contribute to the severity of FASDs. While maternal alcohol consumption during pregnancy has predominantly been studied as a contributor to FASDs, the influence of preconception paternal ethanol exposure (PPatEE) remains unclear. We characterized the effect PPatEE has on the body and behavior of larval and juvenile zebrafish. For seven evenings, between 6:30 and 7:30 pm male zebrafish were exposed to 1% ethanol. On the seventh evening after the final exposure, males were bred with unexposed females. At 3 days postfertilization (dpf) we measured body length, intraocular distance, the area of the eye as well as the length, width and area of the head. Next, using the novel tank test (NTT) on day 65 we measured the total distance moved, the latency to reach the top of the tank, the average velocity as well as the time spent in the bottom, middle and top of the tank. While we did not find a difference in the body length of offspring with PPatEE, we did find significant decreases in intraocular distance, eye area, head area, head length, and head width, demonstrating that PPatEE leads to facial abnormalities. At 6-dpf we found larvae with PPatEE moved more than controls. Furthermore, at 65 dpf, we found juveniles with PPatEE moved more, had an increased velocity, spent less time in the bottom of the tank, and reached the top zone faster than controls, suggesting that PPatEE is associated with hyperactivity and increased risk-taking behaviors. Thus, our results show for the first time that zebrafish can characterize the effects of PPatEE and more importantly suggest that PPatEE is more harmful than previously anticipated.

Craniofacial defects associated with nicotine and nicotine and ethanol co-exposure in zebrafish embryos are mediated by nicotine metabolites generated by Cyp2y3

Presenting Author: Gissela Borrego-Soto - University of Texas at Austin

Co-Author(s): Ritika Ghosal – University of Texas at Austin; Ian Riddington – University of Texas at Austin; Esther Meier – University of Texas at Austin; Aneesha Baral – University of Texas at Austin; Luis Compean – University of Texas at El Paso; Radhika Amin – Univ

Abstract: Nicotine and alcohol are two of the most widely consumed substances among adolescents and adults. Thus, there is worldwide concern regarding the deleterious effects of exposure to these substances during pregnancy. Complex interactions between genes and the environment are thought to influence the severity of birth defects partly by altering the metabolism and rate of elimination of substances. In humans, nicotine is mainly metabolized by the CYP2A6 enzyme and variants within this enzyme associate with nicotine dependence and metabolism. Previously, we showed that embryonic nicotine exposure reduced the size of craniofacial cartilages in zebrafish. Here, we show via mass

spectrometry that zebrafish metabolize nicotine similarly to humans. We demonstrate that it is not nicotine itself, but nicotine metabolites, particularly 4HPBA followed by nicotine-N'-oxide, that are teratogenic. We used CRISPR/Cas9 to generate cyp2y3 zebrafish mutants (the zebrafish homologue of CYP2A6). We found that cyp2y3 mutants have reduced levels of nicotine metabolites and are protected against the craniofacial defects produced by nicotine. However, these mutants are not protected against the teratogenicity of the nicotine metabolites. We also examined the effect of nicotine and ethanol co-exposure in zebrafish embryos. Surprisingly, embryos co-exposed to nicotine and alcohol were significantly less affected than those exposed to nicotine alone. We found that nicotine metabolites were decreased in embryos co-exposed to nicotine and ethanol. However, alcohol was not protective when co-exposed with metabolites. Our results collectively show that nicotine is metabolized into teratogenic substances and that multifactorial interactions can alter this metabolism and subsequent teratogenicity.

The small molecule ML233 is a direct inhibitor of tyrosinase function

Presenting Author: Romain Menard - MDI Biological Laboratory

Co-Author(s): Romain Menard – MDI Biological Laboratory; Caroline Halluin – MDI Biological Laboratory; Dexter Morse – MDI Biological Laboratory; Sadie Kuhn – MDI Bioscience; Joel H. Graber – MDI Biological Laboratory; James Strickland – MDI Bioscience; Romain Madelain

Abstract: Melanogenesis is the biological process regulating the synthesis of melanin pigments in melanocytes. Defective melanogenesis is associated with numerous human skin diseases, including, but not limited to, albinism, vitiligo, melasma, and hypo- and hyperpigmentation disorders. Tyrosinase is the rate-limiting enzyme controlling melanogenesis, and hence tremendous efforts have been made to identify potent and safe inhibitors of tyrosinase function. However, despite decades of research, currently there is no effective treatment that inhibits melanogenesis or tyrosinase activity with no adverse side effects. In the hunt to discover new melanogenesis inhibitors, the zebrafish is a recognized model organism because it alleviates the need for mammalian study during the early stages of drug efficiency and toxicity testing. The zebrafish provides countless advantages for drug screening and/or testing, including drug delivery via solubilization of the drug in the water of the fish, high penetration of the compound through the mouth, gills, and skin, and direct visualization of the pigmentation phenotype in embryos or larvae. In this study, we report the characterization of the ML233 chemical as a potent inhibitor of tyrosinase activity in vivo and in vitro. We demonstrate that ML233 reduces melanin production in the zebrafish model with no observable significant toxic side effects, and in murine melanoma cells. We also predict that these effects are mediated through direct tyrosinase-ML233 interaction, i.e., binding of the ML233 molecule to the active site of the protein to inhibit its function. Together, our results reveal that ML233 plays roles in both healthy and pathological skin cells via inhibition of melanin production. ML233-mediated tyrosinase inhibition is a potentially safe and effective approach to alleviate the symptoms of melanocyte-associated diseases and thereby substantially improve human health.

Estrogen-Mediated Perturbations in Biliary Endothelial Cells: Implications for Liver Homeostasis and Disease

Presenting Author: Patrice Delaney, PhD - Massachusetts General Hospital / Harvard Medical School

Co-Author(s): Wolfram Goessling – Massachusetts General Hospital / Harvard Medical School

Abstract: The liver is comprised of two primary cell types: hepatocytes, which perform diverse metabolic functions, and biliary epithelial cells (BECs), which are crucial for supporting liver function and facilitating bile flow. Together, these cells form a complex network that is vital for the liver's metabolic and physiological integrity. While the effects of excess estrogen on hepatocyte proliferation are well-established, its influence on BECs is still being elucidated, leaving significant gaps in our understanding of the underlying mechanisms and their physiological implications. In this study, we investigate the role of estradiol (E2) in biliary biology and its relevance to liver health and disease using zebrafish larvae. Our findings reveal that developmental exposure to E2 (from 0 to 6 days post-fertilization) significantly reduces the total number of BECs, altering the hepatocyte-to-BEC ratio by six-fold. Similarly, the total volume and complexity of the biliary network are markedly diminished. Intriguingly, livers treated with E2 exhibit a complete absence of bile flow, indicating potential disruptions in the BEC network, hepatocyte function, or both. Live imaging of hepatocyte parameters, including cellular polarity and vesicle trafficking, uncovered profound dysfunction during the developmental E2 challenge. We hypothesized that BECs, which are capable of transdifferentiating into hepatocytes upon substantial hepatocyte damage, may compensate for the loss of hepatocyte function induced by E2. Lineage tracing experiments confirmed that E2 induces BEC transdifferentiation, suggesting that E2-induced hepatocyte dysfunction may trigger a compensatory response from BECs. This highlights the intricate interplay between estrogen and liver cell populations, demonstrating the potential role of BECs in mitigating the dysfunction of hepatocytes induced by estrogen exposure. Further elucidation of these mechanisms holds promise for advancing our understanding of liver pathophysiology and developing targeted therapeutic strategies for liver diseases influenced by estrogen signaling.

