Poster Session I

Themes: Early Development, Morphogenesis & Patterning, Emerging Technologies, Infection & Immunity, Quantitative Biology, and Reproduction

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BMP-Smad signaling is dispensable for PGC identification and migration, but regulates PGC proliferation in zebrafish

Poster Number: 1

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Xiaotong Wu** - Laboratory of Molecular Developmental Biology, State Key Laboratory of Membrane Biology, Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China

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Abstract:

Specification and maintenance of germ cell lineage are vital to the development and heredity. In mammals, bone morphogenetic protein (BMP) signaling induces the germ cell fate in the epiblast cells. However, whether BMP signaling is necessary for zebrafish germ cell development remains unknown. Here, we demonstrate that, in zebrafish, BMP-Smad1/9 signaling is required for primordial germ cell (PGC) maintenance but not the germ cell fate determination and PGC migration. BMP inhibitor treatment or whole-embryos smad1/9 knockdown reduced number of PGCs. Furthermore, to eliminate

the interference of BMP signal in somatic cells and dorsal-ventral axis formation on PGC development, we generate PGC-specific smad1/9 knockout mutant embryos by the double transgenic approach with PGC-specifically expressed Cas9 and ubiquitously expressed gRNAs. Loss of Smad1/9 in PGCs results in impaired PGC proliferation and enhanced cell apoptosis, leading to PGC loss and a male-biased sex determination in adults. Moreover, transcriptome analysis of smad1-deficient PGCs reveals no significant changes in PGC-specific gene expressions, but obvious upregulation of genes related to cell cycle checkpoint signaling and DNA damage repair. Notably, ectopic ATR-pChk1 activation in smad1-cKO PGCs validate cell cycle defects and DNA replication stress. ATR inhibition restores PGC reduction in mutant embryos. Together, our findings highlight that BMP-Smad1/9 signaling in PGCs plays an essential role in zebrafish PGC population maintenance, but is dispensable for PGC cell fate determination and migration.

Physiological cell competition ensures robust tissue patterning

Poster Number: 2

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Kanako Matsumoto** - Research Institute of Microbial Diseases, The University of Osaka

Co-Author(s): Yuki Akieda – RIMD, The University of Osaka; Yukinari Haraoka – RIMD, The University of Osaka; Naoki Hirono – FBS, The University of Osaka; Hiroshi Sasaki – FBS, The University of Osaka; Tohru Ishitani – RIMD, The University of Osaka

Abstract: Animal development is highly reproducible and repeatedly generates tissues and organs with the same function. However, dynamic morphogenesis, including active rapid cell proliferation and migration during development, may induce replication errors and cellular signalling perturbations, generating unfit cells in developing tissues. Nevertheless, the mechanisms underlying robust tissue development overcoming such unfit cell generation are not completely understood. Using zebrafish imaging, we reveal that cell competition ensures robust patterning of the spinal cord and muscle through elimination of cells with unfit sonic hedgehog activity, driven by cadherin-mediated communication between unfit and neighbouring fit cells and subsequent activation of the Smad-Foxo3-reactive oxygen species axis. By analyzing zebrafish and mice transcriptome data, we also identify Foxo3 as a common marker of loser cells in various types of cell competition in zebrafish and mice. Foxo3-mediated physiological cell competition is required for eliminating various naturally generated unfit cells with a variety of abnormality, including unfit Wnt, Shh, Ras, Myc, and ribosome activities, and for the consequent precise patterning during zebrafish embryogenesis and organogenesis. Given the implication of Foxo3 downregulation in age-related diseases, cell competition may be a defense system to prevent abnormalities throughout development and adult homeostasis.

Elucidating the role of apolipoprotein L1 in zebrafish pronephros development

Poster Number: 3

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Tracey Porter** - University of Notre Dame

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Abstract: Chronic kidney disease (CKD) is a significant burden in America. Apolipoprotein L1 (APOL1) mutations have been linked to an increased risk of kidney disease and failure in African and African American populations. APOL1 is highly expressed in various tissues, including the kidney; however, the function of APOL1 in the kidney remains unknown. Here, we utilized the zebrafish to create apol1 loss-of-function models to study its roles in renal development, specifically nephrogenesis. The zebrafish is uniquely suited for this study due to its high conservation of the mammalian kidney, and it is one of the few organisms with apol1 in its genome. Whole-mount in situ hybridization, immunofluorescence, and HCR-IF technology were employed to examine transcripts and cell dynamics of renal progenitors. These studies revealed that loss of apol1 reduced the expression of podocyte regulator wt1a and lineage markers wt1b and nephrin. Furthermore, loss of apol1 dysregulated the interrenal marker nr5a1a. These findings suggest that apol1 is crucial for cell fate choice between the podocyte and interrenal lineages. Ongoing investigations aim to discern the interplay between apol1, retinoic acid, and Notch signaling in renal development. Special thanks to our funding sources, the University of Notre Dame Dean's Fellowship, the Frazier Thompson Research Award, and the Riley Family Endowment Research Award.

Pdgfab/Pdgfra-mediated chemoattraction guides the migration of sclerotome-derived fibroblast precursors in zebrafish

Poster Number: 4

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Emilio Méndez-Olivos, PhD** - University of Calgary

Co-Author(s): Katrinka Kocha – University of Calgary; Shan Liao – University of Calgary; Peng Huang – University of Calgary

Abstract: In vertebrates, the sclerotome is a transient embryonic structure that gives rise to various tissue support cells, including fibroblasts. However, how fibroblast precursors are guided to diverse tissues remain poorly understood. Using zebrafish, our lab has previously shown that sclerotome-derived cells undergo extensive migration to generate distinct fibroblasts subtypes, including tenocytes along the myotendinous junction and fin mesenchymal cells in the fin fold. Interestingly, the pan-fibroblast gene platelet-derived growth factor receptor a (pdgfra), which has been implicated in cell migration across various contexts, is specifically expressed in the sclerotome and its descendants. Loss of functional Pdgfra in a pdgfra gene-trap mutant results in severe defects in the migration of sclerotome-derived cells, leading to a dose-dependent loss of tenocytes and fin mesenchymal cells. By combining live imaging and mosaic labeling with a membrane-bound dominant-negative tool, we demonstrate that Pdgfra acts cell-autonomously to regulate the migration of sclerotome-derived cells. In the absence of ligand pdgfab, which is expressed in the medial somite, sclerotome-derived cells fail to migrate medially, resulting in a loss of tenocytes, although they can migrate normally toward the fin fold and generate fin mesenchymal cells. Strikingly, localized expression of Pdgfab in pdgfab mutants can

direct the migration of sclerotome-derived cells to both normal and ectopic locations, suggesting a chemoattractive role for the Pdgfab ligand. Together, our results demonstrate that Pdgfab/Pdgfra-mediated chemoattraction guides the migration of sclerotome-derived fibroblast precursors to specific locations, where they diversify into distinct fibroblast subtypes.

Mitotic erasure shapes Fgf/Erk signalling dynamics and mesendodermal patterning

Poster Number: 5

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Scott Wilcockson, PhD** - The Francis Crick Institute

Co-Author(s): Caroline Hill – The Francis Crick Institute

Abstract: In zebrafish, specification of the mesendodermal lineages in driven by overlapping gradients of Nodal-Smad2/3 and Fgf-Erk signalling. Recent work has shown that sustained Nodal signalling establishes a bipotential progenitor state from which cells can switch to an endodermal fate or differentiate into mesoderm. The likelihood of these events is negatively modulated by Fgf signalling and yet endoderm cells are found to display variable levels of this inhibitory signal. To investigate a potential role for signalling dynamics in fate switching, we generated a live biosensor of Erk activity (modErk-KTR) and identified Fgf/Erk signalling dynamics linked to the cell cycle, a phenomenon referred to as mitotic erasure. During late G2 phase, Erk activity is extinguished before the onset of mitosis and daughter cells must re-active signalling in G1 phase. Here we present evidence that these pulses of Erk inactivity act to dampen the overall response to Fgf signalling. This therefore links Fgf signalling output to proliferation rate and introduces variable periods of Erk inactivity that drive a heterogeneous population-level response to the same signal. To address how these dynamics might impact downstream patterning, we manipulated proliferation rate with chemical inhibitors and monitored mesendodermal patterning using single molecule FISH (smFISH). This revealed that cell cycle progression is necessary for the induction of sox32, a master regulator of endoderm induction that is inhibited by Fgf/Erk signalling. Therefore, mitotic erasure provides variable windows of repression-relief that allow a small population of mesendodermal cells to undergo fate switching. We thus demonstrate a mechanism of developmental signalling regulation coupled to tissue growth that instructs early tissue patterning.

Embryonic origins of tendon heterogeneity: looking to the eye for differences in tissue-specific attachments

Poster Number: 6

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Cameron Miller** - University of California, Irvine

Co-Author(s): Pavan Nayak – University of California, Irvine; Arul Subramanian – University of California, Irvine; Thomas Schilling – University of California, Irvine

Abstract: Tendons attach muscles to multiple tissue types to accommodate diverse structural and functional needs. Vertebrate tendon fibroblasts (tenocytes) are distinguished by their expression of

scleraxis (scx), an early tenocyte fate determinant transcription factor. However, differences in the genetic programs between tenocytes of varying attachment types remain largely unexplored. We have investigated this heterogeneity through a transcriptomic analysis of craniofacial tendons in zebrafish. The extraocular muscles (EOM) that control eye movements exemplify functional tissue heterogeneity, since they feature both hard tissue attachments to the skull as well as unique soft-tissue tendon attachments to the eye. Single-cell RNA sequencing (scRNA-seq) using sorted mCherry positive cells taken from heads of Tg(scxa:mCherry) embryos at 72 hpf, coupled with quantitative in-situ hybridization chain reaction in whole embryos, identified novel EOM-associated tendon subpopulations. These tenocyte clusters differ in their extracellular matrix components associated with distinct mechanical properties, and express key retinoic acid (RA) synthesis genes (aldh1a2 and rdh10a) and RA signaling effectors (pitx2), implicating RA signaling in their development. Consistent with this hypothesis, blocking RA signaling pharmacologically with DEAB uniquely prevents the formation of EOM tendon insertions on the eye, without affecting their skull attachments. Conversely, elevated RA levels cause an expansion of tenocyte number at EOM-tendon insertions. These results suggest differences in gene regulatory networks involved in EOM tenocyte cell specification at hard versus soft tissue attachments. Studies are ongoing to investigate how RA establishes the pattern of EOM tendon attachments. We are also integrating our scRNA-seq data over multiple stages to identify transcriptional changes during the development of these understudied components of the musculoskeletal system. This work is supported by NIAMS [R01AR67797].

A biological oscillator controls the pace of notochord segmentation

Poster Number: 7

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Priyom Adhyapok** - Duke University

Co-Author(s): James Norman – Cell Biology – Duke University; Jennifer Bagwell – Cell Biology – Duke University; Michel Bagnat – Cell Biology – Duke University; Stefano Di Talia – Cell Biology – Duke University

Abstract: Developmental processes require coordination across multiple cells over time. To achieve this harmony, genetic species act as biological timekeepers to help regulate developmental timing. Here, we present evidence of an oscillatory system in the zebrafish notochord's outermost epithelial sheath layer. This oscillator, reported by an ERK sensor, demonstrates a periodicity of 10 hours, with cells attaining synchronous states across several hundred microns along the notochord length. Using molecular and quantitative approaches, we show that this oscillator is important for the appearance of new domains of Notch activation in the notochord sheath, which eventually results in the formation of mineralized segments in the vertebral column. Tracing the oscillator through development shows the emergence of synchronization and periodicity in the tissue around 4.5 Days Post Fertilization, giving rise to the onset of the segmentation process. We report that the EGF pathway, along with MAPK pathway inhibitors spry2 and dusp6 show dynamical behavior that could serve as the building blocks of the oscillator. Together, our data demonstrate that a tissue-wide oscillator behaves like a 'clock' modulating the rhythm of segmentation in the zebrafish notochord.

Temporal Regulation of Endoderm Convergence and Extension by the BMP Activity Gradient through Mesoderm-Dependent and Independent Mechanisms

Poster Number: 8

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Chia-Teng Chang** - Washington University in St. Louis

Co-Author(s): Lilianna Solnica-Krezel – Washington University in St. Louis; Tony Tsai – Washington University in St. Louis

Abstract: One hundred years ago, Spemann and Mangold identified the organizer, a critical embryonic region that establishes vertebrate body axes by directing cell fate and morphogenesis. A conserved vertebrate mechanism involves the regulation of a ventral-to-dorsal BMP activity gradient during gastrulation by the organizer-expressed molecules. In zebrafish, BMP signaling controls mesodermal cell convergence and extension (C&E) by inhibiting Planar Cell Polarity (PCP) signaling and regulating cell adhesion. This allows lateral cells to converge toward the dorsal midline while directing ventral cells toward the tail bud. However, BMP's role in endodermal movements and the temporal precision of its regulatory functions remain poorly understood. Using optogenetics and other loss- and gain-of-function approaches, we investigated BMP's role in mesoderm and endoderm C&E. We found that low BMP signaling promotes extension in both germ layers, whereas high BMP signaling inhibits their C&E. Remarkably, BMP signaling activation for one hour rapidly redirected dorsal to ventral migration of both mesodermal and endodermal cells. However, when BMP signaling was selectively elevated in endoderm in embryos with reduced BMP signaling, endoderm still mimicked mesodermal movements, indicating that endodermal responses to BMP are non-cell autonomous. We show that movements of endodermal cells in gastrulae with normal or elevated BMP signaling do not entirely dependent on mesoderm or the Cxcl12b/Cxcr4a GPCR pathway, suggesting additional mechanisms underlie endoderm C&E. Our findings highlight the critical role of the BMP morphogen gradient in coordinated C&E movements of mesodermal and endodermal cells. BMP employs both direct and indirect mechanisms to ensure robust embryonic patterning and morphogenesis of germ layers.

Vestigial-Like 3 as a Transcriptional Regulator of Neural Progenitor Cell Fate Pathways

Poster Number: 9

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Cameron Bennett** - University of Colorado Anschutz Medical Campus

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Abstract: Incorrectly timed and placed neural progenitors are associated with neurodevelopmental disorders. One proposed mechanism for controlling spatiotemporal neural development are neuromeres, called rhombomeres in the hindbrain, but how they form remains unknown. Our single-cell RNA-seq analysis identified previously uncharacterized molecular markers for each rhombomere (r) at 13 hours post fertilization (hpf), as well as three pre-rhombomere progenitor populations (PRPD) at 10hpf. Notably, Vgll3, the r2 marker, is co-expressed with r3/r5 marker egr2b at 10hpf in one of the PRPD populations. This work aims to elucidate the molecular mechanism by which Vgll3 promotes an r2 cell identity while suppressing an r3 cell identity. Using HCR RNA-FISH with probes marking irx1b (r1), Vgll3 (r2), and egr2b (r3/r5), we identified and characterized a range of abnormal rhombomere phenotypes in Vgll3-/- and Vgll3+/- embryos. Notably, the majority of the Vgll3-/- embryos presented a spatial collapse phenotype in which r2 appears to be lost, with r1 and r3 directly adjacent. We also observed Vgll3-/- embryos that exhibited a loss of Vgll3 signal but retained the r2 space between r1 and r3. This phenotype was also observed in many Vgll3+/- embryos. We are performing similar experiments with Vgll3's DNA binding partners from the TEAD family of transcription factors, and preliminary analysis supports a similar phenotype. We also observed disordered social behavior in our adult TEAD3b-/- and TEAD3b+/- fish and found that both genotypes exhibit strongly perturbed social schooling behaviors. These results support that Vgll3 is necessary for the correct formation of r2, and perturbation of its expression, through either it or its binding partners, results in abnormal neurodevelopment and behavior.

Embryonic Endoderm Diversifies During Gastrulation

Poster Number: 10

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Stefan Materna** - University of California Merced

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Abstract: During embryonic development, cells are organized into functional tissues through patterning and morphogenesis. Yet, understanding how these processes are controlled and coordinated remains a formidable challenge. We address this question in the zebrafish sox17 lineage during, and immediately after, gastrulation using single cell RNA sequencing and imaging. The sox17 lineage consists of endoderm and dorsal forerunner cells (DFCs), both of which are specified in response to Nodal signaling prior to gastrulation. Endoderm and DFCs initially express similar genes, including several lineage-determining transcription factors. Despite these similarities, we find that gene expression has diverged significantly by mid-gastrulation, in line with their different behaviors. DFCs are a homogenous group of cells throughout our sampling period but undergo a notable shift in gene expression following inflation of KV. In contrast, the endoderm is heterogenous and consists of several subclusters with divergent transcriptional profiles. For the endoderm as a whole, we observe a shift towards genes promoting epithelium formation. More surprisingly, however, the vast majority of genes is restricted to only a subset of endoderm cells. Mapping these subclusters onto the embryo, we find that they occupy discrete and complementary locations in the embryo. These differences are already apparent at mid-gastrulation when cells closer to the margin activate posterior markers. Our data are consistent with the

idea that the endoderm is first patterned, not along the dorsal-ventral axis, but along the animal-vegetal axis. Endoderm cells thus execute a global morphogenetic program while simultaneously adopting distinct regional identities.

Neuronal migration is spatiotemporally regulated by a BMP signaling gradient in the developing zebrafish vagus nerve

Poster Number: 11

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Emma Carlson, BA** - University of Minnesota

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

Abstract: Nervous system function relies on precise organization of neurons and their axon targets. Neural networks are often organized into topographic maps such that the relative positions of cell bodies correspond to the positions of their axons within target tissues. To study the construction of topographic maps during development, we focus on the vagus nerve, a vital component of the parasympathetic nervous system, which is topographically organized along the anterior-posterior (A-P) axis. Vagus motor neurons exhibit temporally distinct behaviors – anterior neurons migrate, extend axons, and, consequently, select targets earlier than posterior neurons. Moreover, disrupting the timing of these events causes aberrant target selection, indicating that their spatiotemporal dynamics are crucial for topography establishment. However, the signals that regulate this timing are unknown. We examined candidate developmental signals and found, via immunostaining for phosphorylated SMAD1 (pSMAD1), that bone morphogenic protein (BMP) activity is concentrated in the posterior region of the developing vagus. This pattern indicated that BMP signaling may promote the observed delay in posterior migration and axon outgrowth. Consistent with this hypothesis, we found that expression of a constitutively active (CA) form of SMAD1 in vagus neurons causes anterior SMAD1-CA-expressing neurons to aberrantly innervate posterior targets. Through timelapse microscopy, we demonstrated that these anterior SMAD1-CA neurons exhibit delayed arrival to the nucleus and delayed axon outgrowth, whereas posterior neurons expressing dominant negative (DN) SMAD1, or neurons treated with the BMP inhibitor dorsomorphin, exhibit precocious migration and axon formation. These results reveal BMP-SMAD1 signaling as a key regulator of spatiotemporal patterning and topographic map development in the vagus nerve.