Environmental Contaminant Exposure During Juvenile Development Induces Sex-Specific Cardiac Dysfunction

Presenting Author: Michelle Kossack, PhD - Brown University

Co-Author(s): Lucy Tian – Brown University; Katrina Albro – Brown University; Kealyn Bowie – Brown University; Joshua Marcus – Brown University; Jessica Wang – Brown University; Cliff Oduor – Brown University; Susan Hall – Brown Unversity; Jeffrey Bailey – Brown Unive

Abstract: Adult cardiovascular disease (CVD) is the leading cause of death worldwide. While adult nutrition and activity levels play important roles in shaping cardiovascular health, early life exposures, stressors, and infections also contribute to the etiology and progression of CVD. Humans are continuously exposed to environmental contamination; however, comparatively little is known about how developmental exposure to contaminants impacts adult heart health. Embryonic and early larval exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a global and persistent environmental contaminant, produces lethal cardiac phenotypes in zebrafish; however, it is not known if juvenile exposures to TCDD can cause long-term, adverse cardiovascular effects. We exposed juvenile zebrafish to an environmentally relevant concentration of TCDD and assessed cardiovascular health between 3 to 12 months post-fertilization using electrocardiograms (ECG), echocardiograms, histology, and RNA sequencing. We discovered that juvenile exposure to TCDD results in adult cardiac dysfunction in an age- and sex-specific manner. Using ahr2 mutants we found that the aryl hydrocarbon receptor, the canonical signaling pathway for TCDD-induced damage, is responsible for mediating changes in the depolarization of the atrium regardless of sex. In females, TCDD exposure is known to cause reduced

fertility. To disentangle the influence of ovarian dysfunction, we used the piwil1:CFP-NTR to ablate oocytes and phenocopy TCDD-specific infertility. We determined that ovarian health mediated changes in the heartrate. Thus, we revealed that the changes in electrical conduction after TCDD exposure are mediated by Ahr2 and ovarian damage. Echocardiograms revealed that TCDD exposure causes cardiac dilation in females and hypertrophic cardiomyopathy in males, phenotypes that were further supported by histological analyses. Finally, using sequencing, we found that vitellogenin genes were upregulated in the TCDD-exposed individuals, which suggests a dysregulation of estrogen signaling. Together, our results indicate that juvenile exposure to TCDD causes lasting effects on adult cardiovascular function and the development of CVD.

Stem Cells **Wisconsin Historical Society | 10:30am-12:00pm**

Session Chairs: Marlies Rossmann - *University of Rochester Medical Center* & Junsu Kang – University of Wisconsin - Madison

Jund orchestrates cis-regulatory element dynamics to facilitate endothelial-to-hematopoietic transition

Presenting Author: Lu Wang - Institute of hematology

Co-Author(s):

Abstract: The tightly controlled temporal-spatial expression of developmental genes depends on the concerted action of cis-regulatory elements (CREs) and transcription factors (TFs) to ensure cell fate decisions. Endothelial-to-hematopoietic transition (EHT) is a cell fate transition process by which endothelial cells acquire hematopoietic identity and become hemogenic endothelial cells (HECs) and then hematopoietic stem and progenitor cells, but the underlying CRE network dynamics and its regulation by TFs remain unclear. In this study, we characterized the dynamics of CRE activation and TF occupancy during zebrafish EHT, and found that the enhancer-promoter distributed collaboration forms the basis for EHT. Moreover, a ubiquitously expressed TF AP-1 collaborated with diverse lineage-specific TFs to remodel enhancer landscape. Deletion of AP-1 family member Jund impaired hematopoietic specification, resulting from the enhanced endothelial identity in the HEC. Mechanistically, Jund and hematopoietic TF Hoxa9 collectively inhibited the activity of an endothelial-related dll4 enhancer through diminishing the active histone modification H3K27ac. Our study provides insights into the cooperative function among ubiquitous TFs and cell type-specific TFs in orchestrating cell fate transition.

A collagen-modifying enzyme is required for vertebrate hematopoietic stem cell specification

Presenting Author: Wilson Clements - St. Jude Children's Research Hospital

Co-Author(s): Hanane Khoury, PhD – Hematology – St. Jude Children's Research Hospital; McLean Willliamson – Hematology – St. Jude Children's Research Hospital

Abstract: Hematopoietic stem cells (HSCs) are the tissue-specific stem cells that continuously regenerate blood and immune cells over the lifetime of an individual. HSCs are the foundational stem cell model and inform understanding of cellular behaviors and decisions including proliferation/quiescence, migration/residency, stemness/differentiation, and the niche conditions that support and direct these outcomes. HSCs are clinically relevant as the therapeutic component of bone marrow transplants, as a vector for gene therapy for hematologic and autoimmune disorders, in disease models, and in drug

studies. To better understand HSC behavior and to inform efforts to maximize efficiency of in vitro directed differentiation, we have been working to better understand the native physiological conditions that lead to emergence of the first HSCs during development. HSCs derive from specialized “hemogenic” endothelium in the primitive trunk vasculature in a manner that is conserved across vertebrate phyla. We computationally identified genes predicted to play central regulatory roles in this process from an in vitro model of hematopoietic commitment. Our findings prioritized an enzyme, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (Plod2), which post-translationally modifies collagen—the central component of the extracellular matrix (ECM)—to stabilize the maturation of collagen networks into higher order structures such as fibrils or basal lamina, as central to HSC emergence. To determine if plod2 is required in a vertebrate for definitive hematopoietic emergence, we performed knockdown of plod2 using morpholino antisense oligonucleotides as well as CRISPR/Cas9-mediated genetic deletion of plod2 in zebrafish. Loss of plod2 drastically reduces or eliminates HSC specification during development. Knockout of Plod2 in mouse indicated that this requirement in hematopoietic development is conserved in mammals. Our results indicate that a collagen-modifying enzyme is required for hematopoietic development in vertebrates and point to the idea that careful modulation of the biomechanical properties of the ECM are crucial to successful physiological HSC specification.

chd2 Mediates crosstalk between Neural Development and Hematopoietic stem and progenitor cell Expansion via Inflammation in Zebrafish Embryos

Presenting Author: Yibo Shao - State Key Laboratory of Organ Regeneration and Reconstruction, Beijing Institute for Stem Cell and Regenerative Medicine, Institute of Zoology, University of Chinese Academy of Sciences, Chinese Academy of Sciences

Co-Author(s): Dongyuan Ma – State Key Laboratory of Organ Regeneration and Reconstruction, Beijing Institute for Stem Cell and Regenerative Medicine, Institute of Zoology, University of Chinese Academy of Sciences, Chinese Academy of Sciences; Feng Liu – State Key Lab

Abstract: Hematopoietic stem and progenitor cells (HSPCs) require a tightly regulated developmental microenvironment for normal functions. While neural signals are known to be critical for HSPC emergence, their role in regulating rapid HSPC expansion remains poorly understood. Here, we reveal that chromodomain helicase DNA binding protein 2 (chd2), a chromatin remodeling factor previously associated with congenital epilepsy and intellectual disability, regulates inflammation levels in the zebrafish caudal hematopoietic tissue (CHT), thereby influencing HSPC development. First, we generated a chd2 mutant zebrafish, which exhibited impaired glutamatergic neuron development, consistent with epilepsy-related phenotypes. Given the link between epilepsy and inflammation, we then analyzed inflammatory responses in chd2-deficient embryos and observed robust CHT inflammation and impaired HSPC development. Specifically, apoptosis of HSPC in the CHT of chd2 mutants was significantly increased, while AGM-derived HSPCs and primitive hematopoiesis remained unaffected. Further analysis demonstrated that HSPCs in the CHT of mutant embryos exhibited ferroptosis. Notably, the aberrant development of glutamatergic neurons in the mutants may influence HSPC development in the CHT via macrophage-driven inflammatory responses.These findings reveal a novel role for the nervous system in regulating HSPC development, providing new insights into the co-occurrence of neurodevelopmental disorders and immune deficiencies.