Signal processing and robustness of the BMP network during zebrafish embryogenesis

Poster Number: 12

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Nissa Larson** - Purdue University

Co-Author(s): Linlin Li – Research Scientist, Weldon School of Biomedical Engineering, Purdue University; Bobby Madamanchi – School of Information – University of Michigan; David Umulis – Senior Vice Provost, Purdue University

Abstract: In developing tissues, signal transduction from morphogen gradients conveys positional information to cells, resulting in cell specification and differentiation. One such morphogen is bone morphogenetic protein (BMP), of the TGF-β superfamily, whose network is highly conserved across many species. In zebrafish species Danio rerio, this signaling pathway directs dorsoventral axis formation during early embryogenesis. Many of the molecules that play a role in this network are known; however, the mechanisms through which they achieve noise attenuation and gradient robustness have not been defined. Specifically, the heterodimer-heterotetramer complex has been shown to be required for signal transduction, but deterministic modeling of the BMP membrane receptors at this stage has not given any insight into evolutionary drivers of the requirement. Building and developing a stochastic, multiscale model of this process will allow us to mechanistically assess zebrafish phenotype variability related to the distributions of noise and stochasticity. We can also analyze time-dependent signaling and frequency metrics that are not available in traditional, deterministic modeling. Fast Fourier Transform and cumulative energy spectral density visualization show that the heterodimer-heterotetramer complex may combine with a low-pass filter in the dorsal-ventral axis formation process, specifically tuned to the noise of the system. To further probe this system, we are working to develop an experimental optogenetics protocol to genetically manipulate dorsoventral signal transduction in vivo and collect and analyze fluorescence intensity of the downstream pSmad gradient. Through these multiscale modeling efforts and experimental coupling, we hope to further understand the noise origins and signal processing of this network. As the BMP signaling pathway is highly conserved and has been implicated in human bone growth and wound healing, its study in simpler systems such as zebrafish stands to accelerate our comprehension of BMP network structure and molecular mechanisms with application in regenerative medical studies.

Investigating the role of cytoskeletal dynamics during epiboly morphogenesis in the zebrafish

Poster Number: 13

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Bakary Samasa** - University of Pennsylvania

Co-Author(s): Linlin Li – Purdue University; Mary Mullins – University of Pennsylvania; David Umulis – Purdue University; Joe Zinski – University of Pennsylvania

Abstract: Epiboly is the first coordinated morphogenetic cell movement during zebrafish development, wherein the cells of the embryo spread over and envelop the yolk. These morphogenetic movements are essential to the transition from a group of individual cells to the formation of a body plan during development. Thus, the dynamics and regulation of epiboly remains a fundamental question in biology. Epiboly is initiated by an expansion of an epithelial enveloping cell layer (EVL) coupled with oriented EVL cell divisions that leads to yolk cell doming and progression to 50% epiboly. However, the mechanism/s of epiboly progression beyond 50% remain less clear. Here, we investigate the roles of p38 mitogen activated kinase (MAPK) and its substrate Mapkapk2 in epiboly progression. Embryos from homozygous maternal-effect mapkapk2 null mutant females and dominant-negative p38 expressing embryos exhibit a defect in epiboly wherein the blastoderm margin constricts circumferentially beginning at the 50% epiboly stage, causing the yolk cell to burst. It has been shown that Mapkapk2 functions in the yolk cell

to regulate epiboly progression, where it is hypothesized to modulate actomyosin-based contractility. Here I use live and fixed imaging of actin to investigate cytoskeletal dynamics to understand the role of actin dynamics in mediating Mapkapk2 and p38 driven epiboly progression. These initial results set the stage for further investigation of epiboly dynamics in other mutants and fluorescent reporter lines of the cytoskeleton and adhesion factors.

Rbm24a dictates mRNA recruitment for germ granule assembly

Poster Number: 14

Theme: Early Development, Morphogenesis and Patterning

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

Co-Author(s): Yizhuang Zhang – School of Life Sciences, Shandong University

Abstract: Ribonucleoprotein (RNP) granules are the most common membrane-less biomolecular condensates. However, mechanisms underlying their assembly are largely unknown. The aggregation of germ granules determines the fate of primordial germ cells (PGCs) and serves as a model for studying RNP granule assembly. Here, we show that maternal RNA binding protein Rbm24a is the key factor governing specific sorting of mRNAs. Mechanistically, Rbm24a complexes and interacts with Buc to dictate the specific grasp of germ plasm mRNAs into phase-separated condensates. Germ plasm particles lacking Rbm24a and mRNAs fail to undergo kinesin-dependent transport toward cleavage furrows where small granules fuse into large aggregates. Therefore, the loss of maternal Rbm24a causes a complete degradation of the germ plasm and the disappearance of PGCs. These findings demonstrate that Rbm24a/Buc complex functions as a nucleating organizer of germ granules, highlighting an emerging mechanism for RNA-binding proteins in reading and recruiting RNA components into the phase-separated protein scaffold.

Cellular Strategies for Interpreting Positional Information Dynamics to Optimize Patterning Precision During Tissue Morphogenesis

Poster Number: 15

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Chia-Teng Chang** - Washington University in St. Louis

Co-Author(s): Julian Renaud – Institute of Science and Technology Austria; Gasper Tkacik – Institute of Science and Technology Austria; Tony Tsai – Washington University in St. Louis

Abstract: How do cells determine their position and identity to form precise patterns within developing tissues? The French Flag model proposes that morphogen gradients encode positional information (PI) essential for accurate tissue patterning. However, many tissues form patterns concurrently with dramatic changes in tissue shape, and how PI dynamics are influenced by such morphogenetic movements remains largely unexplored. To address this gap, we utilized the developing zebrafish spinal cord, in which ventral neural progenitor cell fates are specified by a Sonic hedgehog (Shh) gradient during the morphogenetic transition from neural plate to neural tube. By combining in toto confocal microscopy of transgenic Shh signaling reporter zebrafish with deep-learning-based image analysis, we

achieved error-free tracking of all anterior ventral spinal cord cells across eight embryos from the 0- to 12-somite stages, capturing their complete positional and signaling histories. This approach enabled us to perform the first in vivo quantification of PI dynamics at single-cell resolution during active tissue morphogenesis. Surprisingly, although Shh signaling activity continuously increased throughout the observed developmental window, Shh-encoded PI did not monotonically increase, instead peaking significantly at the 4-somite stage. These PI dynamics were tightly coupled to morphogenesis, as cadherin and integrin mutants that delayed or accelerated morphogenetic progression exhibited corresponding shifts in PI peaks. Remarkably, two critical cell fate regulators, Olig2 and Nkx6.1, reached maximal protein expression at the stage of maximal Shh-encoded PI rather than at the stage of strongest Shh signaling. These results suggest a strategy by which cells preferentially interpret positional information during optimal temporal windows, thereby maximizing the precision of fate specification despite ongoing tissue deformation. This research was funded by NICHD R00 and NIGMS R35.

Elucidating the role of Iroquois transcription factor 5b in zebrafish kidney and multiciliated cell development

Poster Number: 16

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Ashini Kaushik** - University of Notre Dame

Co-Author(s): Rebecca Wingert – Primary Advisor, Biological Sciences, University of Notre Dame; Margaret Buck – Undergraduate Trainee, University of Notre Dame; Hannah Gunn – Undergraduate Trainee, University of Notre Dame; Kathleen Lavelle – Undergraduate Trainee, Boston College

Abstract: Chronic Kidney Disease (CKD) impacts around 10% of the global population, causing millions of deaths each year. Zebrafish, share 70% of their genes with humans and possess similar nephron structures, serve as a valuable model for kidney research. Multiciliated cells (MCCs) play essential roles in fluid clearance, and their dysfunction is linked to conditions such as hydrocephalus and infertility. Recently, their abnormal presence has been associated with various kidney diseases. Despite their significance in health, many aspects of MCC development remain unclear. Previous research identified irx2a, a member of the Iroquois transcription factor family, as crucial for multiciliogenesis in embryonic zebrafish, with its loss leading to fewer MCCs and nephron dysfunction. Here, we propose irx5b may also contribute to MCC formation, given its expression in the distal early nephron segment, corresponding to MCC localization. Our preliminary data shows irx5b loss of function during zebrafish embryogenesis leads to a reduction in MCC numbers. This loss is observed phenotypically with pericardial edema and hydrocephaly, suggesting potential nephron dysfunction. Consistently, irx5b deficient embryos show impaired fluid clearance compared to wild-type controls. The simultaneous knockdown of irx5b and irx2a results in an even greater reduction in renal MCC numbers than the individual knockdown of either gene. We observe segment length alterations in irx5b deficient embryos at 24hpf, including a shortened distal segment and overall nephron compared to controls. These findings indicate that irx5b plays a critical role in zebrafish pronephros and MCC development, collaborating with irx2a to regulate MCC fate. Immunofluorescence analysis further reveals that irx5b morphants exhibit comprised cilia formation in the nephron. Future studies, including gene expression profiling and examining MCCs in other organs like nasal placode and Kupffer’s vesicle in irx5b

morphants, will help to further elucidate the role of Irx5b in multiciliogenesis and implications for kidney diseases.

Regulation of Microtubule Stability by PITP Family Proteins SEC14L2/Sec14l3

Poster Number: 17

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Shunji Jia** - Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

Co-Author(s): lin zhang – Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

Abstract: Cell migration is a pivotal biological process in multicellular organism development, relying on the coordinated regulation of microtubule dynamics and signaling networks. The dynamic instability of microtubules mediates cellular morphogenesis, intracellular transport, and division, with their homeostasis disruption closely linked to neurodegenerative diseases and tumorigenesis. SEC14L2/Sec14l3, members of the phosphatidylinositol transfer protein (PITP) family, are known to regulate Wnt/Ca^{2+} signaling, mediate VEGFR2 endocytic recycling, and facilitate endosomal fission. However, their role in microtubule homeostasis remains unclear. Our preliminary studies have demonstrated that depletion of SEC14L2/Sec14l3 disrupts microtubule networks in both zebrafish embryos and mammalian cells. Given the central regulatory role of microtubule dynamic balance in key biological processes such as cell division, cell migration, and tumorigenesis, this project will employ zebrafish embryos and mammalian cell

lines as models. By integrating molecular biology, cell biology, and developmental biology approaches, we aim to investigate the functional roles and mechanisms of SEC14L2/Sec14l3-mediated microtubule homeostasis during early embryonic development and tumor progression. The findings are expected to provide a theoretical foundation for the treatment of diseases associated with microtubule homeostasis imbalance

Functional study of retinoic acid-activated regulatory sequences and their role in the expression of hox genes in zebrafish

Poster Number: 18

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Caroline Desiront** - University of Liège

Co-Author(s): Colin Lentjes – University of Liège; Bernard PEERS – University of Liège

Abstract: Hox genes are essential for vertebrate embryonic development, particularly for body segmentation and anterior-posterior axis patterning. These genes are notably regulated by retinoic acid, a vitamin A derivative with crucial roles in health and disease. Studies in chick, mice, and zebrafish have shown that RA deficiency leads to malformations affecting several organs, such as the central nervous system, heart, forelimbs (pectoral fins), and pancreas. RA regulates gene expression through that the binding of receptors which recognize specific regulatory sequences (RAREs) near their target genes.

Several RAREs located within murine Hox clusters have been shown to regulate Hox gene expression. RAR ChIP-seq experiments performed by our research groups have revealed the presence of several RAREs far downstream of the hoxbb locus, located in introns of the neighboring skap1 gene. Analysis of mouse RAR ChIP-seq data indicated that similar RAREs are present in the same locations, some showing strong sequence conservation among vertebrates. To investigate their functional role, we first inserted these RAREs upstream the minimal cfos promotor fused to GFP and tested their activity in vivo. GFP reporter expression was very similar to the hoxbb expression profile for three RAREs. Secondly, we removed the RAREs from zebrafish genome by generating various deletion. While a small deletion removing one RARE region had no significant effect on the hoxbb gene expression, larger deletions removing several RAREs resulted in a significant decrease of all hoxbb genes. Interestingly, the spatial expression profile was modified for some hoxbb genes, showing a posterior shift due to the RAREs deletion. These findings highlight the crucial role of distant RAREs in hoxbb gene regulation. Our data support the model in which Hox cluster activation is driven by distant regulatory regions located far on the 3' side of the clusters, potentially contributing to progressive chromatin opening.

Construction of an embryonic caudal organizer by BMP4

Poster Number: 19

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Tao Cheng, PhD** - Women's Hospital, Zhejiang University School of Medicine

Co-Author(s): Yan-Yi Xing – Zhejiang University; Ying Huang – Zhejiang University; Pengfei Xu – nstitute of Genetics, Zhejiang University School of Medicine

Abstract: The caudal part of a vertebrate embryo consists of somites, neural tube, lateral plate mesoderm derivatives and the tailbud. However, the minimal conditions and factors sufficient to induce the caudal region, particularly in humans, remain unresolved. Here, we show that BMP4 alone, when administered at appropriate dosage, is sufficient to induce the formation of an organizer for caudal induction. This organizer induces caudal cell fate specification and morphogenesis in zebrafish embryos. In 3D human pluripotent stem cells (hPSCs) aggregates, BMP4 can induce an elongated embryonic structure characterized by caudal fates. Importantly, hPSCs instructed by BMP4 are sufficient to induce a secondary caudal region when grafted into the animal pole of the zebrafish embryo. Our study thus uncovers BMP4 as the inducer in the formation of a caudal organizer in the vertebrate embryo.

Extracellular Matrix Proteins and Pineal Medial-Lateral Axis Formation

Poster Number: 20

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Brennan Rosenthal** - Integrated Biosciences Department, Univerity of Minnesota Duluth

Co-Author(s): Jennifer Liang – Professor, Integrative Biosciences, University of Minnesota, Duluth

Abstract: Neurulation is an embryological process during which the neural plate folds and closes to form the neural tube. Incomplete closure of the anterior neural tube in mammals leads to anencephaly, a

fatal neural tube defect (NTD). Our lab has found that zebrafish embryos deficient in two extracellular matrix (ECM) proteins, Laminin (Lam) and Fibronectin (Fn), display anencephaly-like phenotypes. Previous work by Araya and colleagues suggests that this NTD could be caused by disorganized apicobasal polarity within neuroepithelium cells (Araya et al., Dev Dyn. 2016, 245:580-9). As cell polarity and axis formation are tightly linked, we hypothesize that axis formation within the anterior neural tube will also be disrupted in ECM-deficient mutants. We assessed medial-lateral axis formation in lamb1 or lamc1 single mutants using the pineal gland. Pineal photoreceptors express the photosensitive protein Exorhodopsin (Exorh) at their apical sides, which maps to midline of the forebrain. lamb1 and lamc1 single mutants display Exorh expression that is scattered throughout the photoreceptor domain. Additionally, melanocytes, which are typically excluded from the pineal region in wild-type embryos, overlap the domain of Exorh expression in lamb1 and lamc1 mutants. These initial experiments suggest that the medial-lateral axis and the boundaries of the pineal gland are disrupted, supporting our hypothesis. Future studies will determine how the left-right and dorsal-ventral axes of the neural tube are impacted in ECM-deficient mutants, providing further insight into the role of ECM in neural tube axis formation.

Whole embryo imaging and cell lineage profiling via ChromaTrace

Poster Number: 21

Theme: Early Development, Morphogenesis and Patterning

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

Co-Author(s): Loic Royer – Chan Zuckerberg Biohub San Francisco

Abstract: Vertebrate embryos share high similarity and conserved structures during early development. Understanding the precise timing and mechanisms governing cell fate determination and lineage development is fundamental to revealing the orchestration of embryogenesis.

We designed ChromaTrace to introduce accumulative genomic barcodes into cells and label nuclei with fluorescent reporters. Using a light-sheet platform, we imaged whole zebrafish embryos injected with ChromaTrace from 6 hpf to 1 dpf, recording the trajectories of all fluorescent cells. Post-imaging, we performed scRNA-Seq on the same embryos, extracting genomic barcodes alongside transcriptomic data.

Combining scRNA-Seq and imaging datasets from twelve embryos, we reconstructed a consensus cell lineage tree across tissues. The scRNA-Seq datasets included over 60,000 high-quality cells, and we captured cell tracks for more than 40,000 cells from imaging data of the same embryos. We characterized 134 cell clusters, revealing lineage relationships among different tissues in 1 dpf embryos, and generated dynamic cell fate maps from epiboly through gastrulation for 14 major tissues. Furthermore, we characterized specific cell movement and division patterns using the live imaging data.

Our results show that multipotent cells are prevalent in pre-gastrulation embryos, and we identified novel stem-like subclusters in neuronal tissues and the tailbud. Neural stem cell clusters in different structures mostly originated from the same ancestors, migrating to distinct destinations from early gastrulation. These clusters contribute directly to body axis development from the epiboly stage and later become the main source for left-right asymmetry in the central nervous system post-gastrulation.

Understanding BMP morphogen gradient interpretation

Poster Number: 22

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Ari Geller** - University of Pennsylvania

Co-Author(s): Parnal Joshi – Iowa State University; Darius Balciunas – Temple University; Iddo Friedberg – Iowa State University; Mary Mullins – University of Pennsylvania

Abstract: Across the animal kingdom, morphogen gradients provide positional information to cells within developing embryos. Bone Morphogenetic Protein (BMP) acts as a morphogen to pattern the dorsoventral (DV) embryonic axis. In vertebrates, high levels of BMP signaling promote ventral fates, while low levels of BMP lead to the development of dorsal fates. Though we generally understand BMP signaling gradient establishment, we lack a mechanistic understanding of how different BMP signaling levels are interpreted to activate specific gene expression programs. BMP signaling begins when BMP ligands bind to and assemble a complex of kinase receptors. Activated receptors then phosphorylate the intracellular effector protein, Smad. In zebrafish DV patterning, phosphorylated Smad5 then accumulates in the nucleus, where it binds and regulates BMP target genes. Our lab recently investigated models of BMP signaling gradient interpretation during DV patterning in zebrafish embryos. We identified 30 direct BMP target genes, many with known roles in DV patterning, and found that they are expressed in 3 distinct DV expression domains. Our results indicate that the boundaries of these expression domains are regulated by pSmad5 concentration thresholds, allowing us to now probe the molecular mechanisms underpinning the formation of these DV gene expression domains. To identify the cofactors that facilitate Smad5 DNA binding and how Smad5 binding patterns differ among the DV gene expression domains, we validated two different internal Smad5 epitope tags. Both triple V5 and single ALFA-tagged Smad5 constructs produced functional protein detectable through immunoprecipitation. ALFA-Smad5 was also detectable by immunofluorescence and by live imaging of ALFA-Nanobody GFP. Using these tools, we plan to describe a mechanism of BMP signaling gradient interpretation. These findings will in turn uncover how different cell fates are specified during embryonic development. This work is supported by NIH grant R35GM131908 to MCM.

Distinct role of protein tyrosine phosphatase receptor type Q (Ptprq) in maintaining glomerulus filtration barrier integrity of the kidney

Poster Number: 23

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Omodasola Adekeye, MSc** - The University of Maine, USA. Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA

Co-Author(s): Caramai Kamei – Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA.; Blanca de Juan Mora – Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA.

Abstract: The glomerulus, a highly specialized filtration unit of the kidney, relies on the integrity of various cell types, particularly podocytes, to maintain selective permeability. Podocytes are epithelial

cells with an intricate structure characterized by numerous interdigitating actin-based projections known as foot processes, interconnected by slit diaphragms. Disruption of this architecture leads to proteinuria, a major contributor to the progression of end-stage kidney disease. This highlights the essential role of podocytes in preserving glomerular barrier function. Through transcriptomic profiling of developing zebrafish glomeruli, we identified protein tyrosine phosphatase receptor type Q (ptprq) as highly enriched in the developing pronephric glomeruli of zebrafish. PTPRQ is a lipid phosphatase receptor known to convert phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to phosphatidylinositol (4,5)-bisphosphate (PIP2), thereby modulating PIP2-dependent signaling pathways and interacting with MYO6, TPRN, and CLIC5 in stereocilia of the ear to maintain cytoskeleton organization. Given its role in other cellular contexts, we hypothesized that Ptprq regulates podocyte morphogenesis and integrity. To investigate this, we first validated ptprq expression in zebrafish glomeruli using whole-mount in situ hybridization and immunohistochemistry. The ptprq protein localized to podocyte membranes and colocalized with the slit diaphragm (a specialized cell-cell junction between podocyte foot processes) marker ZO-1. Functional studies in ptprq-deficient zebrafish (CRISPR-generated knockouts) revealed whole-body edema, a hallmark of kidney dysfunction. Glomerular filtration assays showed significant proteinuria in both ptprq crispants and homozygous mutants (ptprq—/—), indicating compromised barrier function. Further analysis revealed that podocytes in ptprq mutants exhibited reduced calcium signaling, suggesting that Ptprq is essential for calcium-dependent pathways involved in podocyte maturation and maintenance. In summary, our findings establish Ptprq as a key regulator of podocyte morphogenesis and glomerular barrier integrity. Understanding its molecular role not only illuminates glomerular development but may also offer insights into the pathogenesis of genetic glomerular diseases in humans.

MBD3 Regulates Embryonic Body Axis Formation by Modulating Nodal Signaling

Poster Number: 24

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Hai-Rong Pu** - Zhejiang University

Co-Author(s):

Abstract: Establishing the embryonic body axes is a pivotal event in vertebrate development, tightly regulated by genetic and epigenetic mechanisms. Here, we investigate the role of MBD3, a core component of the NuRD chromatin remodeling complex, in early embryogenesis. Using the first zebrafish mbd3 knockout model, we demonstrate that MBD3 loss leads to abnormal anterior-posterior axis elongation, driven by enhanced convergent extension movements. Immunostaining reveals increased nuclear p-Smad2/3 levels, indicating hyperactivation of the Nodal signaling pathway in mbd3 mutants. These findings establish MBD3 as an essential epigenetic regulator that fine-tunes Nodal signaling during axis patterning. Ongoing studies aim to elucidate the molecular mechanisms linking MBD3 to Nodal pathway components. This work underscores the critical role of MBD3-mediated epigenetic regulation in vertebrate embryonic axis formation.

Laminin-111's Role in Mediating Mechanical Stress During Zebrafish Brain Morphogenesis

Poster Number: 25

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Gabriella Voit** - University of Wisconsin-Milwaukee

Co-Author(s): Elizabeth Falat – University of Wisconsin-Milwaukee; Jennifer Gutzman – University of Wisconsin-Milwaukee

Abstract: Brain morphogenesis is a complex process that requires the integration of biochemical signaling and mechanical forces to shape the developing tissue. While molecular pathways guiding brain development are well studied, the biomechanical mechanisms regulating tissue architecture remain less understood. To address this gap, we investigated the role of the basement membrane in mediating neuroepithelial tissue biomechanics using the zebrafish midbrain-hindbrain boundary (MHB) as a model. The basement membrane protein laminin-111 is highly expressed during MHB morphogenesis and is essential for structural integrity. Previous work in our lab has shown that mutations in laminin-111 genes prevent normal basal folding during MHB morphogenesis, leading to structural defects. To further understand the role of laminin-111 in MHB tissue stress, we utilized a biocompatible oil microdroplet-sensor technique. Our findings indicated that laminin-111 mutants exhibit increased tissue stress at the MHB compared to wild-type embryos. N-cadherin immunostaining in laminin-111mutants revealed disrupted apical and basal adhesion, suggesting weakened structural integrity. Barrier integrity defects were confirmed by ventricle injections where leakage of 500 kDa dextran was observed in laminin-111 mutants. Together, our study reveals that laminin-111 is required for optimal tissue stress during basal folding, for cell adhesion, and epithelial integrity, providing new insights into the biomechanical regulation of brain morphogenesis. Uncovering how laminin-111 regulates neuroepithelial biomechanics contributes to the broader understanding of epithelial tissue engineering and organ morphogenesis.

Exploring the effects of redox perturbations on optic tectum development and behavior

Poster Number: 26

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Kevin Gray** - Brigham Young University

Co-Author(s):

Abstract: The zebrafish optic tectum (OT), the non-mammalian counterpart of the mammalian superior colliculus (SC), integrates multisensory information and orchestrates involuntary behavioral responses such as reflexive movements and attention shifts. The anterior-posterior patterning of this midbrain structure is regulated by transcription factors that rely on proper endogenous levels of H2O2, a reactive oxygen species (ROS). Recent studies have implicated the OT/SC and early ROS imbalance in neurodevelopmental disorders such as autism spectrum disorder (ASD). Given the importance of H2O2 in midbrain development, we are investigating how ROS perturbations within the OT may lead to atypical circuitry development and, subsequently, result in aberrant behavioral phenotypes, similar to those associated with ASD. When we previously exposed zebrafish embryos to valproic acid (VPA)—a drug associated with a higher incidence of ASD and known to induce ROS—we observed an increase in

hyperactivity and anxiety. However, whether these effects are specific to ROS induction in the OT remains unclear due to the bath application of VPA. To address this, we are inducing targeted redox changes in the OT using the nitroreductase-metronidazole (NTR-MTZ) system at levels that do not induce apoptosis. Additionally, we will modulate ROS effects by inhibiting the key antioxidant regulators Nrf2a, Keap1a, and Keap1b. In brief, the transcription factor Nrf2a activates antioxidant genes in response to ROS but is ubiquitinated by Keap1a/b when ROS is absent. We anticipate that a nrf2a knockout will potentiate ROS effects, while keap1a/b knockouts will mitigate ROS effects. Subsequent behavioral assays will elucidate the impact of OT-specific redox stress, offering insights into the interplay between redox states, OT development, and behavioral outcomes.

Exploration of the mechanism that ensures robustness of endoderm differentiation

Poster Number: 27

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Juqi Zou** - The Francis Crick Institute

Co-Author(s): Andrew Economou – The Francis Crick Institute; Caroline Hill – The Francis Crick Institute

Abstract: One of the most crucial processes during early embryogenesis is gastrulation, when embryos form three germ layers: endoderm, mesoderm and ectoderm. The specification of endoderm and mesoderm is controlled by the TGF-β family member, Nodal, which is conserved in all vertebrates. Strikingly, recent discoveries in our lab revealed that mesoderm and endoderm specification in zebrafish embryos results from a stochastic process. Nodal signalling is necessary to produce key transcription factors required for both mesodermal and endodermal fates at the zebrafish embryonic margin. Endodermal progenitors, marked by expression of the master transcriptional regulator, Sox32, then emerge randomly, without any temporal or spatial bias in the ~80-min time window of active Nodal signalling. Cells that do not switch to the endodermal fate, differentiate to mesoderm. This stochastic mechanism raises an important question: how is a functional gastrointestinal system formed if the number of endodermal progenitors initially specified is random? We have shown that low doses of the Nodal receptor inhibitor, SB-505124 administered at sphere stage for about 1 hr results in reduced numbers of endodermal progenitors at around 50% epiboly (5 hpf). However, by 24 hpf we nevertheless observed the correct amount of endoderm as measured by endodermal marker gene expression. Indeed, endoderm progenitor numbers were already substantially corrected by 75% epiboly (8 hpf). Thus, a buffering mechanism exists to correct the numbers of endoderm progenitors during gastrulation. In this project, I aim to identify the buffering mechanism that ensures the robustness of endoderm differentiation. By combining advanced multi-dimensional imaging and zebrafish transgenic technology, I am investigating the adjustment process of endoderm progenitor numbers. I will then take both candidate and unbiased approaches to explore the molecular mechanism underlying the adjustment process. Taken together, this study will provide insights into the mechanisms that ensure robustness during early embryogenesis.

Electric Embryos: Characterizing Bioelectric Patterning During Danio rerio Embryogenesis

Poster Number: 28

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Aislinn Lavery** - Smith College

Co-Author(s): Michael Barresi – Smith College; Mercer Kriese – Smith College; Mekhala Mantravadi – Smith College; Narendra Pathak – Smith College; Shirley Song – Smith College; Claire Woppman – Smith College

Abstract: Can electric charge influence cell fate? Bioelectrics, or the flux of ions across membranes of non-excitable cells, is recognized to regulate key developmental processes. Yet bioelectric patterns during early vertebrate embryogenesis remain poorly characterized. We examined bioelectric dynamics across zebrafish embryogenesis. First, we generated a GEVI-Marina reporter transgenic line expressing fluorescence in response to depolarization. Second, we utilized fluorescent voltage reporter dyes DiBAC4(3) and Rhodamine 6G to mark cells experiencing depolarized or hyperpolarized states, respectively. We used in-vivo lightsheet microscopy to document baseline bioelectric patterns and observed changes following depolarizing (4-Aminopyridine) and hyperpolarizing (Ivermectin and NMDG Chloride) treatments. We observe the existence of replicable, non-uniform bioelectric patterns. A depolarization gradient emanates from the embryo's dorsal side, most concentrated in the shield. In contrast, hyperpolarized cells are seen at the leading edge of epiboly and within involuting endomesoderm cells. Pharmacological manipulation of bioelectrics causes predictable polarization changes in the embryo. We also manipulated bioelectrics via GAP 27 peptide injection, blocking between cell flow of Ca2+ through Connexin 43 gap junction proteins. Connexin 43 inhibition reduces endomesoderm involution and slows blastopore closure. We demonstrate that differential, whole-embryo bioelectric states exist and may support proper morphogenesis. We also show that depolarization and hyperpolarization signatures diversify during somitogenesis, indicated by differential patterns seen in the somites, CNS, and tailbud. Future work will determine if bioelectric states intersect with other signaling modules to influence cell fate and form. Funded by NIH R15 [HD060023], the Arnold and Mabel Beckman Foundation, funding from Smith College: SURF, AEMES, STRIDE, and the Nancy Kershaw Tomlinson Memorial fund.

Loss of atp6v1c1b in zebrafish leads to organism-wide mutant phenotypes

Poster Number: 29

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Claire Stockard** - Northwestern University

Co-Author(s): Saba Parvez – Northwestern University Feinberg School of Medicine; Christopher Yates – Baylor College of Medicine

Abstract: The V-ATPase complex is responsible for regulating vesicular pH and thus plays a key role in a variety of cellular processes. In a previous large-scale in vivo CRISPR screen (MIC-Drop), V-ATPase subunit-coding gene atp6v1c1b knockout in F0 zebrafish revealed ventricular arrhythmia. Although many other V-ATPase subunits have been characterized in depth, notably, no other subunit knockout models have displayed a cardiac phenotype. We generated a stable atp6v1c1b mutant zebrafish line via

CRISPR-Cas9 mediated gene knockout in order to observe subsequent organism-wide effects and further characterize the cardiac phenotype. Homozygotic larvae displayed overall reduced structural sizes, including body length, eye size, head size, and otolith area. The lateral line was also affected, with reduced hair cell size and neuromast disorganization. An overall cardiac arrhythmia was identified in addition to bradycardia, contractile defects, and a narrow myocardium. Adult phenotypes included decreased body size, scoliosis, and disrupted patterning. These findings indicate that the atp6v1c1b subunit of the V-ATPase complex is unique in its role in cardiac morphogenesis and is broadly implicated in early development.

Investigating the role of P-bodies in Pnrc2-mediated oscillatory transcript decay

Poster Number: 30

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Clare Austin** - The Ohio State University

Co-Author(s): Sharon Amacher – The Ohio State University; Monica Blatnik – Oberlin; Thomas Gallagher – The Ohio State University; Danielle Pvirre – The Ohio State University; Kathryn Thompson – The Ohio State University

Abstract: The segmentation clock is an oscillatory gene network that regulates somitogenesis, the formation of embryonic segments (called somites) that later develop into vertebrae and musculature. Proline-rich nuclear receptor coactivator 2 (Pnrc2), an mRNA decay adaptor, is necessary for rapid decay of oscillatory gene transcripts in the zebrafish presomitic mesoderm (PSM). When Pnrc2 function is lost, over 1700 transcripts, including known oscillatory gene transcripts her1 and dlc, are overexpressed. Despite overexpression of many transcripts, pnrc2 mutant embryo development is overtly normal, with some developmental delay and reduced post-embryonic viability. We show that lack of an overt embryonic mutant phenotype is likely because accumulated transcripts are poorly translated. The mechanism by which Pnrc2 regulates rapid decay and translational repression of oscillatory gene transcripts is unknown. Among the genes we looked at ddx6 and ddx61genes that encode DEAD-box helicases and are associated with P-body and stress granule assembly are upregulated at both the transcript and protein level in pnrc2 mutant embryos. Pnrc2 is known to interact with P-body-associated proteins in human cultured cells, consistent with our hypothesis that P-bodies contribute to oscillatory gene transcript regulation. To characterize Pnrc2 localization and identify Pnrc2 interactors, we are using CRISPR-mediated knock-in to tag endogenous Pnrc2 with an ALFA epitope sequence, which will allow us to detect the endogenous Pnrc2 protein using the ALFA nanobody. Using an ALFA nanobody-GFP fusion, we will observe Pnrc2 localization during somitogenesis in wild-type and pnrc2 mutant embryos and co-localization with Ddx6 and Ddx61. Using an ALFA nanobody-Halo tag fusion, we will use co-immunoprecipitation and mass spectrometry to identify Pnrc2 interactors. I predict Pnrc2 interacts with P-body proteins in the zebrafish PSM and will colocalize with Ddx6 and Ddx61. This study will identify the role of P-bodies in Pnrc2-mediated oscillatory gene transcript decay during somitogenesis.

Investigating the mechanisms of zebrafish mesendoderm segregation

Poster Number: 31

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Natalia Jaroszynska, MRes, PhD** - The Francis Crick Institute

Co-Author(s): Caroline Hill – The Francis Crick Institute

Abstract: A central question in developmental biology is understanding how cells are specified to become distinct organs and precisely organise themselves along the body axis to give rise to an embryo. During gastrulation, the three major germ layers, endoderm, mesoderm and ectoderm, emerge, and give rise to different organs. In zebrafish, endoderm and mesoderm are specified from bipotential mesendoderm progenitors at the embryonic margin, specified by a brief window of Nodal signalling. Fgf signalling further drives the commitment of mesoderm progenitors, while the mitotic erasure of its activity promotes the stochastic switch to an endodermal fate via the induction of Sox32 transcription factor expression. How these two lineages segregate from one another to form distinct tissue types, while undergoing anterior-posterior patterning and global gastrulation movements remains unclear. Using a transcriptomics, genetics and microscopy approaches we explore early signalling and novel mechanisms by which these cells separate from one another and give rise to specific organs, which form in the right place at the right time.

Spatiotemporal Mapping of Cranial Neural Crest Cell Lineages in the Developing Zebrafish Head

Poster Number: 32

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Hsuan Chen** - Keck School of Medicine of USC

Co-Author(s): Kuo-Chang (Ted) Tseng – Keck School of Medicine of USC; Grace Edmonds – California Institute of Technology; Mark Budde – California Institute of Technology; Michael Elowitz – California Institute of Technology; Gage Crump – Keck School of Medicine of USC

Abstract: Cranial neural crest-derived cells (CNCCs) generate diverse musculoskeletal tissues that support the vertebrate head, enabling essential functions such as breathing and feeding. Although single-cell studies have revealed discrete cell states, how CNCCs adopt specific fates with spatial accuracy remains poorly understood. To comprehensively capture CNCC lineages in the developing zebrafish head, we generated an intMEMOIR zebrafish lineage barcoding system that enables simultaneous readout of gene expression and lineage relationships at single-cell resolution within native tissue environments. This barcoding approach utilizes an inducible Bxb1 integrase to stochastically edit each of a 10-unit barcode array into three distinct states that can be read out by in situ hybridization. By using a highly multiplexed sequential fluorescent in situ hybridization (seqFISH) platform (Spatial Genomics), we have used a 206-gene probe set in combination with intMEMOIR barcode probes to identify over 20 unique cell clusters and their lineage relationships in consecutive sections of the zebrafish head. In preliminary findings, we have compared clonal relationships at one month of age after barcoding at different embryonic stages (20, 36, or 72 hpf). Lineage coupling analysis reveals progressive loss of CNCC multipotency over developmental time points, as well as perdurance of common progenitors for subsets of musculoskeletal cell types, such as bone and tendon. In contrast, we find that cartilage is one of the earliest segregating lineages, consistent with our previous studies showing that cartilage enhancers are some of the first to gain accessibility during CNCC development. In current work,

I am using intMEMOIR to examine how cell fate decisions are altered during musculoskeletal tissue regeneration, and how such lineage plasticity may be fine-tuned for the type of injury.

Pax1a and Pax9 combinatorially regulate sclerotome contribution to the hematopoietic stem cell specification in zebrafish

Poster Number: 33

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Limei Wu** - Department of Hematology, Division of Experimental Hematology, St. Jude Children's Research Hospital, Memphis, TN

Co-Author(s): Clair Kelley – Department of Pathology – St. Jude Children's Research Hospital, Memphis, TN; Nicole Glenn – Department of Hematology, Division of Experimental Hematology – St. Jude Children's Research Hospital, Memphis, TN; Dafne Gays – Department of Molecular Biotechnology and Health Sciences – University of Turin, Italy; Massimo Santoro – Department of Biology – University of Padua, Padova, Italy; Wilson Clements – Department of Hematology, Division of Experimental Hematology – St. Jude Children's Research Hospital, Memphis, TN

Abstract: Hematopoietic stem cells (HSCs) arise during development from hemogenic endothelium in the ventral floor of the primitive dorsal aorta (DA). The complete set of signaling factors regulating HSC specification and emergence from DA remain uncharacterized. We previously showed that experimental manipulations leading to defects in the most ventral compartment of the somite, the sclerotome, are correlated with HSC defects, raising the possibility that sclerotome patterning is required for HSC specification. To address this possibility and examine the geographical relationship between sclerotome and hemogenic endothelium, we generated a novel transgenic zebrafish line in which expression of the Neon fluorophore is driven by the sclerotome marker gene pax1a, allowing us to track sclerotome migration in living animals. We found that sclerotome-derived cells contact the DA immediately prior to the emergence of HSCs in zebrafish. These cells subsequently give rise to vascular smooth muscle cells (VSMCs). Our findings point to a model where sclerotome-derived VSMC precursors function as an embryonic "HSC specification niche." We demonstrate that combinatorial genetic deletion of the paired-box transcription factor genes, pax1a and pax9—classic regulators of sclerotome maturation—results in a loss of initiation of HSC programming in hemogenic endothelial precursors within the primitive dorsal aorta. Thus Pax1a/Pax9 instruction of sclerotome maturation is required for proper development of the niche. Morpholino knockdown of pax1a and pax9 individually—although non-concordant with genetic deletion—causes a widespread abrogation of sclerotome development, and leads to loss of HSCs and VSMCs, indicating that sclerotome generally is required for HSC emergence and integrated development of mature, smooth-muscle invested arterial vasculature. Together our data reveal that sclerotome-derived cells form the HSC specification niche in zebrafish and that this process is regulated cooperatively by Pax1a and Pax9.