Negative regulation of the NLRP3-inflammasome via the aryl hydrocarbon receptor is critical for HSC expansion

Presenting Author: Morgan Walcheck, PhD - Boston Children's Hospital

Co-Author(s): Mindy Leder1 – Boston Children's Hospital; Vivian Taylor – Boston Children's Hospital; Trista North – Boston Childrens Hospital and Harvard Medical School

The ability to produce functional hematopoietic stem and progenitor cells (HSPCs) in vitro offers remarkable therapeutic value to millions of people suffering from blood disorders. However, current de novo differentiation protocols fail to predictably produce multipotent HSPCs with long-term engraftment potential. Therefore, identification of novel spatiotemporal regulators governing HSPC emergence and/or expansion in vivo is a major unmet clinical need. We previously revealed an essential role for inflammatory signaling via the NLRP3-inflammasome in HSPC specification. However, despite the beneficial effects of acute inflammation on HSPC emergence in the dorsal aorta, excess stimulation during HSC expansion in the caudal hematopoietic tissue (CHT) has detrimental impact on maintenance, suggesting inflammatory activity must be tightly spatiotemporally controlled. The aryl hydrocarbon receptor (AHR) is a well-conserved transcription factor, previously shown to inhibit Nlrp3 expression in mouse peritoneal macrophages. To determine if AHR could act as an essential negative regulator of inflammatory activity during HSPC development, we examined the impact of modulation of NLRP-inflammasome activity at each stage of hematopoiesis in the zebrafish embryo. Consistent with a role in inflammatory regulation, we have found that chemical or genetic AHR inhibition enhances NLRP3 reporter activation and inhibits HSPC expansion in the CHT; conversely, AHR activation, reduces inflammatory signaling, allowing for increased HSPC numbers. Importantly, stage-specific AHR-associated inflammasome regulation also had profound effects on lineage commitment, such that both inflammatory stimulation or inhibition via the AHR pathway increased in Rag2+ lymphocytes. Likewise, during ex vivo production of lymphocytes from human induced pluripotent stem cells, we found that AHR-associated inflammasome regulation controlled the balanced production of natural killer cells verses T-cells. Together, this data suggests modulation of inflammasome activity is a critical step in HSC production in vivo which may have direct relevance for improving the formation and maintenance of functional HSPCs ex vivo to treat hematologic disease.

Vcam1 expression in the CHT during the development of hematopoietic stem cells.

Presenting Author: Octavia Santis Larrain - UW-Madison

Co-Author(s): Alice Kadour – Research intern, Cell and Regenerative Biology, UW-Madison; Owen Tamplin – Assistant Professor, Cell and Regenerative Biology, UW-Madison; Nicole Woodhead – Graduate Student, Cell and Regenerative Biology, UW-Madison

Abstract: Hematopoietic stem and progenitor cells (HSPCs) are essential for vertebrate blood systems because they can differentiate into all blood cell lineages. In mammals, the interaction between HSPCs and the fetal liver niche is critical for stem cell programming and expansion. In this niche, the role of integrin alpha 4/beta 1 (Itga4/b1) on HSPCs and vascular cell adhesion molecule (Vcam1) on fetal liver niche cells is required for HSPC function. Zebrafish serve as a powerful model for studying HSPC niche interactions, as the role of Itga4/b1 on HSPCs is conserved. However, the specific niche cells expressing Vcam1 in the zebrafish caudal hematopoietic tissue (CHT), the fetal liver equivalent, remain unclear. Our research aims to determine the critical CHT niche cells expressing vcam1b mRNA and Vcam1 protein. We employed RNAscope for whole-mount fluorescent in situ hybridization (FISH) and immunofluorescence in different zebrafish transgenic lines to identify vcam1b-expressing cells. We

found expression in mesenchymal stromal cells (MSCs) and endothelial cells (ECs) within the CHT but observed no expression in macrophages, contradicting previous reports. We confirmed these findings by reanalyzing published single-cell RNA-seq (scRNA-seq) and bulk RNA-seq datasets from sorted MSC, EC, and macrophage populations across embryonic stages. To assess Vcam1 protein expression, we utilized a custom rabbit polyclonal anti-Vcam1b antibody, validated for flow cytometry. We confirmed Vcam1b protein expression on live MSCs and ECs and its absence from macrophages. We used this antibody to sort Vcam1+ cells for single cell RNA-seq. Cluster analysis and cell-type specific markers validated the isolation of MSCs and ECs but not macrophages. In conclusion, Vcam1b is expressed by MSCs and ECs in the CHT, highlighting the critical role of HSPC-niche cell interactions during development.

Hematopoietic Stem and Progenitor Cell Maturation is Dependent on Integrin α4-mediated Interactions with the Niche During Development

Presenting Author: Nicole Woodhead, MS - University of Wisconsin-Madison

Co-Author(s): Octavia Santis-Larrain – University of Wisconsin-Madison; Sobhika Agarwala – University of Wisconsin-Madison; Wantong Li – Ohio State University; Rodolfo Calderon – Iowa State University; Clyde Campbell – Iowa State University; Raquel Espin-Palazon – Iow

Abstract: Hematopoietic stem and progenitor cells (HSPCs) are regulated by niche support cells. In the mammalian embryo, the fetal liver supports HSPC maturation, however, the genetic regulatory networks involved are poorly understood. To understand these networks, we are using a viable integrin α4 (itga4) mutant zebrafish because HSPCs fail to lodge in the caudal hematopoietic tissue (CHT), the mammalian fetal liver equivalent. To define niche mechanisms that support HSPC maturation, we sorted wild-type (WT) and itga4 mutant Runx:mCherry+ HSPCs via FACS after CHT stage at 5 days post fertilization (5 dpf). Bulk RNA-seq detected 286 up- and 49 down-regulated transcripts in itga4 mutant versus WT ($q < 0.05$), and GSEA revealed upregulated inflammatory pathways in itga4 mutant HSPCs. To validate inflammatory signaling changes, we compared itga4 or control morpholino injected into NFκB:d2eRFP;CD41:GFP+ reporters, and NFκB activity was increased 1.5-fold in itga4 morphant HSPCs versus WT at beginning of CHT stage at 3 dpf. To determine if itga4 mutant HSPCs have an altered chromatin landscape, we performed bulk ATAC-seq and discovered 84,236 peaks unique to WT and 16,875 peaks unique to itga4 mutant ($q < 0.05$). Motif analysis identified ETS and AP-1 as potential HSPC maturation regulatory factors. To understand loss of itga4 in HSPCs through development and into adulthood, we used split-pool barcoding single cell RNA-seq of whole kidney marrow (WKM) from young (6-month-old) WT and itga4 mutant adults. We also included aged (24-month-old) WT adults to represent an “inflammaging” phenotype. Consistently, factors increased in aged WT WKM (e.g., cebpa, il1b, nfkbiab), were also increased in young itga4 mutant WKM. Furthermore, young adult itga4 mutant WKM had more myeloid-biased HSPCs (4.46% vs 1.63%, $p=0.0432$) and mitotic HSPCs (47.42% vs 3.83%, $p < 0.001$) versus young WT. Together, our results suggest itga4-dependent niche interactions are required for HSPC stability through development and into adulthood.