Dystroglycan regulates lens development

Poster Number: 34

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Alexys Berman** - University of Utah

Co-Author(s): Jason Presnell – University of Kansas; Kristen Kwan – University of Utah

Abstract: Eye development is executed via a series of precise and coordinated events, and genetic conditions can disrupt eye development and can be severe and blinding. Disruptions to dystroglycan result in muscular dystrophies that are associated with various eye conditions, including those that affect the lens like congenital cataracts. However, the pathological mechanism of lens defects in affected individuals remains poorly understood. Dag1 has previously been implicated in maintaining the integrity and composition of extracellular matrices (ECMs), which are dynamic protein lattices that surround many tissues and mediate cell behaviors. Dag1 may impact lens development via ECM integrity and other mechanisms such as modulation of TGFβ signaling or lens cell junctions. However, it is currently unknown how Dag1 influences lens development. First, to understand where and when Dag1 may be necessary for lens development, we investigated its gene expression and protein localization in the wild type zebrafish eye over the first 5 days post fertilization (dpf). We determined that dag1 is expressed by lens epithelial cells and retinal cells, and Dag1 protein is found in multiple locations in the eye including the basal surface of the lens epithelium. Next, to understand whether dag1 may be necessary for lens development, we generated a dag1 mutant zebrafish and examined the lens. The mutant lens phenotype appears to diverge from siblings by 4 dpf when the lens epithelial cells of mutants are larger and abnormally shaped, and the lens core exhibits craters in its surface. By 5 dpf, the lens core of mutants lacks tightly packed lens fiber cells found in wild type embryos. Our results suggest zebrafish are a suitable model for investigating dystroglycan's role in lens development. Further research will determine the mechanisms by which Dag1 regulates lens development.

Integrated Genomic and Proteomic Analysis of Zic2a Function During Development

Poster Number: 35

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Sarika Kedar Marathe** - UW-Madison

Co-Author(s): Lauren Braun – Integrative Biology – UW-Madison; Yadwinder Kaur – UW-Madison; Fang Liu – Iowa State University; Zhitao Ming – Iowa State University; Maura McGrail – Professor, Genetics, Development, and Cell Biology, Iowa State University; Yevgenya Grinblat – Professor, Departments of Integrative Biology and Neuroscience, UW-Madison

Abstract: In humans, mutations in Hedgehog signaling pathway genes and ZIC2, a zinc-finger transcription factor, are linked to congenital malformation of the forebrain, holoprosencephaly (HPE). Zebrafish possess two paralogs of ZIC2, zic2a and zic2b, and we have shown that zebrafish zic2a modulates Hh signaling during forebrain patterning. We hypothesize that Zic2a directly binds regulatory regions of a core subset of Hedgehog target genes to modulate Hh pathway. To test this hypothesis, it is necessary to map Zic2a binding sites across the zebrafish genome and to define its protein partners. We are generating zebrafish lines that express peptide-tagged Zic2a, to use with the CUT&Tag workflow and in-vivo TurboID proximity labeling. We have chosen to tag endogenous Zic2a with the rationally designed ALFAtag peptide using CRISPR/Cas9-mediated HDR. Anticipating that zic2aALFA allele may

yield low levels ALFA-tagged protein, we are also generating a Tol2-based 14XUAS:zic2a-ALFA transgenic line as a signal-enhancing alternative. 14xUAS:zic2aALFA transgenic line has been crossed to our recently generated KalTA4 knockin allele of zic2a, zic2aKalTA4, ensuring that ALFA-tagged Zic2a is restricted to its endogenous spatial and temporal domain of zic2a. CUT&Tag with a highly specific anti-ALFA-nanobody (NbALFA) will result in a high-resolution, genome-wide map of Zic2a binding sites. Next, to identify the proteins that interact with Zic2a in vivo, we will use TurboID, an engineered biotin ligase that rapidly biotinylates all proteins within ~10 nm of its fusion partner, thereby "tagging" transient and low-affinity binding proteins that conventional co-IP often misses. Using TurboID fused to NbALFA, we will dock the ligase precisely onto Zic2aALFAexpressed by zic2aALFA (physiological levels) or Tg(14XUAS:zic2aALFA); zic2aKalTA4 (signal-boosted levels). Biotinylated species will then be subjected to mass-spec analysis. Together, these genomic and proteomic maps will define the first cis-regulatory network governed by Zic2a during embryogenesis and reveal its points of crosstalk with the Hedgehog signaling pathway.

Identification of Evolutionarily Conserved Regulatory Elements of the Zebrafish col2a1b Gene

Poster Number: 36

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Angelina Carcione, M.S.** - Loyola University Chicago

Co-Author(s): Rodney Dale, PhD – Associate Professor, Biology, Loyola University Chicago; Bridget Baumbich – Undergraduate Researcher, Biology, Loyola University Chicago

Abstract: Type II Collagens (Col2) are essential proteins providing flexibility and structure to vertebrate animals. During development, Type II Collagens play a vital role forming many structures, such as the cartilage of skeleton, the notochord, the retina and many other tissues. Conserved throughout vertebrates, cartilage is composed of two main cell types: chondrocytes, which make up the bulk of the structure, and perichondral cells, which form the epithelial layer that surrounds chondrocytes and is essential for bone growth and joint protection. While much is known about the expression and regulation of Type II collagens in chondrocytes, specifically the regulation and secrete Type II Collagen Alpha-1 (Col2a1) little is known about perichondrium's genetic regulation and expression of Col2a1. This study will utilize the benefits of the vertebrate Danio rerio, the common zebrafish, to elucidate the genetic regulation and expression of the evolutionarily conserved Col2a1 in the perichondrium. Specifically, we will focus on the zebrafish gene col2a1b, one of two paralogs to the human COL2A1, which is expressed in the perichondrium but not the chondrocytes of cartilage structures. Utilizing bioinformatic and comparative genomic techniques, we have been able to fill in gaps missing in the published zebrafish genome around the telomeric end of chromosome 11 where col2a1b resides. We have identified evolutionarily conserved putative transcription factor regulatory elements around the col2a1b first exon. Generation and imaging of reporter plasmids of our putative regulatory elements has shown expression patterns that support the functional conservation of col2a1a and col2a1b. Overall, these findings provide evidence that the paralogs col2a1a and col2a1b share similar genetic regulation and understanding these mechanisms may provide insight into understanding therapeutic strategies for diseases and disorders involved with COL2A1.

Somite inwardly rectifying potassium channels regulate zebrafish fin size.

Poster Number: 37

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Sung Jun Park** - Department of Comparative Pathobiology, Purdue University

Co-Author(s):

Abstract: Understanding how organ size and shape are determined is a fundamental question in developmental biology. Although significant findings have been made in zebrafish fin development, the detailed mechanisms of fin patterning in zebrafish remain largely unknown. In our study, we analyzed two long-finned insertional zebrafish mutants, Dhi862 and Dhi4458, identified through retroviral insertional mutagenesis. Both mutants carry insertions in the non-coding region of kcnj10a (kir4.1) gene, an inwardly rectifying potassium channel. The two mutants are dominant, exhibiting elongated fins with altered fin ray segments. During early development, we found ectopic expression of kcnj10a in somites between 18-32 hours post-fertilization (hpf), and the larval fish undergo allometric growth. Furthermore, inactivation of kcnj10a rescued the elongated fins in Dhi862 mutant, confirming that kcnj10a ectopic expression is responsible for the long fins. Finally, we identified other potassium channels, such as kcnj2a, that act as an endogenous bioelectric regulator in the somite involved in fin patterning. Our results suggest that the expression of inwardly rectifying channels in the somite is responsible for the zebrafish fin pattering and size determination.

Functional analysis of ultraconserved sequences in Zebrafish

Poster Number: 38

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Braveen Joseph** - National Human Genome Research Institute

Co-Author(s): Kevin Bishop – National Human Genome Research Institute; Blake Carrington – National Human Genome Research Institute; Ava Edison – National Human Genome Research Institute; Bayu Sisay – National Human Genome Research Institute; Abdel Elkahloun – National Human Genome Research Institute; Raman Sood – National Human Genome Research Institute; Shawn Burgess – National Human Genome Research Institute

The term, “Ultraconserved sequences” (UCs) was coined in 2004 for contiguous DNA sequences that are at least 200 bp long and exhibit 100% conservation (no deletions, insertions, or single nucleotide polymorphisms), among the human, rat and mouse genomes. There are 481 human-mouse-rat UCs identified. A large percentage of the UCs are in non-coding regions (77%: 371/481) of the human genome. Studies in mice using the LacZ transgenic reporter assays has showed that these regions function as regulatory elements. Mutagenesis of a few of these non-coding regions in mice neither dramatically altered their regulatory functions nor elicited any abnormal phenotypes at early developmental time points. Larger-scale analysis of the all the UCs in vivo would give a more complete picture of the function of these regions and whether the high degree of conservation has a significant explanation. Notably, multispecies sequence alignments of UCs exhibit strong conservation across all vertebrates. Zebrafish (Danio rerio) represents an ideal candidate for in vivo testing the roles of UCs,

due to its low cost and well-established CRISPR/Cas9 mutagenesis and genetic strategies. Through sequence alignments, we identified zebrafish genomic regions that exhibited high sequence similarity to known UCs and designed guide RNAs (gRNAs) targeting these non-coding regions. gRNA templates were synthesized as oligo pools, then amplified and transcribed and injected them individually into zebrafish eggs and document observed phenotypes. We have mutated and screened 96 loci out of 367. In total 45% of the loci tested showed developmental defects: 22 loci (22.9%) resulted in severe phenotypes, while 21 loci (21.8%) caused moderate defects. Mutations at three loci caused dorsalization defects, while others exhibited various phenotypes, including tail deformities, edema, cyclopia, and early mortality. Notably, these regions are clustered near genes involved in early development. In future, we will validate and investigate the mechanistic functions of these regions.

Tracing the Current: cacna1c Mutation and its Effect on Neural Crest Cell Dynamics and Early Bioelectric Pattern in Danio rerio

Poster Number: 39

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Ruiyi Song** - Smith College

Co-Author(s): Mercer Kriese – NYU Grossman School of Medicine; Narendra Pathak – Smith College; Michael Barresi – Smith College

Abstract: Timothy Syndrome (TS), an autosomal dominant disorder caused by a heterozygous de novo mutation in cacna1c, disrupts voltage-gated calcium (Ca^{2+}) signaling through dysregulation of the Cav1.2 L-type Ca^{2+} channel. This dysregulation perturbs Ca^{2+}-dependent bioelectric patterning, resulting in developmental anomalies, such as craniofacial malformations, including reductions of the mandible. The craniofacial structures are derived from cranial neural crest cells (CNCCs), a subset of the migratory, multipotent progenitor neural crest cell population, yet the mechanistic link between cacna1c-mediated Ca^{2+} disruption and CNCC behavior remains unresolved. Leveraging the zebrafish (Danio rerio) model, which offers well-defined CNCC-derived craniofacial structures and robust cellular analysis and imaging tools, we investigated cacna1c expression and function during embryogenesis. Hybridization chain reaction (HCR) revealed cacna1c expression in CNCC populations during segmentation. The knockdown of cacna1c using two splice-blocking morpholinos induced physiological defects, including somitic truncation and cardiac dysfunction. Genetic encoded voltage indicator (GEVI) Marina transgenic embryos demonstrated regional depolarization that is time sensitive, potentially demonstrating a physiological link to CNCC dysregulation. Immunofluorescence (IF) further identified perturbations in CNCC dynamics that primarily affected proliferation rates and migratory patterns. Together, these preliminary results showcase that Ca^{2+}-dependent bioelectric signaling is disrupted in cacna1c knockdown, which alters CNCC dynamics. This work expands our understanding of how ion channel dysfunction, as seen in Timothy Syndrome, could influence NCC-driven processes and their broader developmental outcomes on morphogenesis. This work is supported by NIH R15 [HD060023], Smith College SURF, and the Nancy Kershaw Tomlinson Memorial fund.

Generating new transgenic lines via CRISPR-based knock-in tagging

Poster Number: 40

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Claudia Carugati** - Duke University

Co-Author(s): Michel Bagnat – Distinguished Professor of Cell Biology, Cell Biology, Duke University; Daniel Levic – Assistant Research Professor of Cell Biology, Cell Biology, Duke University

Abstract: Modern approaches in cell and developmental biology rely heavily on live imaging of fluorescent reporters encoded by transgenes. In the zebrafish field, transgenic lines have traditionally been generated using overexpression approaches via promoter fragments to drive exogenous expression. These types of transgenic lines, while useful, are prone to expression differences due to positional effects and copy number variation. Therefore, my work is focused on generating modern, state-of-the-art transgenic lines using CRISPR-based approaches to report expression from endogenous loci. Here, we present novel transgenic lines to study organogenesis. These include: A) knock-in fusion lines by inserting fluorescent protein (FP) sequences at the N- or C-terminus; B) transcriptional reporters via p2A-FP insertion at the C-terminus; and C) tissue-specific expression lines through integration of reporters within endogenous promoter regions. Collectively, these tools provide a framework for investigating cellular and molecular mechanisms underlying development in a variety of organs.

Interrogating the role of Nodal positive feedback in embryonic patterning and robustness

Poster Number: 41

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Alison Guyer** - University of Pittsburgh School of Medicine

Co-Author(s): Caleb Dobbs – Computational and Systems Biology – University of Pittsburgh School of Medicine; Nathan Lord, PhD – Computational and Systems Biology – University of Pittsburgh School of Medicine

Abstract: In principle, simple diffusion of a signaling molecule from a localized source is sufficient to create a signaling gradient. However, many natural patterning systems seem to require complex signaling feedback, suggesting that simple ligand diffusion is not sufficient for developmental pattern formation. The model morphogen Nodal patterns mesendodermal cells in vertebrate embryos. Nodal signaling is shaped by both positive and negative feedback. Nodal signaling activity induces the expression of more Nodal (positive feedback), as well as Lefty proteins, diffusible inhibitors of its own signaling (negative feedback). Though deeply conserved, the specific roles of these feedback loops in Nodal patterning remain a matter of debate. Previous work in zebrafish found that Lefty-mediated negative feedback is dispensable for development under normal conditions but is required to correct unexpected signaling perturbations. In this project, we will investigate the role of Nodal positive feedback in zebrafish embryogenesis. We sever positive feedback by generating a zebrafish mutant that produces a functional Nodal gradient but cannot produce more Nodal in response to signaling. I will present preliminary evidence that suggests the removal of Nodal positive feedback does not compromise mesendodermal patterning. In the future, these feedback-compromised embryos will be used to systematically investigate the requirement for positive feedback in Nodal patterning and robustness.

tcf15/paraxis is Necessary for Co-Migration of Peripheral Axons and Neural Crest Cells

Poster Number: 42

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Bennett Andrassy** - Kenyon College

Co-Author(s): Maximos McCune – Kenyon College; Sarah Petersen, PhD – Ashby Denoon Associate Professor of Neuroscience; Principal Investigator at the Petersen Lab, Neuroscience; Biology, Kenyon College

Abstract: Peripheral nerve development requires coordinated migrations of nascent axons and neural crest cells (NCCs), which aid axonal guidance and stabilization. NCCs later differentiate into multiple cell types, including Schwann cells (SCs), the myelinating glia of the peripheral nervous system (PNS). In tcf15 mutant zebrafish, the posterior lateral line nerve (PLLn), a major mechanosensory nerve, exhibits mispatterning and hypomyelination, suggesting disrupted axon/NCC co-migration throughout early neurodevelopment. We utilized in vivo time-lapse imaging of mutant and wild-type (WT) zebrafish harboring transgenic constructs labeling peripheral axons (nbt:DsRed) and NCCs (sox10:GFP) to ascertain effects of tcf15 dysfunction on the co-migration of these cell types. We found that NCCs frequently fail to permanently associate with the PLLn in tcf15 mutants, remaining in a migratory state and destabilizing nerve development. Additionally, NCCs frequently failed to elongate along the PLLn, suggesting defects in directed migration and contact-mediated differentiation into SC precursors. The total abundance of NCCs in tcf15 mutants was comparable to WTs, showing that tcf15 influences NCC migration, adhesion, and differentiation to a greater degree than proliferation or survival. Together, our findings indicate that tcf15 regulates PNS development by promoting NCC adhesion and co-migration with developing nerves. As tcf15 is a basic helix-loop-helix transcription factor expressed exclusively in muscle, we hypothesized that tcf15 non-cell autonomously promotes ErbB signaling in NCCs, allowing proper PNS development. To verify this mechanism, we are using HCR to examine mRNA expression in mutant and WT fish, as well as assessing effects of pharmacological ErbB inhibition on PNS development across tcf15 mutants, heterozygotes, and WT fish. Preliminary data indicates that ErbB inhibition in WTs and heterozygotes recapitulates aspects of the mutant phenotype, implicating reductions in ErbB signaling in the observed defects. Our work characterizes genetic determinants of peripheral neurodevelopment, contributing to an increased understanding of mechanisms underlying demyelinating disorders.

Regulators of Kidney Progenitor Development Impact Neighboring Vessel and Blood Progenitor Specification

Poster Number: 43

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Elliot Perens, MD PhD** - University of California, San Diego

Co-Author(s): Jasmine Hernandez – Pedaitrics – University of California, San Diego; Lisanne Stouthart – School of Biological Sciences – University of California, San Diego; Matthew Nunes – School of Biological

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Abstract: Organ development depends on the establishment of well-defined domains of progenitor cells. Progenitor domain formation often follows a similar sequence: morphogen gradients differentially impact the expression of activating and repressing transcription factors (TFs); pairs of TFs in adjacent domains reciprocally repress each other to refine their boundaries; and, finally, the distinct combination of TFs expressed in a domain determines its fate. In the context of kidney development, kidney progenitors reside within the intermediate mesoderm (IM), a pair of bilateral tissue stripes in the middle of the posterior lateral mesoderm, bounded by hemangioblast progenitor domains. Consistent with a model of reciprocal repression, we previously showed that the TFs Hand2 and Npas4l inhibit IM fate and promote endothelial progenitor fate, while the TF Osr1 has the opposite effect. Surprisingly, our findings indicate that, in addition to their well-known roles in promoting IM fate, the TFs Pax2a and Pax8 also promote the formation of medially adjacent primitive hematopoietic progenitors and laterally adjacent endothelial progenitors, while inhibiting the expansion of medially situated endothelial progenitors. Consistent with a loss of primitive hematopoietic progenitors and an expansion of medial endothelial progenitors (which give rise to the dorsal aorta), pax2a;pax8 mutants lack erythrocytes and exhibit a striking increase in hematopoietic stem cells emerging from the dorsal aorta. Intriguingly, we find that pax2a and pax8 are transiently expressed within the medially and laterally adjacent hemangioblasts before expression is refined to the IM. In contrast, while hnf1ba and hnf1bb are also expressed in and required for IM formation, they are not expressed in adjacent hemangioblasts and do not impact their formation. Together, these findings suggest new roles for pax2a and pax8 in modulating the descendants of hemangioblasts and further illuminate the relationships coordinating the specification of the IM and neighboring hemangioblast lineages.

Quantitative Analysis of Shape and Topological Changes in EVL Cells during zebrafish epiboly

Poster Number: 44

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Linlin Li, PhD** - Purdue University

Co-Author(s): Mary Mullins – University of Pennsylvania; David Umulis – Purdue University; Aakarsh Rai – Purdue Univestiy; Bakary Samasa – University of Pennsylvania

Abstract: Epiboly in zebrafish is a critical morphogenetic process that depends on the coordinated thinning and spreading of the enveloping layer (EVL), deep cells, and the yolk syncytial layer. Underlying these tissue-wide rearrangements are tightly regulated mechanical and molecular cues. In this study, we employ an imaging-driven computational approach to investigate EVL cell morphology and topology during epiboly in wild-type zebrafish. By using high-resolution confocal microscopy of Tg(actb2:lifeact-gfp) embryos, we capture detailed spatiotemporal data of EVL cells. We then apply advanced image-processing pipelines to map three-dimensional volumetric data onto two-dimensional projections while preserving depth information for robust cell segmentation and tracing in 3D. Through these computational methods, we extract morphometric descriptors—cell area, aspect ratio, and circularity—to quantify changes in cell shape during epiboly. We also evaluate topological parameters such as

junctional angles, cell–cell border lengths, and neighbor exchange rates to gain insight into tissue-level packing geometry. This integrated methodology provides a quantitative framework for understanding the dynamic cellular reorganization that underlies epiboly. Furthermore, our imaging-based computational approach highlights the power of combining live imaging with quantitative analytics to elucidate how cellular behaviors drive tissue morphogenesis.

A dorsally labeled, BMP-deficient zebrafish line to study embryonic patterning with optogenetics

Poster Number: 45

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Matthew Monaghan** - NICHD/NIH

Co-Author(s): Leanne Iannucci – NICHD/NIH; Katherine Rogers – NICHD/NIH

Abstract: Bone morphogenetic protein (BMP) establishes a ventral-to-dorsal signaling gradient across the early zebrafish embryo that is key to proper development. It remains unclear how exactly this spatial signaling gradient prescribes cell fate to naive cells. An ideal experiment would be to introduce different signaling gradient shapes and examine the developmental consequences to understand gradient-mediated tissue patterning. To achieve this, we will optogenetically manipulate BMP signaling in early-stage zebrafish embryos to generate various signaling distributions. Our lab has an “Opto-tool”, bOpto-BMP, that activates BMP signaling in response to blue light and is not responsive to light wavelengths above 495 nm. To perform gradient replacement experiments, we will combine bOpto-BMP with a zebrafish line that 1) lacks the endogenous signaling gradient and 2) can be oriented along the dorsoventral axis at early stages. To knock out endogenous BMP signaling, we will use the BMP ligand mutant swirl. To orient the dorsoventral axis, we will place a fluorescent protein behind the goosecoid promoter, which is active on the dorsal side. To avoid inadvertently activating bOpto-BMP, we need a red fluorescent protein with excitation and emission wavelengths above 495 nm. To identify an ideal red fluorescent protein, we tested three candidates in vivo: mApple, mScarlet3, and mScarlet-I3. We identified mScarlet-I3 as the brightest and fastest maturing FP tested. We are currently generating a stable transgenic line with Tol2 and pIGLET systems. mScarlet-I3 will be placed behind a goosecoid promoter and then crossed with swirl mutants to create our desired line. With the ability to consistently orient these embryos in optogenetic experiments, we hope to be able to learn more about how information is encoded in signaling gradients by “painting” various gradient distributions and assessing developmental outcomes.

Correlation between the neural tube phenotypes of Nodal signaling and Extracellular matrix mutants

Poster Number: 46

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Jennifer Liang, PhD** - University of Minnesota Duluth

Co-Author(s): Kirsta Olson – Biology – University of Minnesota Duluth; Ava Kloke – Biology – University of Minnesota Duluth; Anika Jilek – Biology – University of Minnesota Duluth; Madison Gohman – Biology – University of Minnesota Duluth; Angelique Bernik – Biology – University of Minnesota Duluth

Abstract: During primary neurulation, a flat neural plate folds to form a closed tube, which then develops into the brain and spinal cord. Failures in neurulation, or neural tube defects (NTD), are some of the most common causes of human birth defects. We found that zebrafish embryos with mutations in genes encoding components of the Nodal signaling pathway or the extracellular matrix (ECM) have several overlapping neural tube phenotypes, suggesting they work together to promote neural tube closure. First, both Nodal signaling and ECM mutants have anterior NTD analogous to the fatal human birth defect anencephaly. In both cases, the NTD is dose dependent. Mutant embryos with mild decreases in Nodal signaling or ECM have a closed anterior neural tube, but the neural tube is often twisted relative to the axis of the embryo. Embryos with moderate decreases have an incompletely penetrant anterior NTD and those with severe deficiencies a severe, completely penetrant NTD. The function of Nodal signaling in anterior neurulation has been mapped to its role inducing mesoderm formation during blastula stages. Thus, we propose that Nodal signaling and mesoderm activate the production of the ECM that lies between the mesoderm and developing neural tube. The ECM then serves to anchor the neuroepithelium during the movements of neurulation. This project is supported by a UMN Grant-in-Aid to JOL, an Undergraduate Research Opportunities Fellowship to AK and Biology Undergraduate Research in Science and Technology Fellowship to MG.

Loss of wdr37 in zebrafish leads to variable developmental anomalies and reduced survival

Poster Number: 47

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Elena Sorokina** - MCW

Co-Author(s): Sanaa Muheisen – MCW; Elena Semina, Dr. – MCW; Samuel Thompson – MCW

Abstract: Human WDR37 is associated with neurooculocardiogenitourinary syndrome (NOCGUS) characterized by ocular coloboma and Peters anomaly, poor growth, structural brain defects, cardiac and genitourinary abnormalities, and dysmorphic facial features. The WDR37 protein includes seven WD40 domains that are known to act as protein interaction scaffolds; however, the precise function of WDR37 is currently unknown. A zebrafish line carrying c.284_291del (p.Gln95Argfs*4) frameshift variant in wdr37 was generated using CRISPR/Cas9 technology; the mutant transcript is predicted to undergo nonsense-mediated decay or, if expressed, to be truncated at ~20% of normal length. Homozygous fish displayed increased lethality and poor growth, while heterozygous animals appeared normal. Morphological examination of homozygous embryos identified the following features at 3-5-dpf: a visible accumulation of red blood cells in the lumen of the enlarged caudal vein (in ~70% of mutants), as well as variable craniofacial and ocular anomalies (in ~15% of mutants). Histological analysis of 3-dpf eyes identified an abnormal cell mass in the anterior region of the developing lens. Further evaluation of craniofacial anomalies using Alcian blue staining identified narrow and malformed lower jaw. In situ hybridization studies confirmed broad embryonic expression of wdr37 in zebrafish, consistent with reports from other species. Semi-quantitative RT-PCR analysis using whole embryos demonstrated

comparable levels of wdr37 transcripts in 1-cell and 4-, 8-, 24-, 48- and 72-hpf embryos. To investigate the possible effect of maternally provided wdr37 on the mutant phenotype, homozygous progeny of heterozygous and homozygous females was compared. Overall, similar anomalies were observed, but with higher frequency in the progeny of homozygous females (~90% for caudal vein and ~57% for lens anomalies). RNAseq and qRT-PCR analyses are underway to investigate the transcriptomic changes in wdr37 mutants. In summary, the phenotype associated with wdr37 loss-of-function in zebrafish is consistent with features observed in human NOCGUS syndrome.

Zebrafish Kcnh genes' expression in early embryonic development

Poster Number: 48

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Kuangyi Wu** - Department of Comparative Pathobiology, Purdue University

Co-Author(s): Dingxun Wang – Department of Comparative Pathobiology, Purdue University; GuangJun Zhang – Department of Comparative Pathobiology, Purdue University

Abstract: KCNH genes encode voltage-gated potassium channels, which consist of three subgroups: EAG (ether à go go), ERG (EAG related gene), and ELK (EAG like K) channels. KCNH channels are essential for neuronal excitability and cardiac repolarization, and mutations in their human orthologs cause epilepsy and arrhythmia, yet their role in embryonic development, especially non-excitable tissues or organs, remains largely unknown. Using whole-mount in situ hybridization, we examined the temporal and spatial expression of 13 zebrafish kcnh genes at 12-72 hours post fertilization (hpf). As expected, nearly all of them were already transcribed by 24 hpf and expressed in the central nervous system. In addition, we found that some of the kcnh genes were expressed in non-neural tissues. Notably, kcnh6a displayed a robust expression in the developing heart, aligning with its conserved role in cardiac repolarization. In contrast, several transcripts of other kcnh genes showed transient signals in somites and fin buds. These kcnh expression provides a clue for their functions during embryonic development and may help us better understand human KCNH channelopathies.

Live imaging and perturbation of the myotendinous junction using vinculin knock-in lines

Poster Number: 49

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Kaelyn Owen** - Duke University

Co-Author(s): Daniel Levic – Duke University; Michel Bagnat – Duke University; Brenton Hoffman – Duke University

Abstract: The myotendinous junction (MTJ) serves as the primary site of force transmission at the muscle-tendon interface. This structure consists of extracellular matrix, basement membrane, and cytoplasmic protein complexes. MTJs harbor two adhesion structures: the dystrophin glycoprotein complex (DGC) and the integrin-vinculin-talin complex. In zebrafish, the MTJ, often referred to as the myoseptum, is the boundary that separates adjacent myotomes. The myosepta are functionally

homologous to human tendon, contain conserved adhesion structures, and serve as an in vivo model for studying the MTJ. A key MTJ protein is Vinculin, which acts as a linker to connect the adhesion structure to the actin cytoskeleton and is critical for force transmission. To monitor MTJ dynamics during development, we used our CRISPR-based approach to generate transgenic knock-in (KI) fusion lines for both vinculin a, TgKI(vcla-eGFP), and vinculin b, TgKI(vclb-mSc) and TgKI(eGFP-vclb). Using these novel KI lines, we found that Vcla is expressed specifically in myocytes and localizes to myosepta, while Vclb displays more ubiquitous expression. To study the role of MTJ components on vinculin localization, we performed CRISPR knock down (KD) of Dystrophin (dmd), the main component of the DGC, and Focal Adhesion Kinase (ptk2ab), a key component in integrin signaling. Dystrophin KD results in wavy myosepta, indicated by increased tortuosity, while focal adhesion kinase knockdown results in similar but milder phenotypes. Surprisingly, dmd KD did not alter Vinculin's MTJ enrichment. Overall, these new Vinculin KI lines provide a tool for studying the architecture of the MTJ in vivo, which in combination with perturbation through KD of genes of interest, introduces a platform for screening proteins involved in force transmission.

Deficiency in top2b leads to craniofacial and cardiac anomalies in zebrafish

Poster Number: 50

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Justin Freestone** - Medical College of Wisconsin

Co-Author(s): Sarah Seese – Medical College of Wisconsin; Sanaa Muheisen – Medical College of Wisconsin

Abstract: Topoisomerase II Beta (TOP2B) is the second member of the topoisomerase II family. Collectively, the primary role of topoisomerases is to relieve supercoiling during transcription or replication by inducing DNA breaks, however, the exact function(s) of TOP2B, like that of other topoisomerases, are not fully elucidated. Zebrafish top2b was previously identified as a potential interacting partner of MAB21L2. Interestingly, a human coloboma-associated mutant MAB21L2-p.R51G protein demonstrated a weaker interaction with top2b, suggesting a possible role in disease mechanism. In situ hybridization studies confirmed broad expression of top2b in zebrafish eyes. To investigate effects of top2b deficiency, two genetic lines were obtained: top2bsa11711 (c.1044 T>A; p.Tyr348*) from ZIRC, and c.2235_2236delCCinsGTGGAAGGAAG; p.Gln746Trpfs*13 that was generated using CRISPR-Cas9 technology. Both variants are expected to trigger nonsense-mediated mRNA decay leading to reduced or no protein expression; however, if expressed, p.Tyr348* variant is predicted to prematurely truncate top2b in the middle of the DNA gyrase domain while p.Gln746Trpfs*13- prior to the DNA topoisomerase IV domain. Gross morphological examination of progeny produced by heterozygous crosses identified ~25% of embryos with visible craniofacial abnormalities w/o heart edema for each line. Both phenotypes were first noticeable at ~5-dpf and gradually worsened leading to 100% larval lethality by 12-dpf. Genotyping of abnormal embryos from 4 clutches (38 embryos) for p.Tyr348* and 6 clutches (52 embryos) for p.Gln746Trpfs*13 lines confirmed homozygosity for the corresponding mutations for all malformed embryos. Heterozygous larvae demonstrated normal appearance and survival to adulthood. Alcian blue staining identified irregular development of the ceratohyal and Meckel's cartilage in both lines. Histological H&E staining of transverse sections at the eye level did not detect any consistent structural ocular abnormalities, however, additional studies are

underway. Deficiency in top2b leads to severe defects in craniofacial and heart development, as well as early larval lethality in zebrafish.

RB determines ascl1b neural progenitor cell division symmetry through negative regulation of Notch

Poster Number: 51

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Lakpa Sherpa** - Iowa State University

Co-Author(s): Fang Liu – Iowa State University; James Preston – Iowa State University; Sekhar Kambakam – Iowa State University; Zhitao Ming – Iowa State University; Maura Mcgrail – Iowa State University

Abstract: The retinoblastoma (RB) tumor suppressor is a central regulator of cell cycle progression in neural progenitors, but its role in controlling cell division symmetry and cell fate during brain development is not well understood. We previously generated Cre and floxed GeneWeld CRISPR knock-in alleles that demonstrate a cell autonomous requirement for RB in limiting ascl1b neural progenitor proliferation. Recently, we discovered RB inactivation in ascl1b progenitors alters not only proliferation but also the symmetry of apical neural progenitor cell division in the developing forebrain. The RB floxed allele is designed to induce mRFP expression after Cre mediated inactivation, allowing visualization of neural progenitor cell division via live confocal imaging. Live imaging reveals wild type ascl1b progenitors undergo symmetric divisions oriented parallel to the apical surface, with polarity marker Pard3 localized symmetrically across the cell apical cortex. ascl1b-RB knock-out leads to a significant increase in the number of dividing progenitors and a dramatic shift in the orientation of division relative to the apical surface, from parallel to perpendicular. Preliminary results show ascl1b-RB KO dividing progenitors show asymmetric localization of Pard3 at the apical cell membrane. The switch from symmetric to asymmetric division correlates with increased expression of Notch pathway genes and an increase in mature TH+ neurons in the larval forebrain. Treatment of ascl1b-RB KO embryos with the Notch gamma-secretase inhibitor LY411575 suppresses the increase in pH3 cells in the forebrain. These results demonstrate increased ascl1b progenitor proliferation after RB loss is dependent on the Notch pathway. Together, this supports our hypothesis that RB controls the symmetric division of ascl1b progenitors and suppresses progenitor daughter cell fate through negative regulation of Notch. Experiments are underway to investigate the mechanism by which RB determines ascl1b cell division symmetry through apical Pard3 localization and control of Notch signaling.

Using a zebrafish model to investigate a role of PFKFB1 in ocular development and disease

Poster Number: 52

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Megan Fischer** - Medical College of Wisconsin

Co-Author(s): Sanaa Muheisen – Medical College of Wisconsin; Elena Sorokina – Medical College of Wisconsin; Samuel Thompson – Medical College of Wisconsin; Ross Collery – Medical College of Wisconsin; Elena Semina – Medical College of Wisconsin

Abstract: Purpose: A candidate variant in PFKFB1 (NM_002625.4:c.318-2A>G) predicted to lead to loss of function was recently identified in a family affected with a complex retinal phenotype. PFKFB1 encodes a member of an enzyme family involved in the regulation of glycolysis and gluconeogenesis, with no human disease association currently reported. Here we present the generation and characterization of a zebrafish model of pfkfb1 deficiency and studies into a possible role of this gene in a human retinal phenotype. Methods: RNAScope in situ expression studies were performed. CRISPR-Cas9-mediated gene editing was used to generate mutant lines, which were evaluated by gross examination under a microscope, optical coherence tomography (OCT), and histology. Results: Expression studies showed strong expression of pfkfb1 in ocular structures, including the developing retina. A zebrafish line carrying a c.173_177del;p.(Thr58Serfs*3) frameshift variant was generated in the AB strain background; mutant embryos and adult fish demonstrated normal overall development and survival. OCT/histological examination of adults of all genotypes revealed variable retinal abnormalities/RPE disorganization that, to the best of our knowledge, has not previously been reported in wild-type AB animals. To further investigate, we generated two new pfkfb1 frameshift mutations (c.108_111del;p.(Ala37Glyfs*10), c.1002_1003del;p.(Glu335Valfs*8)) in a mixed Tü/Ekwill background. Similar to c.173_177del, both lines demonstrated normal development and survival; however, no retinal phenotype was detected by OCT or histological studies in the c.108_111del adults, while characterization of the c.1002_1003del fish is underway. Additional studies using light damage are ongoing to assess the impact of pfkfb1 deficiency on oxidative stress tolerance in the eye. Conclusions: Expression of zebrafish pfkfb1 in the developing eye provides support for a role of this gene in eye development. However, initial characterization of pfkfb1-deficient zebrafish lines does not reveal a clear ocular phenotype, and further studies are needed to understand the effects of pfkfb1 deficiency on ocular function.

UHRF1 is required for cell-cycle progression in developing hepatocytes

Poster Number: 53

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Tijana Randic** - Department of Biology, NYU Abu Dhabi

Co-Author(s): Bhavani Madakashira – NYU Abu Dhabi; Kirsten Sadler – NYU Abu Dhabi

Abstract: Cell cycle progression during organogenesis is tightly regulated to ensure developing tissues achieve proper size and shape. The Ubiquitin-Like with PHD and Ring Finger Domains 1 (UHRF1) is a key regulator of DNA methylation, and we have shown that zebrafish uhrf1 mutants exhibit global DNA hypomethylation. However, there is evidence that UHRF1 has an additional role in cell cycle regulation. We examined this using uhrf1 mutants, which are characterized by a small liver that is patterned appropriately but fails to acquire a mature hepatic identity. uhrf1 mutant hepatocytes display an aberrant cell cycle phenotype, including persistent DNA re-replication and activation of pro-proliferative transcriptional programs. Single-cell transcriptomics of livers at 120 hours post-fertilization (hpf) revealed distinct hepatocyte subtypes in uhrf1 mutants, marked by elevated expression of DNA damage

markers and enriched immune response gene signature, with most cells expressing high levels of either S- or G2/M-phase-associated genes. EdU labeling and immunofluorescence analysis showed an increased number of hepatocytes in both S- and M-phase in uhrf1 mutants during liver development. At 96 and 120 hpf, EdU-positive cells constituted 55% and 43% of the mutant liver, respectively, compared to 25% and 10% in control larvae. The number of hepatocytes in M-phase increased approximately 4.2- and 5-fold in uhrf1 mutants relative to controls at these time points. Notably, at 120 hpf, 47.6% of mitotic cells in mutants accumulated in metaphase, indicative of altered mitotic dynamics. Despite enhanced mitotic entry, mutant hepatocytes exhibited pronounced mitotic aberrations, including abnormal spindle assembly and chromosome misalignment. Collectively, our findings uncover a requirement for UHRF1 in two distinct stages of the cell cycle: DNA replication and mitosis. We hypothesize these represent an important role of uhrf1-mediated DNA methylation maintenance and a separate role in progression through mitosis during liver development.