Keynote Session II: Shannon Hall | 5:00-6:00pm

Dissecting Human Hematopoietic Stem Cell Development, Self-Renewal and Transformation

Hanna Mikkola, MD, PhD – *University of California Los Angeles*

Sunday, July 13, 2025

Plenary Session VI:

Regeneration Across the Lifespan **Shannon Hall | 8:30-10:00am**

Session Chairs: Ellen Lien – *Uniersity of Southern California* & Jing-Wei Xiong

Platelet-derived Hb-egfb regulates zebrafish heart regeneration

Presenting Author: Yuanyuan Sun - Peking University

Co-Author(s): Chenglu Xiao – School of Basic Medical Sciences, Jiangxi Medical College, Nanchang University, Nanchang, China; Junjie Hou – School of Basic Medical Sciences, Jiangxi Medical College, Nanchang University, Nanchang, China; Jing-Wei Xiong – School of Basic

Abstract: Zebrafish heart regeneration is involved in an orchestra of intercellular signaling from different heart cell types. However, it remains poorly understood how platelets and related signaling participate in heart regeneration. Here, we report that platelet-derived ligand Hb-egfb is critical for zebrafish heart regeneration. By using global hbegfb knockout mutants (hbegfb KO), we found that hbegfb deficiency interfered with epicardial invasion into the infarct area, decreased injury-induced cycling cardiomyocytes, and increased cardiac fibrosis and collagen deposition, while the deficiency of hbegfb paralogue gene, hbegfa, had minimal impact on cardiomyocyte proliferation and heart regeneration after ventricular resection. Interestingly, we found that Hb-egfb was highly enriched in platelets in the heart after injury, and conditional hbegfb knock-out in platelets (hbegfb cKO) had heart regeneration defects that were similar to hbegfb KO mutants, suggesting that Hb-egfb in platelets play essential function during cardiac regeneration. Ongoing studies focus on how Hb-egfb in platelets interacts with its receptors in the epicardium to coordinate heart regeneration. Therefore, this work present the critical role of Hb-egfb as a novel platelet-derived factor during zebrafish heart regeneration.

Sonic hedgehog signaling upregulates basal epidermal fibrillin 3 to support guided osteoblast movements underlying zebrafish fin ray branching

Presenting Author: Sam Horst - University of Oregon

Co-Author(s): Gabriel Yette – University of Oregon; Astra Henner – University of Oregon; Scott Stewart – University of Oregon; Kryn Stankunas – University of Oregon

Abstract: Adult zebrafish fins rapidly and robustly regenerate, including restoring their characteristic branched bony ray skeletons. Ray branching proceeds by splitting of progenitor osteoblast (pOb) pools adjacent to sonic hedgehog a (shha)-expressing basal epidermal cells (bEps). Sonic hedgehog / Smoothened (Shh/Smo) signaling is active transiently in both cell types within a narrow, distal domain of the fin regenerate. Inhibiting Shh/Smo signaling specifically blocks ray branching. However, how Shh/Smo signaling promotes pOb pool splitting is poorly understood. We established an intubated adult live imaging method to visualize pOb and bEp behaviors for up to 24 hours during fin regeneration. shha-expressing bEps moved collectively over adjacent pOb pools. pObs varied in morphology and directional cell movements. Membrane reporters showed distal pObs cell protrusions that contact passing-by bEps, followed by their co-movement. Smo inhibition increased the bEp movement rate and disrupted interactions between bEps and pObs. In some cases, Smo-inhibition caused partially split pOb

pools to re-fuse. We conclude Shh/Smo signaling likely promotes transient, heterotypic interactions between pObs and passing bEps that ratchets pObs into split pools. We then used transcriptomics and CRISPR mutagenesis to identify and functionally screen candidate Shh/Smo target genes. Germline mutation of one candidate, fibrillin 2b, disrupted ray branching during fin regeneration. fibrillin 2b is orthologous to fibrillin 3 in humans, so we will refer to it as fbn3, and fibrillin 2a as fbn2. The branching phenotype was exacerbated in fbn2/fbn3 double mutants. Smo inhibition decreased fbn3 expression specifically in shha-expressing bEps. fbn3 mutants showed elevated or aberrant microfibrils at the interface between pObs and bEps. We propose Shh/Smo-upregulated Fbn3 reorganizes the extracellular environment between pObs and bEps to enable their co-movements underlying ray branching morphogenesis. R01GM149999 and F31HD113401 provided support.

Regeneration programs buffer defects in development

Presenting Author: Kazunori Ando, PhD - Morgridge Institute for Research, University of Wisconsin-Madison

Co-Author(s): Sushant Bangru – Postdoctoral Fellow, Regenerative Biology, Morgridge Institute for Research, University of Wisconsin-Madison; John Welsby – Duke University; John Thompson – Duke University; Colin Dolan – Research Technician, Duke University; Zihan Wang

Abstract: Genetic disorders of human development are common. Mutations in more than 4,000 genes are responsible for over 6,500 rare diseases, affecting an estimated 300 million people worldwide. Zebrafish is a common vertebrate model for understanding development and disease, sharing genome similarity with humans and with an elevated capacity for regeneration of damaged or lost body parts. To identify mutations that modify regeneration programs, we performed a standard N-ethyl-N-nitrosourea (ENU) mutagenesis screen using transgenic zebrafish with a permissive promoter and EGFP cassette inserted in the vicinity of the pro-regenerative factor gene, fgf20a. We then analyzed F3 larvae expected to be homozygous for ENU-induced mutations for changes in finfold amputation-induced fgf20a:EGFP expression, and we isolated one line with heritable, elevated fgf20a:EGFP presence in the absence of injury. Whole-genome sequencing (WGS) identified a mutation within exon 27 of the fraser syndrome 1 (fras1) gene, mutated in patients with inherited skin disease, and engineered fras1 mutations complemented/reproduced this phenotype. fras1 mutant larvae spontaneously displayed elevated expression of other known injury/regeneration-responsive reporter lines in fin fold, while mutations in zebrafish homologues of other genes mutated in human developmental diseases also displayed elevation of regeneration programs. Tempering Fgf signaling by transgenic expression of a dominant-negative Fgf receptor in fras1 mutants exacerbated the finfold degeneration phenotype. Our findings support a hypothesis in which regeneration programs respond to developmental defects caused by genetic mutations, potentially as a means to buffer deleterious phenotypes.