Imaging in vivo transcription to study fate restriction mechanisms in zebrafish neural crest

Poster Number: 54

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Michael Wen** - University of Chicago

Co-Author(s): Andrew Gillis – Bay Paul Center – Marine Biological Laboratory; Victoria Prince – Organismal Biology and Anatomy – University of Chicago

Abstract: The neural crest (NC) is a transient stem cell-like population of cells found in vertebrates that is characterized by its multipotency and differentiation potential. Neural crest cells (NCCs) give rise to a large array of tissues and cell types including craniofacial cartilage and bone, neurons and glia of the peripheral nervous system, pigment cells, and components of the cardiovascular system. Although NC differentiation has been well-studied over the past few decades, the mechanism which results in the diversity of cell types is still highly debated. One reason for this controversy is due to the lack of methods to follow transcription dynamics of single NC progenitors over time. To resolve this, we have applied the PP7 RNA-labeling system to zebrafish as a parallel method to the previously applied MS2 RNA-labeling system. By using PP7 and MS2 RNA-labeling methods together, we will be able to image the transcription dynamics of two different genes within the same specimen at single-cell resolution. We have generated a stable transgenic line with red fluorescently labeled PP7 bacteriophage coat protein (PCP) and have validated the PP7-PCP interaction. Further, we have established a near infrared neural crest reporter transgenic line, to allow specific imaging of NCC transcription dynamics using both the green fluorescently labeled MS2 bacteriophage coat protein (MCP) line and the red fluorescently labeled PCP line. The application of these live transcription imaging technologies to a vertebrate model provides a new way to study differentiation that could resolve the long-standing debate of fate restriction mechanisms within the neural crest. This work is supported by the American Heart Association (25PRE1362838).

Mechanisms of neurogenesis in the developing posterior lateral line ganglia

Poster Number: 55

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Tara Cerny** - Washington University School of Medicine in St. Louis

Co-Author(s): Mark Warchol – Otolaryngology – Washington University School of Medicine in St. Louis; Lavinia Sheets – Otolaryngology – Washington University School of Medicine in St. Louis

Abstract: The lateral line system in fish is used to sense water flow. It consists of clusters of sensory cells, called neuromasts, and innervating neurons. The posterior lateral line ganglion (pLLG) contains the cell bodies of afferent neurons that contact sensory hair cells in neuromasts of the posterior lateral line system. These neurons share many developmental and functional similarities with the afferents that innervate the cochlea and vestibular organs in mammalian ears. The postembryonic development of the pLLG is poorly understood, but prior studies have shown that between 5 and 7 days post fertilization (dpf), about 15 neurons are added to the ganglion (M Haehnel et al., JCN, 2012). Our studies aim to characterize the mechanism of postembryonic neurogenesis in the pLLG. We have used both transgenic fluorescent reporter lines and fluorescent in situ labeling to profile the expression of neuroD, a transcription factor expressed in sensory neurons of the inner ear, and nestin, a known marker for neural progenitors. Our preliminary data show that both neuroD and nestin are expressed in cells of the pLLG. At 2-3 dpf, we observe some overlap in the expression of these markers. At later developmental stages, these transcripts become spatially segregated. Decreased levels of nestin appear throughout the ganglion, slightly favoring the ventral end. NeuroD expression remains robust and concentrated near the ventral pole. We are also using EdU labeling to identify precursor cells that give rise to newly generated ganglion cells. Initial observations show that exposing fish to EdU between 5 and 6 dpf and fixing them at 7 dpf yields approximately 10 EdU-labeled nuclei per ganglion, none of which are neurons. We are currently applying EdU at earlier developmental stages to trace the lineage of ganglion cells added after 5 dpf.

Proton Portraits: Mapping Dynamic Acidification Microdomains in Transgenic Zebrafish

Poster Number: 56

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Charles Williams, PhD** - Michigan State University

Co-Author(s): Charles Hong – Michigan State University/HFH; Leif Neitzel – Michigan State University; Maya Silver – University of Maryland; Aaron Wasserman – Michigan State University

Abstract: Extracellular proton gradients regulate protein conformation, ion channel activity, and cell signaling, yet tools to monitor extracellular pH dynamics in vivo remain limited. Here, we report a novel transgenic zebrafish (Danio rerio) line ubiquitously expressing a genetically encoded, ratiometric fluorescent pH sensor, pHluorin2, anchored to the extracellular plasma membrane via a glycosylphosphatidylinositol (GPI) motif. The sensor, with a pKa of \~7.2, enables detection of proton fluctuations between with real-time visualization of extracellular acidification in intact embryos. Using dual excitation ratiometric confocal imaging, we mapped extracellular acidification across tissues during the first 72 hours post fertilization. Analyses revealed discrete acidification microdomains, including periodic low pH at notochord cell intercalations during axis elongation, transient otic placode

acidification before sensory vesicle maturation, and sustained myotomal interstitial acidification. Intriguingly, these patterns diverged from T tubule acidification, highlighting compartmentalized acid–base regulation in developing muscle. To investigate mechanisms, we used morpholino knockdown of Bin1b and MTM1, essential for T tubule biogenesis. Loss of either gene disrupted T tubule architecture and reduced myotomal extracellular acidification, implicating T tubule surface area and proton exchange as key determinants of the extracellular pH landscape. Together, our findings establish the first ubiquitous GPI-anchored pHluorin2 reporter for monitoring extracellular proton dynamics in vivo and delineate tissue-specific acidification events during zebrafish embryogenesis. This versatile platform enables exploration of extracellular pH roles in development, disease, and proton-sensitive signaling pathways.

Determining The Causation of Microphthalmia in Cannabidiol Treated Zebrafish Emrbyos

Poster Number: 57

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Nicolas Zuniga** - University of Northern Colorado

Co-Author(s): Andrea James, PhD – Associate Professor, Biology, University of Northern Colorado

Abstract: Cannabidiol (CBD) is a cannabinoid derived from the plant cannabis sativa. Multiple studies on CBD show that it is used as an anti-psychotic, an anti-inflammatory for pain relief, and an antinausea agent. The Agriculture Improvement Act (2018) played a role in removing hemp and hemp derivatives like CBD from being categorized as a Schedule I substance, which led to recreational usage in America. In 2003, there was an increase in cannabinoid consumption during pregnancy to alleviate morning sickness along with distress. Prior studies on CBD-treated zebrafish embryos have demonstrated phenotypes of microphthalmia, scoliosis, fewer neuronal connections, and less motor neuron activity. While previous research identified effects of CBD exposure like microphthalmia, the mechanism behind the reduction in eye size remains unclear. Are there fewer cells, or are they smaller? Zebrafish are utilized as a model organism for early human development. Human and zebrafish have 70% gene similarity for cannabinoid receptors, along with similar cellular signaling and tissue locations of cannabinoid receptors. Using a previous experimental design that observed microphthalmia, we aimed to determine how CBD affects early eye development. [High] CBD-treated embryos had a significant decrease in survival rate when compared to wild-type survival ($p < 0.05$). To determine if a decrease in cell count was associated with microphthalmia, we counted cells using DAPI and measured cell size using phalloidin in [high] CBD-treated versus untreated embryos via confocal microscopy. Confocal images were cropped to equal sizes and adjusted equally for brightness and contrast. Channels were split to show each stain individually for 1 optical micron section. [High] CBD had fewer cells without a decreased cell size, particularly at 4mg/L. It is unclear if this reduction in cell count is due to a lack of migration, increased cell death, or decreased mitosis.

Investigating the role of arhgap18 in ocular development

Poster Number: 58

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Samuel Thompson** - Medical College of Wisconsin

Co-Author(s): Sanaa Muheisen – Medical College of Wisconsin; Linda Reis – Ophthalmology – Medical College of Wisconsin; Elena Semina – Ophthalmology – Medical College of Wisconsin

Abstract: Coloboma is an ocular condition where the optic fissure fails to close resulting in a gap in the ocular tissue(s). Coloboma is frequently associated with microphthalmia (small eye) or anophthalmia (absence of eye). Diagnostic rates for microphthalmia, anophthalmia, and coloboma (MAC) are currently only 15-30%, highlighting the need to identify novel factors for improved diagnosis. We identified a homozygous premature termination variant in ARHGAP18 in a 6-year-old female with congenital coloboma involving the iris, choroid and optic nerve and no other health issues. ARHGAP18 encodes GTPase-activating protein for RhoA that is known to act as a downstream effector of YAP1, which is associated with colobomatous microphthalmia in humans. The zebrafish genome has a single ortholog of human ARHGAP18; the two proteins show high identity suggesting functional conservation. This study is aimed to characterize the phenotypic outcome of arhgap18 deficiency in zebrafish, with a focus on eye development. We hypothesized that zebrafish homozygous for a loss-of-function variant of arhgap18 will display ocular features consistent with MAC spectrum. A zebrafish line carrying a nonsense variant in arhgap18, (c.541C>T, p.Gln180*), was obtained from ZIRC. Expression of arhgap18 in the embryonic eye and other developing systems was validated via in situ hybridization. Examination of progeny produced by heterozygous crosses did not identify a clear structural phenotype in either heterozygous or homozygous animals; however, homozygous embryos were more likely to have smaller eyes. Overall, homozygous and heterozygous fish demonstrated normal survival and maturation into adults. Since arhgap18 had been previously reported as a maternally provided transcript, additional experiments are underway to generate and examine homozygous mutants lacking any arhgap18 mRNA. ARHGAP18 represents a strong candidate for human MAC phenotype. Identification of MAC features in a zebrafish model of arhgap18 deficiency will further support its possible role in human disease.

Characterization of a retinoic acid response element controlling cx43 expression and skeletal patterning

Poster Number: 59

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Alexander Seaver, PhD** - Lehigh University

Co-Author(s): Xinzhao Li – Undergraduate Researcher, Biological Sciences, Lehigh University; Kathy Iovine – Professor, Biological Sciences, Lehigh University

Proper patterning of vertebrate skeletal systems is governed by complex and sensitive network of molecular and cellular signals. Zebrafish regenerate amputated fins to restore bone structure and function following injury. Our lab has identified gap junction protein Cx43 as integral to regulating the fate of skeletal precursor cells during regeneration. Depletion of Cx43 gap junctional intercellular signaling results in decreased length of bony fin ray segments due to premature activation of the joint transcription factor evx1 which in turn causes premature joint formation. Indeed, prior findings indicate that oscillations of Cx43 contribute to the stereotypical patterning of alternating segments and joints. Recently, we found that the morphogen retinoic acid (RA) appears to regulate cx43 oscillations during

regeneration. For example, reducing levels of RA synthesis by morpholino KD leads to decreased cx43, increased evx1, and shortened bone segments. To determine if RA regulates cx43 transcription directly, we sought to identify possible retinoic acid response elements (RAREs) near cx43. Using a combination of CiiiDER to identify predicted transcription factor binding sites and aligning these with published ATAC-seq data, we identified a predicted RARE that is accessible during regeneration located ~15 kb downstream of cx43. Deletion of this putative RARE leads to shortened segments, decreased cx43, and increased evx1. Continuing work includes testing these RARE deletion mutants for responsivity to alterations in RA signaling by injecting exogenous RA and inhibiting RA synthesis with pharmacological agent WIN18446. Additionally, we plan to test the responsivity of this putative RARE by luciferase assay in cultured cells. By identifying this element, we present previously undescribed evidence that RA acts directly on cx43 levels. While it is not the sole regulator of cx43 expression, deletion of this RARE is strong enough to induce a skeletal patterning phenotype, further establishing a cell-fate role of RA signaling during regeneration.

Defining the Role of Histone H2B Monoubiquitylation in Early Embryonic Cell Cycle Regulation and Totipotency

Poster Number: 60

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Jackson Wilborn** - University of Utah/Huntsman Cancer Institute/HHMI

Co-Author(s): Graham Hickey – University of Utah/Huntsman Cancer Institute/HHMI; Aneasha Whittaker-Tademy – University of Utah/Huntsman Cancer Institute/HHMI

Abstract: Following fertilization, metazoan embryos undergo extensive epigenetic reprogramming to establish totipotency—the capacity to generate all cell types from a single-cell zygote. This critical developmental window coincides with zygotic genome activation (ZGA), when the embryo transitions from maternal to embryonic control of gene expression. Intriguingly, several canonical histone post-translational modifications (PTMs) are notably absent prior to ZGA, prompting our investigation into which chromatin marks are present, their genomic distribution, and their functional contributions to early development. We propose a model in which a histone monoubiquitylation dichotomy plays a key role in gene expression regulation and gene class partitioning up through ZGA. Previous work found H2AK119ub as a critical mark for designating developmental genes and repressing their expression until canonical bivalent chromatin marks appear. Our current data suggest that H2BK120ub marks housekeeping genes by ZGA. Surprisingly, our preliminary evidence also suggests that H2BK120ub may be necessary as early as the first blastomere division and essential for embryonic viability. Disruption of maternally deposited rnf20 and rnf40—the E3 ligases responsible for H2BK120ub—leads to immediate and persistent cleavage defects, underscoring a critical maternal effect. Our results raise the possibility that H2BK120ub could be necessary for the unique cell cycle demands of cleavage-stage embryos in either mitosis or DNA replication. Ongoing work employs novel approaches, including CUT&Tag, genetically-encoded affinity reagents, and high-sensitivity DNA replication assays, to resolve the spatial and functional dynamics of H2BK120ub during these formative stages.

Zebrafish with different rates of postembryonic development have different intestinal microbiota

Poster Number: 61

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Samia Sadaf, Master's Degree** - Department of Biology

Co-Author(s): Pijush sutradhar, Master's Degree – Graduate Position, Department of Biology, Clarkson University

Abstract: During embryonic zebrafish development, fish develop at a constant rate when raised at the same temperature and time can be used to stage fish. However, as zebrafish transition into the postembryonic stage, they develop at different rates even when kept in similar conditions. We suggest that there are observable differences between post embryonic fish passing through developmental stages at different rates. Fish during this period obtain energy from digestion of food within the intestine and we hypothesize that some of the differences in rate of development may be due to differences in the digestive system. We have observed differences in intestinal motility, microbiome, and inflammation within the intestine between fish growing at a faster compared to slower rates during the first weeks of the post embryonic period. Regardless of the rate that the fish are growing, we find that when fish reach the previously characterized stage of Standard Length (SL) 5.5, they begin growing at the same rate and will reach an adult stage within two weeks. We suggest that after SL 5.5, the digestive system undergoes maturation that enables the fish to better regulate the internal environment, more consistently obtain energy from ingested food, and grow at a consistent rate. Funding from National Institutes of Health (NIH), Eunice Kennedy Shriver National Institute of Child Health and Human Development, Grant: 1R15HD108689-01.

Retinoic acid signaling pathway members are present in cranial tenocytes inhabiting the zebrafish lower jaw

Poster Number: 62

Theme: Early Development, Morphogenesis and Patterning

Presenting Author: **Alia Segura** - University of Texas at Austin

Co-Author(s): Johann Eberhart – University of Texas at Austin

Abstract: Retinoic Acid (RA) is a key signaling pathway with various roles across embryonic development. RA embryopathies encompass a multitude of birth defects resulting from an excess or lack of maternal diet-derived RA exposed to fetuses. Craniofacial malformations have been identified due to the loss of functioning members from the cyp26 and aldh families required in degrading and synthesizing RA, respectively. Our lab has identified muscle and tendon defects in zebrafish branchial region resulting from a mutation in cyp26b1. In humans, loss of RA synthesizing and catabolizing genes have demonstrated clinical relevance with hearing loss and craniofacial defects identified in individuals carrying CYP26B1 variants. Detailed analyses of craniofacial defects due to teratogenic and suboptimal doses of RA are necessary to understand the underlying disease mechanisms. The RA pathway members

present during tendon condensation are currently unknown. To identify the source RA in the lower jaw, I performed in situ hybridization for known RA synthesizing genes aldh1a2, aldh1a3 and aldh8a1. I found that aldh1a2 was expressed by a larger population of cranial tenocytes prior to their organization and was spatially restricted over the course of tendon condensation. To identify the RA sink, I analyzed the expression of cyp26a1, cyp26b1 and cyp26c1. I found that cyp26b1 was the main driver of RA degradation in the lower jaw and was expressed by cartilage, mesenchyme and cranial tendons themselves. To determine if tenocytes are receptive to RA signaling, I analyzed the expression of RA receptors and found they were expressed by cranial tenocytes among other tissues in the head. These findings demonstrate that cranial tenocytes dynamically express pathway members essential in RA signaling. Future work will determine the functionality of these pathway members in tendon morphogenesis through phenotypic analysis of CRISPR knockouts and analysis of genetic landscapes in cranial tendons with altered RA signaling.

17β-Estradiol Disrupts Lipoprotein Homeostasis in Zebrafish Larvae

Poster Number: 63

Theme: Reproduction: Germline, Sex Determination & Reproductive Health

Presenting Author: **Hannah Hirsch** - Mass General Brigham and Harvard Medical School

Co-Author(s): Patrice Delaney, PhD – Postdoctoral Fellow, Mass General Brigham and Harvard Medical School; Wolfram Goessling, MD, PhD – Mass General Brigham and Harvard Medical School

During pregnancy, maternal estrogens steadily increase, peaking in the third trimester. This hormonal surge is correlated with several liver-specific pathologies, including acute metabolic dysfunction-associated steatotic liver disease and intrahepatic cholestasis of pregnancy, both of which typically emerge in late gestation and resolve postpartum. Although clinical correlations are well established, the causal link between estrogen and liver-mediated lipid metabolism and packaging remains unclear—largely due to the confounding physiological changes that occur during pregnancy. The underlying molecular mechanisms by which estrogen disrupts hepatic function are still not well understood. To investigate how excess estrogen alters lipid dynamics, we used the LipoGlo zebrafish model fus(ApoBb.1-nluc), in which endogenous apolipoprotein B (Apo-B), a key structural component of lipoprotein assembly in hepatocytes, is fused to luciferase. This system enables real-time, high sensitivity quantification of liver derived lipid particles and distribution in vivo. We found that chronic exposure to 5 µM 17β-estradiol (E2) during development significantly decreased Apo-B expression by 0.63-fold in the liver and 0.65-fold in the carcass at 5 days post fertilization. Notably, this reduction in liver mediated lipid packaging is not simply the result of a non-functional liver, as live imaging of hepatic glycoprotein glycosylation was increased in E2 treated tg(fabp10a:Gc-EGFP) fish. Additionally, live imaging of the fus(ApoBb.1-Dendra2) zebrafish model, which expresses a Dendra2-tagged endogenous Apo-B, revealed that E2 treatment alters Apo-B distribution, reducing lipid particle presence in the liver and causing aberrant accumulation in the tail region. Taken together, this work demonstrates that excess estrogen disrupts hepatic packaging and systemic distribution of apolipoprotein B–containing lipid particles, highlighting a direct hormonal modulation of lipid trafficking pathways that may underlie pregnancy-associated liver dysfunction. Future work will focus on comparing chronic, developmental versus acute exposure of mature livers to E2 to differentiate the effects of developmental and functional exposure on the liver.