Molecular characterization and regeneration of the aging zebrafish retina

Presenting Author: Leah Campbell, PhD - University of Notre Dame

Co-Author(s): Pin Lyu – Johns Hopkins University; Isabella Palazzo – Johns Hopkins University; Yang Jin – Johns Hopkins University; Jiang Qian – Johns Hopkins University; Seth Blackshaw – Johns Hopkins University; David Hyde – University of Notre Dame

Abstract: The zebrafish retina is a powerful model for neuronal regeneration. Following acute damage, Müller glia reprogram and divide asymmetrically to produce a neuronal progenitor cell, which continues to proliferate into a cluster of cells that differentiate and replace the neuronal cells lost to damage. This regenerative capacity is retained in the old zebrafish retina when it is acutely damaged; however, the chronic, progressive degeneration that occurs during aging is not sufficient to induce regeneration. We sought to define molecular aging of the zebrafish retina and test a model of multiple acute damage that would mimic a chronic degeneration phenotype. Single-nuclear RNA sequencing (snRNA-Seq) and ATAC sequencing (snATAC-Seq) were combined for a multiomics approach to examine retinas from zebrafish ranging from 1 month to 48 months old. A molecular clock was generated from the multiomic data that corresponded to the biological age of different retinal cell types. Differentially expressed genes were examined in retinal sections from a range of aged zebrafish by in situ hybridization, including secreted protein, acidic, cysteine-rich (sparc), which is more highly expressed in Müller glia of young retinas than old retinas and dickkopf WNT signaling pathway inhibitor 1b (dkk1b), which is more highly expressed in aged retinas as compared to young retinas with spatial specificity in the ventral periphery. We also acutely damaged retinas with three rounds of N-methyl-D-aspartate (NMDA), allowing for three weeks of regeneration between each round of damage. Multiomics snRNA-Seq and snATAC-Seq were performed after each round of damage. The multi-damaged retinas resulted in a reduced number of neuronal progenitor cells, a reduced number of regenerated neurons, and altered gene expression profiling compared to retinas damaged a single time. These results are essential for identifying potential targets and developing strategies to repair and rejuvenate the aged and chronically damaged retina.

Small molecule augmentation of Notch signaling rescues regeneration in models of liver and muscle disease

Presenting Author: Duc Dong, PhD - Sanford Burnham Prebys Medical Discovery Institute

Co-Author(s): Shiv Kumar – Sanford Burnham Prebys Medical Discovery Institute; Joseph Lancman – Sanford Burnham Prebys Medical Discovery Institute; Sophie Hao – Sanford Burnham Prebys Medical Discovery Institute

Abstract: Loss of Notch signaling is attributed to regenerative failure of liver ducts in Alagille Syndrome (ALGS; JAG1-/+; 1/50,000 births) and skeletal muscles in Duchenne Muscular Dystrophy (DMD; DYSTROPHIN XdY; 1/5,000 male births), leading to over 50% lethality by early adulthood. To address these urgent, unmet therapeutic needs, we identified a first-in-class Notch agonist compound NoRA1 and determined that it directly binds to the Notch receptor to increase its cleavage and nuclear accumulation via a novel regulatory node of Notch pathway activation. In vivo treatment of zebrafish and mouse mutant ALGS models with NoRA1 enhances liver duct network regeneration, liver function, and survival. Consistently, human primary liver cholangiocytes treated with NoRA1 increases Sox9 expression and cell migration in a wound healing assay. In DMD, muscle wasting results from exhausted muscle stem cells (MuSCs), which are normally maintained in a quiescent/renewal state by Notch targets including PAX7. We find that NoRA1 robustly increases expression of PAX7 and other key stem cell regulatory genes such as COL5A1 and SPRY1 in primary mouse MuSCs and is orally available to limb, diaphragm, and heart muscles in mice. Promisingly, in these models, as well as in human ventricular cardiomyocytes derived from iPSCs, NoRA1 also increases the expression of Utrophin, a Dystrophin compensatory protein. Zebrafish dystrophin homozygotes, treated with NoRA1 after structural skeletal muscle damage is observed, show increased Notch signaling in Pax7+ MuSCs and a rescue of locomotor

behavior. In models of FSHD, the third most prevalent form of muscular dystrophies, NoRA1 rescues survival and differentiation of patient myoblasts and increases muscle force in mice. Together, these studies using zebrafish, mouse, and human disease models demonstrate the therapeutic potential of NoRA1, a small molecule Notch receptor agonist, for enhancing regeneration in ALGS and muscular dystrophies.

Regeneration in the face of DNA stress

Presenting Author: Gilbert Weidinger, PhD - Ulm University

Co-Author(s): Denise Posadas – Ulm University; Hossein Mohammadi – Ulm University

Abstract: An important question in regeneration research is whether species and organs that regenerate well are immune against challenges that inhibit regeneration in other contexts. Zebrafish achieve complete heart regeneration via dedifferentiation and proliferation of cardiomyocytes and efficient bone regeneration in the fin via dedifferentiation of osteoblasts. Surprisingly, we found that regenerating cardiomyocytes experience DNA replication stress, which represents one reason for declining tissue regeneration during aging in mammals. Likewise, dedifferentiating osteoblasts in regenerating fins upregulate gene signatures indicative of DNA damage responses as well. Pharmacological inhibition of ATM and ATR kinases reveals that DNA damage response signaling is essential for zebrafish heart and fin regeneration. This suggests that regenerating fins and hearts are not immune against DNA stress that limits regeneration in aged mammals. Rather, the ability to overcome DNA stress appears to represent a key factor for the elevated regenerative capacity of these organs. Manipulation of Bone Morphogenetic Protein (BMP)-Smad signaling using transgenics and mutants showed that BMP signaling alleviates cardiomyocyte replication stress. BMP signaling also rescues neonatal mouse cardiomyocytes, human fibroblasts and human hematopoietic stem and progenitor cells (HSPCs) from replication stress. DNA fiber spreading assays indicate that BMP signaling facilitates re-start of replication forks after replication stress-induced stalling. Our results reveal a conserved role for BMP signaling in promotion of stress-free DNA replication and more broadly suggest that a thorough understanding of zebrafish regeneration could inspire anti-aging interventions.

Concurrent Session IV

Advanced Imaging & Engineering

Shannon Hall | 10:30am-12:00pm

Session Chairs: Kaite Drerup - *University of Wisconsin-Madison* & Daniel Levic - *Duke University*

MCA: MultiCellular Analysis calcium imaging toolbox for ImageJ

Presenting Author: John Hageter, PhD - West Virginia University

Co-Author(s): Andrew Dacks – Case Western Reserve University; Audrey DelGaudio – West Virginia University; James Holcomb – West Virginia University; Eric Horstick – West Virginia University; Braxton Johnson – West Virginia University; Julius Jonaitis – Case Western Re

Abstract: Functional imaging using genetically encoded indicators, such as GCaMP, have become a foundation tool for in vivo experiments and allow for the analysis of cellular dynamics, sensory processing, and cellular communication. Large scale or complex functional imaging experiments pose analytical challenges, and many programs have worked to create pipelines to address these challenges,

however, most platforms require proprietary software, impose operational restrictions, offer limited outputs, or require expertise in command line coding, which collectively can limit utility. To address this, we designed MCA (a Multicellular Analysis toolkit) to work with ImageJ, a widely used open-source software which has been the standard scientific imaging analysis program for the last 30 years. We developed MCA to be visually responsive, utilizing ImageJ's platform to generate new images based on the functions completed so users can visually see each step in the analysis pipeline in real time. MCA also implements a user-friendly GUI providing a simple interface which resembles other native ImageJ plugins. We incorporated functionality for rigid registration to correct for motion artifacts, multiple algorithms for nuclei or cell body detection, and methods for annotating cells and exporting data. Additionally, we also integrated a custom model trained in Cellpose 2.0 for segmentation of nuclei expressing pan-neuronal nuclear localized GCaMP. We validated the accuracy of MCA output to previously published calcium imaging data which elicited visually evoked neuronal responses demonstrating that MCA produces results consistent with established findings. We further show the versatility of MCA, by demonstrating that our software can be utilized for multiple sensory modalities and various model organisms. Together these results establish that MCA is robust and viable for extracting calcium dynamics in a user-friendly environment for multiple forms of functional imaging.

pIGLET: safe harbor landing sites for reproducible transgenesis applications

Presenting Author: Christian Mosimann, PhD, MSc - University of Colorado School of Medicine, Anschutz Medical Campus

Co-Author(s): Robert Lalonde – Department of Pediatrics, Section of Developmental Biology – University of Colorado School of Medicine, Anschutz Medical Campus; Harrison Wells – Department of Pediatrics, Section of Developmental Biology – University of Colorado School

Abstract: Transgenic zebrafish strains are critical for live imaging of developmental processes, lineage tracking, and disease modeling. However, standard transgenesis with random DNA integration remains time-, labor-, and resource-intensive. Targeted transgenesis into established landing sites using phiC31 integrase was transformative for mouse and Drosophila; while functional in zebrafish, phiC31 integrase-compatible, validated genomic loci as safe harbors for transgenesis applications remain limited. We recently generated and benchmarked two landing sites we refer to as phiC31-Integrase Genomic Loci Engineered for Transgenesis (pIGLET). Substituting the highly Cre recombinase-responsive, Tol2-based ubi:Switch and hsp70l:Switch loxP transgenes with attP landing sites using CRISPR-Cas9, we demonstrated that the resulting landing sites called pIGLET14a and pIGLET24b are well-suited to predictably generate transgenes with faithful activities. Beyond fluorescent reporter transgenes for different cell types, re-responsive loxP Switch transgenes show predictable activity patterns and recombination efficiencies when introduced into either landing site, overcoming a major hurdle in creating Switch transgenes by random integration. The predictable transgenesis into pIGLET landing sites enables qualitative and quantitative testing of developmental and disease-associated enhancers. For reporter transgenes of standard vector sizes, we routinely achieve germline transmission with 50-90% efficiency, drastically reducing animal numbers needed for transgenesis. We are now generating and validating additional pIGLET lines for universal transgenesis applications towards expanding the repertoire of available landing sites for increased flexibility for experiments and husbandry. We are further validating first attP landing sites suitable for tissue-specific expression of transgenes as quasi-enhancer traps, enabling the generation of reporter strains for previously inaccessible expression

patterns and cell types. Do date, we have distributed the initial pIGLET14a and pIGLET24b lines to nearly 100 facilities in the US, Canada, Europe, Taiwan, and New Zealand to support easy accessibility to the landing site lines. Altogether, our pIGLET landing sites with associated protocols enable community-accessible, reproducible transgenesis for numerous applications.

Precise control of secreted signaling protein activity using a synthetic protease library

Presenting Author: P. C. Dave Dingal, PhD - The University of Texas at Dallas

Co-Author(s): Sadhya Achanta – Bioengineering – The University of Texas at Dallas; Nitin Chikkodi – Biological Sciences – The University of Texas at Dallas; Jaideep Kaur – Bioengineering – The University of Texas at Dallas; Mustafa Alrawi – Biological Sciences – The U

Abstract: Cell signaling is essential for coordinating tissue morphogenesis. Embryonic cells deploy signaling proteins to guide the differentiation of nearby cells, ultimately forming various tissues such as muscle, bone, and blood. Secreted signaling proteins must undergo proteolytic activation. To precisely control the activity of signaling proteins, we created the Synthetic Processing (SynPro) system, an engineered library of secreted proteases that are functional in the secretory pathway of zebrafish embryonic cells. Here we used proteases from the Potyviridae family, a class of enzymes that can cleave a unique seven-amino-acid sequence. We demonstrate the ability of SynPro proteases to cleave key signaling proteins during early embryogenesis: Vg1, Lefty1/2, BMP2/4/7, and Toddler, with concomitant phenotypes when these proteins are active. We further engineered the SynPro system for optogenetics to artificially induce embryonic signaling with light. To develop a light-inducible SynPro system, we fused each half of a SynPro protease with iLID and SspB proteins, which dimerize upon blue-light stimulation. We observed light-induced reconstitution and activity of SynPro proteases and downstream activation of secreted Lefty1. Overall our findings suggest that we can engineer sequence-specific enzymes to control secreted proteins and their downstream signaling pathways at the cellular and whole-animal levels.

See-through science: Danionella cerebrum as a model for CNS regeneration

Presenting Author: Pui-Ying Lam, PhD - Medical College of Wisconsin

Co-Author(s): Mallika Khurana – Medical College of Wisconsin; Joseph Austin – Medical College of Wisconsin; Noelle Walechka – Medical College of Wisconsin; Juliet Peterka – Medical College of Wisconsin; Matthew Veldman – Medical College of Wisconsin; Pui-Ying Lam – Me

Abstract: Rebuilding functional neuronal circuitry after injury in the central nervous system (CNS) is unachievable for many mature vertebrates. It has been difficult to study dynamic cellular interactions and topography of long-distance axon regeneration within an intact adult vertebrate brain because of its size and opacity. Here, we took advantage of the small, transparent, and pro-regenerative adult Danionella cerebrum and performed high-resolution confocal longitudinal imaging of retinal ganglion cell (RGC) axon regeneration, correlating cellular events with functional recovery. We observed that during early RGC axon regeneration, an extensive network of axons formed with some following the path of the degenerating myelin from injured RGCs. Later, at a phase that coincided with vision recovery, axon bundling and re-wiring occurred which resulted in a new axonal topography distinct from what was present before injury. Our model provides a unique opportunity to visualize the spatial and temporal events that occur during CNS regeneration in intact adult vertebrates.