Investigating the role of Kif2c in transforming the microtubule cytoskeleton at the oocyte-to-embryo transition

Poster Number: 64

Theme: Cell Biology

Presenting Author: **Allison Marvin** - University of Pennsylvania

Co-Author(s): Ricardo Fuentes – Universidad de Concepción; Mary Mullins – University of Pennsylvania

Abstract: The oocyte-to-embryo transition encompasses several developmental events that engage with transformations of the microtubule (MT) cytoskeleton. Two such events are 1) oocyte maturation, when the prophase-arrested oocyte resumes meiosis, completing meiosis I until arresting in metaphase II, and 2) egg activation, when the dormant egg initiates processes crucial for early embryogenesis. We identified a zebrafish maternal-effect mutant that reveals maternal-specific control of MT organization in the oocyte and egg. During egg activation, offspring of mutant females display numerous ectopic aster-like MTs throughout the cytoplasm and subsequently fail to complete the first cell division. We identified the mutated gene as kif2c, a kinesin-13 family MT depolymerase also called mitotic centromere-associated kinesin (MCAK). Previous studies have shown chromosome alignment defects and ectopic MT assembly upon loss of kinesin-13. We found that the ectopic asters in mutant embryos arise during oocyte maturation, as part of the recently discovered phenomenon of cortical maturation asters in zebrafish. I found that cortical maturation asters do not dissociate at the end of oocyte maturation in kif2c mutant oocytes and instead persist into the egg. My results further indicate that Kif2c functions during a second period at egg activation to regulate MT dynamics. Thus, we hypothesize that Kif2c is precisely regulated to control cortical maturation asters during oocyte maturation before depolymerizing MTs at egg activation. Zebrafish represents a powerful tool to uncover novel maternal functions of Kif2c, and together with cutting edge live imaging and chemical dimerization tools, these studies will reveal novel mechanisms governing cytoskeletal transformations at the oocyte-to-embryo transition.

Macrophage Ca2+ transients and their role in pro-inflammatory activation

Poster Number: 65

Theme: Infection & Immunity

Presenting Author: **Jordan Munos** - Medical College of Wisconsin

Co-Author(s): Simon Glabere – Department of Cell Biology, Neurobiology & Anatomy – Medical College of Wisconsin; Pui-Ying Lam – Assistant Professor, Department of Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin

Abstract: When injury occurs, macrophages are among the first responders and associate with the wound for the entirety of the healing process. Ca2+ is thought to regulate a wide variety of immune cell functions. It however remains unclear what the functional role of Ca2+ signaling is in the macrophage wound response in vivo. Danionella cerebrum (DC) is a close relative to zebrafish which remains transparent throughout life. We generated transgenic DC expressing GCaMP6s specifically in

macrophages and performed live confocal imaging of macrophage Ca2+ signaling during the injury response. We found that wounding results in macrophage migration to the injury site where cells exhibit a robust and repeating cycle of increasing and decreasing levels of intracellular Ca2+, which persists for hours after injury. The frequency and duration of these transients do not align with previous reports focused on Ca2+ signaling in macrophages engaged in chemotaxis and phagocytosis, suggesting an unreported role for Ca2+ signaling during an injury response. Of the two major sources for Ca2+, extracellular and the endoplasmic reticulum (ER), inhibition of different Ca2+ permeable channels reveals that ER sources of Ca2+ play a major role in sustaining the observed transients. Inhibiting Ca2+ permeable channels on the ER membrane results in a reduction of TNFα expression, a well-known marker of pro-inflammatory activation, suggesting that Ca2+ transients play a role in macrophage activation.

A Q system-based reporter reveals zebrafish picornavirus infection dynamics in vivo

Poster Number: 66

Theme: Infection & Immunity

Presenting Author: **Jared Nigg** - Chan Zuckerberg Biohub

Co-Author(s): Shiloh Kluding – Chan Zuckerberg Biohub; Jennifer Doherty – Chan Zuckerberg Biohub; Keir Balla – Chan Zuckerberg Biohub

Abstract: Zebrafish picornavirus (ZfPV) is a naturally infecting virus of zebrafish that is prevalent in laboratory settings worldwide. In contrast to most viral infection models that have been established in zebrafish, research with ZfPV presents the possibility to examine co-evolved host-virus interactions in a natural setting. Although many tools exist to study host-virus interactions during ZfPV infection, methods to identify infected cells in vivo are lacking. To address this, we developed a transcription-based reporter system to drive expression of fluorescent proteins in ZfPV-infected cells. Here we describe a Q system-based reporter construct in which the chimeric transcription factor QFGal4 is tethered to a transmembrane anchor via a linker containing a cleavage site for the ZfPV-encoded 3C protease. Viral protease activity liberates QFGal4 in ZfPV infected cells, enabling its translocation to the nucleus, where it activates transcription of target genes. We initially tested this reporter system in transient assays by injecting reporter construct mRNA at the single-cell stage into transgenic embryos harboring GFP under the control of a QFGal4 binding site. GFP expression was specifically induced by co-injection of the reporter construct with purified ZfPV, ZfPV RNA, or mRNA encoding the ZfPV 3C protease. Natural ZfPV infections in larvae and adults are believed to be asymptomatic, with no clear pathology in infected tissues. In contrast, our reporter assays reveal that ZfPV infections initiated at the single-cell stage result in high rates of viral replication, yolk degradation in developing embryos, and severe developmental abnormalities in larvae. Together our findings demonstrate the effectiveness of a transcription-based reporter for detecting viral protease activity in vivo and uncover novel aspects of ZfPV infection dynamics.

Neutrophils disrupt the intestinal barrier via IL-22/TGF-β/Mmp9 axis in the zebrafish model of inflammatory bowel disease

Poster Number: 67

Theme: Infection & Immunity

Presenting Author: **Yiqing Yang** - South China University of Technology

Co-Author(s): Peixian Huang – South China University of Technology; Junwei Lian – South China University of Technology; Tao Yu – Shenzhen Peking University-the Hong Kong University of Science and Technology Medical Center; Gaofei Li – South China University of Technology; Yiyue Zhang – South China University of Technology

Abstract: Inflammatory bowel disease (IBD) is characterized by an abnormal inflammatory response against commensal bacteria, which is associated with a marked increase in neutrophil counts within the colonic mucosa. However, the precise role and mechanisms underlying neutrophil involvement in IBD remain elusive. Here, we utilize zebrafish as the model to explore the roles of neutrophils in IBD. Our findings indicate that Interleukin-22 (IL-22) is significantly upregulated in the intestine after dextran sodium sulfate administration, facilitating the enrichment and activation of neutrophils. Further analysis reveals that the transforming growth factor-β (TGF-β) pathway is activated in intestinal neutrophils, which is critical for intestinal barrier disruption during IBD. Moreover, we identify that neutrophil-derived Mmp9, induced by the TGF-β signal pathway, exacerbates colitis severity. This study demonstrates a distinct pattern of neutrophil enrichment and activation in inflammatory responses associated with IBD, emphasizing the critical role of IL-22 in modulating neutrophil activation via the TGF-β signaling pathway. These insights enhance our understanding of neutrophil-related pathophysiology and could inform the development of targeted therapies for IBD.

Deficiency of Trif Weakens Innate Immune Responses Against VHSV in Zebrafish

Poster Number: 68

Theme: Infection & Immunity

Presenting Author: **Hyerim Ha** - Department of Marine Life Sciences & Center for Genomic selection in Korean Aquaculture, Jeju National University

Co-Author(s): K.P Madushani – Marine Life Research Institute, Kidang Marine Research Institute – Jeju National University; Sumi Jung – Marine Life Research Institute, Kidang Marine Research Institute – Jeju national university; Jehee Lee – Department of Marine Life Sciences & Center for Genomic selection in Korean Aquaculture – Jeju National University

Abstract: Infectious diseases are a growing problem in aquaculture, especially viral diseases with no effective treatment. Prevention is the only effective approach. To support this, studying immune-related genes in fish is essential. Zebrafish are widely used in disease research due to their genetic similarity to humans. As a teleost, they are also ideal for fish immunity studies. In this study, we created a knockout zebrafish model to investigate the function of an immune gene. Our target gene was trif (TIR-domain-containing adapter-inducing interferon-β), which is an adaptor molecule of the TLR3 signaling pathway and plays a key role in antiviral immunity. To assess its function, we focused on VHSV (Viral Hemorrhagic Septicemia Virus), a major viral threat in aquaculture. First, in silico analysis and tissue distribution analysis were performed to identify the basic characteristics of the trif gene in zebrafish.

Afterwards, we established a trif knockout zebrafish line to evaluate its in vivo function. Using this model, we performed VHSV challenge experiments and compared clinical symptoms, viral load, and survival rates between WT and trif-/- zebrafish. Furthermore, gene expressions were analyzed to confirm the effects of trif deficiency on innate immunity. As a result, trif-/- zebrafish showed more severe symptoms, higher viral load, and lower survival rates than WT. In addition, the expression levels of inflammatory cytokines, antioxidant gene, and immune cell-related markers were generally reduced in trif-/- zebrafish. Additionally, to visualize viral infection, we used recombinant VHSV, expressing green fluorescence. in an injury immersion model. This confirmed that the virus dissemination speed was faster in trif-/- zebrafish. This series of studies demonstrated that Trif deficiency impairs TLR3-mediated antiviral signaling, making the host more susceptible to viruses, and that Trif plays an important role in innate immunity and antiviral defense in fish.

Elucidating the Role of MAVS in Antiviral Immune Responses Using a Zebrafish knockout Model

Poster Number: 69

Theme: Infection & Immunity

Presenting Author: **Sumi Jung** - Marine Life Research Institute, Kidang Marine Research Institute, Jeju National University

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Abstract: The RIG-I-like receptor (RLR) family recognizes viral RNA during virus infection, mediates innate antiviral immunity, and regulates the inflammatory response to infection. The mitochondrial antiviral signaling protein (MAVS) is a key component of the RLR pathway, which is activated when retinoic acid inducible gene-I (RIG-I) or melanoma differentiation gene-5 (MDA5) recognizes viral RNA, mediates antiviral immune responses, and promotes the production of antiviral cytokine such as interferon. In this study, we established Mavs knockout (KO) zebrafish using the CRISPR/Cas9 system and performed virus infection experiments to analyze the impact of Mavs deficiency on immune responses to viral infection in vivo. Analysis of mavs expression in zebrafish revealed that at 24 hours post-fertilization (hpf), expression was concentrated in pronephric duct and later expanded to the liver and gut. In adult zebrafish, mavs mRNA expression was highest in the liver, a major immune organ in vertebrates, suggesting that Mavs plays a critical role in antiviral defense. Using the CRISPR/Cas9 system, we designed sgRNA targeting the sequence in exon 4 of the mavs gene and established a Mavs KO zebrafish with an 11-bp deletion at the target site, resulting in a premature stop codon. In the viral hemorrhagic septicemia virus (VHSV) infection experiment using WT and Mavs KO zebrafish (larvae and adults), the Mavs KO zebrafish exhibited higher mortality and increased virus replication in both larvae and adult zebrafish. Analysis of downstream genes expression following VHSV infection revealed that pro-inflammatory cytokines and interferons were upregulated from early phase in WT zebrafish, whereas their responses were attenuated in the early phase of virus infection in Mavs KO zebrafish, failing to support effective virus clearance. Collectively, our research emphasizes the pivotal role of the Mavs in

antiviral defense mechanisms, providing valuable insights into its essential function in the respond against viral infections.

Characterizing Virally-Induced Interferon Signaling in Zebrafish

Poster Number: 70

Theme: Infection & Immunity

Presenting Author: **Shiloh Kluding** - Chan Zuckerberg Biohub

Co-Author(s): Keir Balla – Chan Zuckerberg Biohub; Deepika Sundarraman – Chan Zuckerberg Biohub

Abstract: Interferons (IFNs) are a diverse group of secreted cytokines that activate antiviral immune responses in vertebrates. Despite their known importance, the spatio-temporal patterns of IFN expression, tissue specificity, and the functional roles of IFN signaling in host-virus interactions have not been thoroughly characterized. As the most species-rich vertebrate group, fish offer additional context to expand our understanding of the induction, function, and evolution of antiviral immunity. The optical transparency and genetic tractability of the zebrafish model uniquely enables live-imaging of immune activity at the organismal scale with single-cell resolution. We previously generated transgenic zebrafish that expresses green fluorescent protein (GFP) under the control of an interferon-stimulated gene promoter (isg15:GFP) and observed seemingly spontaneous isg15 expression that led us to discover a naturally occurring zebrafish picornavirus (ZfPV) primarily infecting the gut. Leveraging this novel host-virus model to investigate IFN functionality and specificity during homeostasis versus viral invasion, we interrogated the expression patterns of all zebrafish IFNs and found that ifnphi1 and ifnphi4 were differentially expressed during ZfPV infection. Subsequently, overexpression of ifnphi1 elicited immune stimulation in most tissues of uninfected isg15:GFP zebrafish larvae, whereas ifnphi4 overexpression elicited isg15 expression that was restricted to mucosal tissues, revealing a tissue specific element in specific IFN-mediated immune responses. By combining imaging and sequencing modalities and harnessing co-evolving viruses as tools of immune perturbation, we hope to reveal how specific IFNs spatially and temporally restrict viral infections – such as ZfPV – to defined tissues, balancing the protective and pathological effects of immune activation during zebrafish development and infection.

Behavioral responses to viral infection in larval zebrafish

Poster Number: 71

Theme: Infection & Immunity

Presenting Author: **Claire Tobin** - University of Utah

Co-Author(s): Hailey Hollins – University of Utah; Mai Tran – University of Utah; Bradley Cutler – Ohio State University; Martin Haesemeyer – Ohio State University; James Gagnon – University of Utah

Abstract: Animals exhibit a variety of sickness behaviors including lethargy, anorexia, and warmth-seeking. These behaviors are not mere side effects of sickness, but important adaptations that fight infection. For example, generating a fever by seeking warmth can enhance the innate immune response to infection and improve survival. Fever has been used by warm-blooded and cold-blooded animals to fight infection for over 600 million years. Cold-blooded animals rely on heat-seeking behaviors, such as

swimming to warmer water or moving into the sun, to increase their body temperature and fight infection. This is called behavioral fever. Although behavioral fever was first described over 50 years ago, we know little about the underlying mechanisms coordinating behaviors that fight infection. I aim to understand how immune signaling choreographs behavioral responses that fight infection. In this project, we demonstrate that larval zebrafish infected with zebrafish picornavirus-1 (ZfPV1) exhibit behavioral changes, including behavioral fever. We found that ZfPV1 infection causes larval zebrafish to seek warmer temperatures, generating a behavioral fever of over two degrees Celsius. Additionally, single-cell RNA sequencing data indicates that ZfPV1 infection induces a robust immune response in larval zebrafish that includes interferon signaling and immune cell activation. We are currently characterizing how behavioral modifications can fight ZfPV1 infection and hope to understand how immune signaling drives this response.

Mylpf is produced near its saturation point for promoting myofibril growth in the zebrafish embryo

Poster Number: 72

Theme: Quantitative Biology

Presenting Author: **Tayo Adekeye** - University of Maine

Co-Author(s): Maddison Coffin – university of maine; Emily Teets – The Ohio State University; Emily Tomak – university of maine; Angelina White – university of maine; Daniel Tanaka – university of maine; Sadie Waterman – university of maine; Sharon Amacher – The Ohio State University; Joshua Kelley – university of maine; Jared Talbot – university of maine

Abstract: Muscle cells grow stronger by assembling sarcomeres, the fundamental contractile unit, which function together in myofibrils. Little is known about how myofibril growth is controlled. Abundance of contractile genes and proteins has been proposed to regulate growth; however, it remains unclear how changes in their transcript and protein levels correspond to phenotypic effects in vivo. Here, we investigate how the dosage of Myosin-Light-Chain-Phosphorylatable-Fast (Mylpf) impacts myofibril growth. In zebrafish, Mylpf is encoded by two paralogs, mylpfa and mylpfb, which are expressed only in fast-twitch muscle. We generated mutant alleles in both genes that cause full loss of protein production without causing compensation in the opposite gene. Using these alleles, we show that Mylpf function is essential to sarcomere and myofibril formation, failing in the mylpfa-/-;mylpfb-/- double mutant at the step where myosin-rich thick filaments colocalize with actin-rich thin filaments. The single mutants exhibit starkly different muscle phenotypes. Whereas the mylpfb-/- mutant develops normally, the mylpfa-/- mutant can form myofibrils at only one-third the wild-type width. Correlating with the strong mylpfa-/- mutant phenotype, mylpfa mRNA and protein are expressed more abundantly than mylpfb throughout embryonic development; six times higher 24 hours post-fertilization (hpf), and two times higher by 72 hpf. We can accurately (R2=0.94) model phenotypic severity by the protein abundance in each genotype using a logistic regression, which predicts function to be near the saturation point in wild-type. Consistent with this model, transgenic expression of mylpfa-GFP, mylpfb-GFP or human MYLPF-GFP efficiently rescues mylpfa-/- mutants along a linear trendline, but do not increase myofibril size in wild-type siblings. These findings suggest that Mylpf expression levels are just past the saturation point needed for efficient myofibril assembly in the zebrafish embryo, where loss (but not gain) strongly

affects phenotype. This research was funded by NIH grants GM117964, R15AR081019, R15GM140409, and 1P20GM144265.

Single-cell characterization of cell fate decisions in a ribosomopathy model

Poster Number: 73

Theme: Reproduction: Germline, Sex Determination & Reproductive Health

Presenting Author: **Arish Shah, PhD** - University of Heidelberg

Co-Author(s): Frieda Leesch – IMP, Vienna; Laura Lorenzo-Orts – IMP, Vienna; Lorenz Grundmann – IMP, Vienna; Maria Novatchkova – IMP, Vienna; David Haselbach – IMP, Vienna; Eliezer Calo – Massachusetts Institute of Technology; Andrea Pauli – IMP, Vienna

Ribosome biogenesis is essential for all proliferative cells, yet disruptions in this universal process often lead to strikingly lineage-specific developmental phenotypes. In vertebrates, mutations in ribosome biogenesis factors frequently impair cranial neural crest cell (cNCC) derivatives, while sparing mesodermal tissues. Using a zebrafish model lacking polr1a, the catalytic subunit of RNA polymerase I, we examine how differential lineage requirements for ribosome production shape craniofacial development. Despite a global reduction of rRNA transcription in polr1a-CRISPant embryos, mesoderm-derived cartilage persists while cNCC-derived cartilage is entirely lost. Single-cell RNA sequencing of wildtype and polr1a CRISPants reveals widespread activation of the p53 stress response across lineages, but apoptotic gene signatures and loss of differentiation trajectories are specific to neural crest–derived mesenchyme.

Intriguingly, inhibition of apoptosis in the polr1a CRISPants fails to rescue cartilage production, indicating that cNCC failure is not due to apoptosis alone. At 24 hours post fertilization, cNCC-derivatives in polr1a CRISPants are transcriptionally distinct from wild type counterparts, revealing an uncoupling between modules involved in differentiation potential and other cell type-specific functions under conditions of ribosome insufficiency. Future comparative analysis with medaka, which has different maternal ribosome provisioning, will inform how early ribosome abundance buffers lineage-specific sensitivity to zygotic ribosome loss. Our results reveal that the coordination between ribosome biogenesis and cell fate is not uniform across embryonic lineages, and that cNCCs are uniquely dependent on sustained ribosome capacity to execute cartilage differentiation. This work defines a molecular framework for understanding how growth and fate are coupled during vertebrate craniofacial development and provides a developmental rationale for the tissue-specificity of ribosomopathies.