High throughput in vivo mapping of signaling histories with CRISPR barcodes

Presenting Author: Bushra Raj, PhD - University of Pennsylvania

Co-Author(s): Jessie Greenslade – Cell and Developmental Biology – University of Pennsylvania; Abigail Siniscalco – Cell and Developmental Biology – University of Pennsylvania; Hemagowri Veeravenkatasubramanian – Cell and Developmental Biology – University of Pennsylv

Abstract: Cell signaling regulates embryonic patterning, proliferation, migration, and cell fate decisions. Traditional reporters of pathway activity, such as fluorescent transgenes coupled to receptor or ligand activation, lack scalability and offer limited molecular resolution of cell states. To overcome these constraints, we developed SABER-seq, a CRISPR-based molecular recorder that converts transient signaling events into heritable genomic barcodes, enabling retrospective readout of signaling history at single-cell resolution via transcriptomics. SABER-seq comprises two modular components: a signaling-responsive Cas9 sensor and a CRISPR barcode recorder. Upon pathway activation, Cas9 is induced to edit a barcode array, irreversibly marking cells that experienced signaling. These edits are stably inherited, providing a lineage-traceable record of signaling events.We applied SABER-seq to record binary Notch signaling activity in the zebrafish juvenile brain. We calibrated and validated the specificity of Notch recording in brain tissues. Next, we integrated SABER-seq with a single-cell brain atlas comprising 137 transcriptionally distinct cell types. From ~5,000 single cells, we reconstructed Notch signaling histories and mapped them onto molecular cell identities. Our analysis revealed widespread Notch activity across brain cell types, but surprisingly, the majority of neuronal subtypes showed no enrichment for Notch-edited barcodes, suggesting that Notch signaling is not broadly required for binary neuronal fate decisions in vertebrates. However, two neuronal subtypes exhibited differential Notch barcode editing, highlighting rare populations where Notch may play a critical role in fate specification. Together, our work establishes SABER-seq as a powerful tool for high-throughput, high-resolution mapping of signaling events in vivo at relatively low costs. By enabling retrospective signaling analysis at single-cell scale, SABER-seq opens new avenues for dissecting how transient molecular cues shape long-term developmental outcomes in the zebrafish brain and beyond.

A High-Throughput Imaging and Analysis Pipeline for Phenotyping MIC-Drop-Generated Zebrafish Mutants in 96-Well Plates

Presenting Author: Abhinav Bachu - Northwestern University

Co-Author(s): John Efromson – Ramona Optics; Claire Stockard – Northwestern University; Saba Parvez – Northwestern University

Abstract: Zebrafish (Danio rerio) serve as a key model for high-throughput genetic screening, particularly with CRISPR-based strategies like the Multiplexed Intermixed CRISPR Droplets (MIC-Drop), which simultaneously induces multiple mutations alongside unique barcodes, enabling large-scale generation of mutant lines with a single injection. One significant advantage of the zebrafish system is the ability to produce numerous larvae that can be arrayed in multi-well plates for high-throughput screening of gene function. However, traditional workflows for simultaneously assessing morphology, behavior, and genotype remain limited by low-throughput approaches dependent on standard microscopes and manual data processing. Using a newly developed, parallelized microscope, the Multi-Camera Array Microscope (MCAM™), we have optimized a rapid, high-resolution algorithmic pipeline to integrate

morphological segmentation, behavioral fingerprinting, and DNA barcode demultiplexing in vivo. By employing MIC-Drop–derived zebrafish larvae, we captured bright-field images and short video clips across a full 96-well plate, and this datastream was then processed to obtain a comprehensive profile of each larva, including quantitative anatomical metrics (e.g., head and tail measurements) and a detailed behavioral fingerprint—a multidimensional representation of locomotor activity and stimulus responses. This fingerprint normalizes differences across various behavioral metrics, such as swim bouts, velocity, and stimulus responses, allowing for direct comparisons between mutant cohorts and wild-type baselines. This automation is facilitated by a convolutional neural network that identifies larval features and a pose estimation algorithm that extracts movement parameters, both of which are combined with a barcode demultiplexing workflow that links each phenotype to the specific CRISPR edit introduced by MIC-Drop. Using the MCAM™, we have been able to, within minutes, generate enough data to support statistical analyses of both phenotype and genotype in large cohorts. Finally, we present this open-source software package, which offers a user-friendly platform for end-to-end zebrafish mutant screening, and can be adapted for other transgenic lines or related model organisms.

Evolution & Comparative Biology — Play Circle Theater | 10:30am-12:00pm

Session Chairs: Shunji Jia - *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences* & Ji-Feng Fei - *Guangdong Provincial People's Hospital*

Evolution and regulation of diapause in the African killifish

Presenting Author: Param Priya Singh - University of California, San Francisco

Co-Author(s): Christopher He – University of California, San Francisco; Stephanie Gagnon – University of California, San Francisco; G. Adam Reeves – Stanford University; Kévin Contrepois – Stanford University; Katharina Papsdorf – Stanford University; Anne Brunet – St

Abstract: Extremophiles—species that live in extreme environments—have evolved unique adaptations for survival. Understanding how extreme adaptations evolve can reveal new pathways with important ramifications for survival in all organisms. The African killifish Nothobranchius furzeri is an extremophile for embryo survival. Killifish lives in ephemeral ponds that completely dry up for ∼8 months each year. To survive this annual drought, they have evolved a form of long suspended animation, with embryos entering diapause and subsisting in the mud during the dry season. Diapause embryos survive for months, even years—longer than adult life—without any detectable tradeoff for future life. Remarkably, diapause embryos already have complex organs and tissues, including a developing brain and heart. Hence, diapause provides long-term protection to a complex organism. However, the mechanisms underlying the evolution and regulation of diapause are unknown. To understand diapause evolution and identify key regulators of cell type specific regulatory mechanisms in diapause, we performed integrative multi-omics (gene expression, chromatin accessibility, and lipidomics) in the embryos of multiple killifish species during diapause and development states. We find that diapause evolved by a recent remodeling of regulatory elements at very ancient gene duplicates (paralogs) present in all vertebrates. By integrating chromatin accessibility and gene expression dynamics at the single cell level, we identified cell type specific transcription factors underlying diapause entry and maintenance. CRISPR-Cas9-based perturbations identify the transcription factors REST/NRSF and FOXOs as critical for the global regulation of diapause gene expression program, including genes involved in lipid metabolism. Diapause embryos show a distinct lipid metabolic profile, with an increase in triglycerides with very-long-chain fatty acids. Overall, our work identifies mechanism for the evolution

and regulation of complex adaptations and offers strategies to promote long-term survival by activating suspended animation programs in other species.

Zebrafish models of duplicated genes implicated in human brain evolution

Presenting Author: Megan Dennis, PhD - University of California, Davis

Co-Author(s): José Uribe-Salazar – University of California, Davis; Daniela Soto – University of California, Davis; Gulhan Kaya – University of California, Davis; Nicholas Haghani – University of California, Davis; Gabriana La – University of California, Davis; Aidan

Abstract: Duplicated genes expanded in the human lineage likely contributed to brain evolution, yet challenges exist in their discovery due to sequence-assembly errors. We used a complete telomere-to-telomere genome sequence to identify 213 human-specific gene families. From these, 362 paralogs were found in all modern human genomes tested and brain transcriptomes, making them top candidates contributing to human-universal brain features. Choosing a subset of gene families, we generated zebrafish CRISPR “knockout” models of nine orthologs and introduced mRNA-encoding human-specific paralogs, effectively “humanizing” larvae, to understand their roles in brain development. As a proof-of-principle, we first characterized SRGAP2C, a gene previously studied in mice and implicated in altered synaptogenesis and neuronal migration by antagonizing its ancestral gene functions. Morphometric, behavioral, and single-cell transcriptome analyses collectively suggest SRGAP2C similarly antagonizes the zebrafish Srgap2 and impacts axonal guidance, synaptogenesis, and seizure susceptibility. Beyond neurons, we discovered a novel Srgap2 function in controlling membrane dynamics and maturation of microglial cells, possibly leading to altered axonogenesis in the developing retina and increased sensitivity to broad and fine visual cues. Applying these same tools to other gene families suggests that an additional four paralogs (ARHGAP11B, FAM72B, FRMPD2B, and PDZK1B) similarly antagonize their ancestral counterparts, while surprisingly only one gene exhibits gene dosage effects (GPR89B). These findings align with recent human data showing opposing copy-number variants, which decrease or increase gene expression, often impact complex traits in the same direction. Generating stable mutant lines of two gene families in zebrafish validated the results and implicated GPR89B in dosage-mediated brain expansion and FRMPD2B in altered synapse signaling, both features altered in the human brain when compared to other non-human primates. Together, our holistic approach provides new insights and a comprehensive resource for studying gene expansion drivers of human brain evolution.

Uncovering cryptic genetic variants that contributed to eye-loss in the evolution of the blind cavefish, Astyanax mexicanus

Presenting Author: Hannah Grunwald - Boston Children's Hospital

Co-Author(s):

Between canalization of phenotypes, low penetrance diseases, and knock-out gene models with no detectable phenotype, it is clear that ‘cryptic’ hidden genetic variation is common. Nevertheless, we don’t understand the mechanism(s) by which cryptic variants are obscured, the conditions under which they are revealed, or the consequences of exposed variation. The Hsp90 family of chaperones may act as ‘capacitors’ of cryptic variation, buffering and obscuring missense variants in clients under homeostatic conditions and exposing them in the form of novel phenotypes in response to

environmental stressors. The blind cavefish, Astyanax mexicanus, evolved dramatic cave-adapted traits, including complete loss of eyes, over only ~200,000 generations. Eyeless fish evolved in >30 caves, despite the fact that troglomorphic traits are never seen in the 'surface' morphs of Astyanax that inhabit the rivers, suggesting that evolution of these traits cannot be explained by selection on standing phenotypic variation. Did these highly complex traits evolve from numerous novel mutations that arose convergently in dozens of caves? Or is it possible that they evolved instead from standing genetic variation that was hidden before exposure to the cave environment? To identify Hsp90-clients that may have contributed to eye-size evolution in Astyanax, I developed a list of candidate genes which are Hsp90 clients and fall under Astyanax eye-size QTLs. I have systematically knocked out these genes using CRISPR/Cas9 in zebrafish to assess their impact on eye size in a teleost. I have identified genes not previously associated with eye size whose loss results in larger and smaller eyes and lenses. Treatment with an Hsp90 inhibitor has revealed that some of these phenotypes are potentiated by Hsp90. Collectively, this work is a platform for discovery of novel eye-size determinants, expands our understanding of evolution in extreme environments, and provides a launchpad to investigate buffered and exposed Hsp90-dependent cryptic variation.

Exploring the cellular and molecular mechanisms underlying indeterminate skeletal muscle growth in teleosts.

Presenting Author: Yansong Lu - Monash University - Australian Regenerative Medicine Institute

Co-Author(s): Peter Currie – Monash University - Australian Regenerative Medicine Institute; Avnika Ruparelia – University of Melbourne

Abstract: In vertebrates, growth strategies are remarkably diverse. While mammals and birds exhibit determinate growth, ceasing around sexual maturity, most teleosts display indeterminate growth, continuing to grow throughout life. To better understand the mechanisms underlying indeterminate muscle growth in teleosts, we compared three species with distinct growth capacities: zebrafish (Danio rerio), giant danio (Devario malabaricus), and African killifish (Nothobranchius furzeri). Using standard histological techniques alongside novel image analysis workflows, we identified differences in muscle stem cell (MuSC) localisation, proliferation, and developmental timing that contribute to variation in muscle growth capacity. These differences result in determinate growth in zebrafish and indeterminate growth in giant danio and killifish. Additionally, scRNA-sequencing revealed conserved MuSC-specific expression of extracellular matrix (ECM) genes as key regulators of MuSC dynamics. Consistent with this, CRISPR/Cas9-mediated disruption of these genes in zebrafish altered MuSC behaviour and muscle growth, supporting their functional role. Collectively, these findings underscore the importance of MuSCs in shaping their own niche through ECM gene expression to regulate their dynamics and, ultimately, muscle growth.

Odyssey of Strange Fish: Holostean Fishes Inform the Developmental Evolution of Vertebrates and Bridge Zebrafish to Human

Presenting Author: Ingo Braasch, PhD - Michigan State University

Co-Author(s):

Abstract: The macroevolution of vertebrates has been accompanied by gains and losses of genes, regulatory elements, and developmental processes, contributing to major evolutionary transitions such

as the emergence of tetrapods from fishes or the unmatched biodiversity of the teleost fishes. Teleosts like zebrafish are derived from a teleost-specific genome duplication that had major impact on their genome and gene function evolution. Together with the earlier two vertebrate genome duplications, this complicates comparative studies across vertebrates due to lineage-specific genome reshuffling and gene losses, obscuring the distinction of orthologs vs. paralogs, and hiding the origins of vertebrate developmental programs. To overcome these challenges, we are developing “ancient fishes” of the holostean lineage, i.e., gars and bowfin, as new model species for Evo-Devo and comparative developmental genomics. Holosteans serve as the closest ‘unduplicated’ outgroup to the fast-evolving 30,000+ living teleost species as well as outgroup to lobe-finned vertebrates including tetrapods and humans. Holosteans have slowly evolving and thus highly informative genomes, deeply conserved developmental programs, and archaic body plans that provide unique opportunities for wide-ranging comparisons among vertebrates. Using examples from diverse developmental pathways and processes, we demonstrate that comparative genomic analyses and transcriptomic and epigenomic profiling through holostean development are powerful for connecting the disparate sets of genes, gene regulatory elements, and morphologies among distant vertebrate lineages. The ‘evolutionary inertia’ of holostean genomes facilitates the identification of ancestral cis-regulatory elements, revealing hidden orthology of gene regulatory programs across vertebrates. Developing gars as functional genomic models for comparisons to zebrafish and other biomedical fish models, we developmentally test hypotheses about the evolutionary origins of vertebrate gene functions. Holosteans are thus powerful as "bridge model species" that illuminate vertebrate evolution, development, disease, and regeneration and thereby connect zebrafish to human biology.

Disclosing the role of muscle stem cells during tail regeneration in the axolotl

Presenting Author: Ji-Feng Fei, PhD - Guangdong Provincial People’s Hospital

Co-Author(s):

Abstract: The Mexican axolotl, a tetrapod vertebrate, possesses remarkable regenerative abilities, making it an ideal model for studying the mechanisms of tissue and organ regeneration. For a deeper investigation of this fascinating regeneration phenomenon, we previously established a series of genetic resources and tools in the axolotls, including sequencing and assembling the axolotl genome and developing CRISPR/Cas9 based gene knockout, gene knock-in approaches, and inducible cell-ablation system. In recent years, we have established a series of injury and disease models in axolotls to elucidate the mechanisms involved in organ regeneration, via single-cell sequencing, spatial transcriptomics and functional genomics. To this end, we have compared the role of muscle stem cells in the tail and limb regeneration and found that muscle stem cells are capable of being committed to multipotent to generate fibroblast, and chondrocytes, in addition to muscle tissues. Furthermore, we have identified Tgf-beta as the key signaling governing such a cell fate switch of muscle stem cells during tail regeneration. Our study revealed that primary body axis and appendage regeneration may hold different principles during regeneration.

and regulation of diapause in the African killifish
Param Priya Singh - *University of California, San Francisco*

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