Breaking Down the Balbiani Body and Germplasm: An Endogenous Structure-Function Analysis of the Buc Protein

Poster Number: 74

Theme: Reproduction: Germline, Sex Determination & Reproductive Health

Presenting Author: **Megan Guerin** - University of Pennsylvania

Co-Author(s): Manami Kobayashi – Postdoctoral Researcher, Cell and Developmental Biology, University of Pennsylvania; Mary Mullins – Principal Investigator, Cell and Developmental Biology, University of Pennsylvania

Most vertebrate oocytes are polarized by the Balbiani body (Bb), a conserved aggregate of maternal RNAs, proteins, and membranous organelles. During early oogenesis, the Bb recruits and asymmetrically localizes RNA-protein granules required for embryonic germline specification and axial patterning at the cortex, thereby polarizing the oocyte and establishing the animal-vegetal axis. Deposited RNA-protein granules at the vegetal cortex are transported towards the blastomeres upon fertilization, and inheritance of these maternal factors are sufficient to specify primordial germ cell fate and induce the signaling center that mediates dorsal-ventral patterning throughout gastrulation. In a maternal-effect forward genetics screen, our lab isolated null alleles of the zebrafish buc gene, which is the only known gene to be required for Bb formation. Mutant oocytes lack animal-vegetal polarity, do not form the Bb and therefore cannot asymmetrically localize RNA-protein granules at the cortex, resulting in embryonic lethality. The buc locus encodes a disordered protein with a prion-like domain (PrLD) in the N-terminus, which is conserved in the Xenopus homolog (Xvelo). Mutating aromatic residues in the PrLD of the full-length Xvelo protein reduces Bb localization, but does not abolish it. It has been demonstrated that disordered regions in other granule-organizing proteins positively influence granule phase-separation within the cytoplasm. I will address whether disordered regions also contribute to Bb localization and aggregate assembly. This project aims to understand the role of disordered regions in Buc protein self-aggregation and Bb assembly during early zebrafish oogenesis. I am utilizing TALENS and the CRISPR-Cas9 system to endogenously remove disordered regions from a V5-tagged buc allele generated in our laboratory. I will report on my characterization of the V5-buc allele, novel V5-tagged buc alleles that I generate, and my initial analysis regarding Bb assembly and germplasm aggregation in these mutant lines. This research is supported by NIH NIGMS award number R35GM131908.

3D Zebrafish Microanatomical Atlas as an Integrative Research Resource

Poster Number: 75

Theme: Emerging Technologies

Presenting Author: **Khai Ang, PhD** - Penn State College of Meicine

Co-Author(s): Jean Copper – Penn State College of Medicine; Mee Ngu – Penn State College of Medicine; Jessica Christ – Penn State College of Medicine; Alex Lin – Penn State College of Medicine; Sayed Reza – Penn State Harrisburg; Daniel Vanselow – Penn State College of Medicine; Keith Cheng – Penn State College of Medicine

Abstract: Whole-organism phenotyping is vital for understanding how genetic and environmental factors determine normal and disease phenotypes, and atlas resources that integrate unbiased “-omics” and tissue morphology would facilitate this goal. The pathology of cells and their arrangements remain fundamental endpoints in a wide variety of fields that require the study of gene function and organismal toxicity. To achieve more comprehensive insight, there is a compelling need to integrate multi-imaging technologies and molecular “-omics” data on a single atlas that enables unbiased phenotypic interrogation across all cell types. While traditional 2D histology provides subcellular detail, its inherent limitations include its destructive, two-dimensional perspective, and is prone to sampling artifacts,

which restrict insights into the 3D structure and organization of cells within tissues. To overcome these limitations, we propose to add a microCT component to atlases that will add 3D representations of cells and organs, allowing researchers to interrogate the spatial relationships between the structures and digital exploration of microanatomy in virtual environments. MicroCT images will serve an integrative role, anchoring histology, fluorescence, and ultrastructural imaging, and large-scale “-omic” data across length scales. We imaged zebrafish at an unprecedented combination of 5 mm field-of-view (FOV) with 0.5 μm isotropic resolution at Lawrence Berkeley National Laboratory. Data are presented using the 4-planar viewer (sagittal, transverse, coronal, and 3D), Neuroglancer. Segmentation of the organ of interest can be done and incorporated into the 3D file for visualization. By incorporating molecular and morphological data within a unified, organism-wide context, this 3D atlas platform enables multi-scale interrogation of how genetic variation and environmental perturbations drive phenotype. The tools of the 3D zebrafish atlas resource are being created as a foundation for future applications across model organisms and human atlases to anchor research and educational efforts to increase understanding of biological structure, disease, and development.

Efficient Knock-In Approaches for High-Precision Genome Editing in Zebrafish

Poster Number: 76

Theme: Emerging Technologies

Presenting Author: **Anjelica Rodriguez-parks, M.S.** - University of Wisconsin-- Madison

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Abstract: Precise genome editing remains a major challenge in functional genomics, particularly for generating knock-in (KI) alleles in model organisms. Here, we present the mini-Golden system, a versatile Golden Gate-based subcloning platform that enables rapid assembly of a donor construct containing homology arms and a gene of interest. This system includes a library of middle entry vectors, enhancing the preparation of donor minicircles for KI applications. Using the mini-Golden system, we efficiently generated a foxd3CreER KI zebrafish line, enabling conditional recombination in neural crest cells. To further improve genome editing precision, we developed a synthetic exon-based donor template strategy combined with fluorescence-based screening to introduce a single amino acid substitution into the zebrafish genome. Using this approach, we successfully engineered a targeted Ile-to-Val substitution in hbaa1.2, one of the two adult hemoglobin alpha genes in zebrafish. This strategy minimized undesired recombination and significantly improved the identification of lines carrying edited genome. Together, these approaches provide a robust toolkit for efficient and precise genome engineering in zebrafish, with broad applicability to other model systems.

MIC-Drop-seq: Scalable single-cell phenotyping of mutant vertebrate embryos

Poster Number: 77

Theme: Emerging Technologies

Presenting Author: **James Gagnon** - University of Utah

Co-Author(s):

Abstract: Pooled perturbation screens can reveal cellular regulatory networks, yet scaling these techniques for large-scale screens in animals remains challenging. To address this, we developed MIC-Drop-seq, which combines high-throughput CRISPR gene disruption in zebrafish embryos with phenotyping by multiplexed single-cell RNAseq. In one MIC-Drop-seq experiment, we simultaneously identified changes in gene expression and cell abundance across 74 cell types resulting from loss of function of 50 transcription factors. These observations recapitulated many known phenotypes, while also uncovering novel functions in brain and mesoderm development. A key advantage of whole-animal screens is that they reveal how changes in one cell type affect the development of other cell types. Surprisingly, such cell-extrinsic phenotypes were abundant, indicating that transcription factors frequently exert effects beyond the cells where they are expressed to adjacent cells. We propose that MIC-Drop-seq will facilitate efforts to dissect the complete gene regulatory networks that guide animal development.

Non-Destructive Larval Genotyping of Danio rerio for Mitochondrial DNA Genetics

Poster Number: 78

Theme: Emerging Technologies

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

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Abstract: The rapid advancement of nuclear and mitochondrial genomic editing tools has created an urgent need for efficient, non-lethal larval genotyping methods in zebrafish (Danio rerio) research. This study optimizes and validates a non-destructive proteinase K digestion method for mitochondrial DNA genotyping while characterizing its impact on larval survival and gene expression. Using optimized protocol parameters, we demonstrate successful amplification of different mitochondrial genetic loci with consistently high sensitivity. Molecular validation through PCR, restriction fragment length polymorphism analysis, and Sanger sequencing confirmed the specificity and reliability of the extracted DNA, while RNA sequencing analysis revealed no significant transcriptional differences between genotyped and unshaken control larvae, indicating minimal physiological impact. The method successfully detected C-to-T base edits in the mt-tl1 gene introduced using the FusX TALE Base editor system, demonstrating its applicability to gene editing studies. Both 48-well and optimized 96-well formats were used, enabling this approach to be deployed at scale. This optimized method enables researchers to correlate genotypes with phenotypes in longitudinal studies while maintaining specimen

viability. It is particularly valuable for investigating early-onset mitochondrial diseases and utilizes standard laboratory equipment and reagents, facilitating widespread adoption in zebrafish research while adhering to ethical principles in reducing animal mortality.

Genetically Encoded Affinity Reagents (GEARs) uncover spatiotemporal requirements of higher-order genome organization during embryogenesis

Poster Number: 79

Theme: Emerging Technologies

Presenting Author: **Curtis Boswell** - Developmental and Stem Cell Biology Research Program, The Hospital for Sick Children and Department of Molecular Genetics, University of Toronto

Co-Author(s): Caroline Hoppe – Yale University School of Medicine; Liyun Miao – Yale University School of Medicine; Mina Kojima – Yale University School of Medicine; Antonio Giraldez – Yale University School of Medicine

Abstract: Probing endogenous protein localization and function in vivo remains challenging due to limitations with gene targeting in certain model organisms and once established, the inflexibility of precision targeted alleles. To overcome these challenges, we developed a toolkit called Genetically Encoded Affinity Reagents (GEARs), which leverages affinity-based reagents—such as nanobodies and single-chain variable fragments (scFvs)—along with their cognate binding tags to rapidly probe and manipulate protein function in zebrafish. Using a streamlined protocol for installing short GEARs epitope tags into genes of interest, we generated multiple knock-in lines that take advantage of this system. Notably, we demonstrate that these tagged alleles enable visualization of native protein behavior, functional perturbation via degron GEARs to recapitulate loss-of-function phenotypes, and interrogation of protein interaction networks using proximity-based identification methods. Most notably, maternal protein erasure can be effectively performed using this system that can recapitulate maternal-zygotic phenotypes, particularly in situations where germline transplantation fails. To illustrate the utility of GEARs, we applied this methodology to study the genome architecture regulator CTCF during zebrafish development. Our findings reveal a critical role for maternal CTCF in establishing chromatin competency for neural development, as well as unexpected genomic consequences resulting from its loss. Together, the GEARs system provides a rapid and versatile means to interrogate protein function and can provide novel insights to maternal proteins and their roles in embryogenesis. This work is supported by the Canadian Institutes of Health Research (CIHR).

Circadian effects in optogenetic experiments: Considerations for investigating FGF-BMP signaling interactions with light

Poster Number: 80

Theme: Emerging Technologies

Presenting Author: **Micaela Murphy** - NICHD | NIH

Co-Author(s): Velanganni Selvaraj Maria Thomas, PhD – Post-doctoral fellow, NICHD, NIH; Katherine Rogers, PhD – Principal Investigator, NICHD, NIH

Abstract: Signaling gradients direct distinct spatial gene expression patterns during gastrulation. To investigate the relationships between signaling input and gene expression output, we recently established a zebrafish-optimized suite of blue-light-responsive (455 nm) optogenetic (bOpto) FGF, BMP, and Nodal signaling activators. bOpto tools are an attractive strategy to manipulate signaling because they enable wavelength-dependent, pathway-specific activation with fast on/off kinetics. However, blue light exposure could affect circadian gene activity, confounding interpretation of optogenetic experiments. Here, using bulk RNA sequencing and hybridization chain reaction fluorescence in situ hybridization (HCR-FISH), we found that a subset of circadian-associated genes is modestly upregulated by short exposures to 455 nm or 495+ nm light in gastrulating zebrafish embryos. We therefore recommend including blue-light-exposed controls in optogenetic experiments. However, as we have not observed notable changes in developmental signaling or morphology following light exposure, we are now applying these optogenetic tools to investigate interactions between BMP and FGF signaling. In response to an optogenetically-generated FGF signaling pulse, we observed downregulation of a subset of BMP target genes ("class I"), whereas other BMP target genes were unaffected ("class II"). This is surprising because known mechanisms for FGF-mediated inhibition of BMP signaling should downregulate all BMP target genes. Our observations are consistent with endogenous BMP target gene expression patterns, where class II genes (and not class I) are expressed in a region with high FGF signaling. Our working hypothesis is that a transcriptional repressor activated by FGF signaling differentially interacts with BMP target genes: Class I genes are transcriptionally inhibited but class II are not. To investigate the mechanisms behind this gene-specific inhibition, we are now using single nucleus multiomics (ATAC/RNAseq) to evaluate changes in BMP target gene expression and chromatin accessibility following optogenetic FGF signaling activation.

Funding: NIH Intramural ZIAHD009002-01 to KWR

Developing an aspartate sensor for in vivo visualization of metabolic changes during cell lineage differentiation

Poster Number: 81

Theme: Emerging Technologies

Presenting Author: **Carl Berggren** - University of Rochester

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Abstract: Aspartate plays numerous critical roles within cells, serving as the major nitrogen donor in nucleotide synthesis as well as acting as a key intermediate in central metabolic pathways such as the urea and TCA cycles. Aspartate biosynthesis has also been shown to represent a metabolic vulnerability for cancer cells. Furthermore, aspartate plays a vital role in the healthy function of neurotransmitters, and its dysregulation has been linked to neurodegenerative disorders like Alzheimer's disease. As such, monitoring aspartate metabolic flux in vivo represents a critical tool for researchers to interrogate its role in both physiological and pathological processes. In this work, we adapt the genetically encoded fluorescent biosensor jAspSnFR3 for in vivo visualization of aspartate concentrations. The sensor

integrates into the genome through the Tol2 transposon system and is expressed under the control of a ubiquitous promoter. To establish a more robust baseline expression, the sensor has been codon optimized for use in zebrafish. We also present two complementary approaches to test the functionality of the sensor through either the pharmacological inhibition or induction of aspartate biosynthesis in zebrafish embryos. Finally, we propose an approach to express the sensor in a cell-specific manner through the utilization of mpeg and lmo2 Cre-drivers. Together, this work demonstrates the potential for future uses of the jAspSnFR3 metabolic sensor for in vivo monitoring of aspartate concentration changes in diverse developmental and disease conditions.

High-throughput multi-camera array microscope platform for automated 3D behavioral analysis of freely swimming zebrafish larvae

Poster Number: 82

Theme: Emerging Technologies

Presenting Author: **Kevin Li** - Duke University

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Abstract: Understanding the behavioral and morphological dynamics of freely moving zebrafish larvae requires accurate, high-throughput 3D analysis. However, traditional single-view, 2D video tracking fails to capture the full scope of their natural 3D movements and postural dynamics. Here, we present a novel high-throughput 24-camera array microscope with a plug-and-play mirror well plate for snapshot imaging of up to 48 wells over a 118 x 82mm field of view from two orthogonal directions to enable accurate and high-throughput 3D estimation. Integrated with advanced machine learning algorithms, our approach automates parallel 3D behavioral analysis, providing 3D skeletal tracking, swim bladder morphological dynamics, and kinematics of freely swimming zebrafish larvae. Our approach provides an efficient, scalable solution for high-throughput 3D behavioral studies with broad compatibility with standard workflows across laboratories and procedures, facilitating reproducible large-scale research in pharmacology, toxicology, and neuroscience

Zebrafish Community Cre/lox Resource zebrafishccr.org

Poster Number: 83

Theme: Emerging Technologies

Presenting Author: **James Preston** - Iowa State University

Co-Author(s): Fang Liu – Postdoctoral Fellow, Iowa State University; Astha Tuladhar – Iowa State University; Tessa Bierbaum – Iowa State University; Lakpa Sherpa – Iowa State University; Karl Clark – Texas A&M; Stephen Ekker – University of Texas at Austin; Iddo Friedberg – Iowa State University; Jeffrey Essner – Iowa State University; Maura McGrail – Iowa State University

Abstract: The mission of the Zebrafish Community Cre/lox Resource is to provide the Zebrafish community with Cre resources that open new areas of investigation and promote novel discoveries in human health, development, and disease. With CRISPR/Cas9 precision targeted integration, we can generate Cre knock-ins and floxed alleles in any gene of interest, unlocking previously inaccessible cell lineages and cell type specific gene studies to broaden the scope and impact of Zebrafish research. With our GeneWeld CRISPR knock-in technology we've isolated endogenous Cre and tamoxifen regulated-CreERT2 lines for spatial and temporal analysis of proneural, mesoderm, endoderm, vascular, blood and hematopoietic stem cell lineages. We're currently generating floxed conditional alleles in genes that regulate the epigenome and mitochondrial function, rapidly emerging areas essential to development, disease, and regeneration. Floxed alleles allow simultaneous gene inactivation and mRFP labeling for live imaging of mutant cells. Visit our website at zebrafishccr.org to find out more about our KI technology, available lines, lines in progress, inspiring images, and a user friendly web page for placing line orders. The Zebrafish Community Cre/lox Resource is funded by the National Institutes of Health Office of Research Infrastructure Programs NIH R24 OD020166 and NIH R24 OD036201.

High-throughput fluorescence heart imaging of freely swimming zebrafish larvae

Poster Number: 84

Theme: Emerging Technologies

Presenting Author: **Jennifer Bagwell** - Duke University

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The measurement of zebrafish heart rates has proved to be an important tool for studying cardiovascular diseases and advancing drug discovery, as well as a crucial indicator for fish health during experimental procedures. However, current imaging methods such as mounting and anesthesia may inadvertently affect both the fish's heart rate and long-term health. Other methods are restricted to early embryonic stages in which the fish are still in their chorion. Combining our 48-camera array microscope with machine learning algorithms, we introduce a high throughput method to track and segment freely swimming zebrafish from early embryonic to juvenile stages. This large field of view setup allows users to rapidly image up to 48 zebrafish at a time in a standard 96 well plate or dish and perform semi-automatic fluorescence analysis on the segmented zebrafish. We imaged temperature, time, and drug trials of cmlc2:GCaMP zebrafish to demonstrate the effectiveness of our method, with notable results showing the distinct effects the stimuli had on the heart rates. Our robust free swimming tracking capabilities enables novel long term in vivo fluorescence imaging experiments, allowing for new developmental questions to be analyzed.

A Fluorescence Integrated Zebrafish Larvae Screening Platform for Whole Body Imaging

Poster Number: 85

Theme: Emerging Technologies

Presenting Author: **Giuliano Ferrero** - Union Biometrica

Co-Author(s): Yongwoon Kim – Union Biometrica; Deborah Frenkel – Union Biometrica; Nate Portier – Union Biometrica; Francis Smet – Union Biometrica; Yifei Wang – Union Biometrica; Mariya Lomakina – Union Biometrica; Tom Mullins – Union Biometrica; Bogan Chris – Union Biometrica

Abstract: Traditional techniques of preparing zebrafish larvae for imaging involve mounting them in agarose and manually rotating them to the desired viewing angle for microscopy. This process can be time consuming and tedious. Consistency is often dependent on the scientists' dexterity and focus. The throughput rate then limits the type and scale of possible experiments. The VAST BioImager is a high throughput screening platform capable of automatically handling and rotating larvae to desired positions and angles at a rate of 1 larva every 1.0 - 1.5 min at its fastest. A limitation of this system is the lack of fluorescence imaging. Typically, this is dealt with by combining the VAST BioImager with a fluorescent microscope. However, there are difficulties latent in integrating these two systems together such as microscope platform and software compatibility, and hardware constraints which can restrict activating or changing fluorescence filters to be a manual process rather than an automatic one.

Improving on the previous design of the VAST BioImager, zebrafish larvae can now be imaged with automated 3 color fluorescence plus brightfield and a 2.5x lens, enabling an all-in-one whole body imaging platform. This type of platform can be used to screen large numbers of zebrafish larvae as well as to evaluate any fluorescence properties across the entire length of the body. Rapid, repeatable rotations enable high throughput generation of optical projection tomography datasets (3D volumes of regions of interest). This whole-body imaging platform can then be used in a number of ways such as assaying development in an environmental contaminant screen or tracking cancer xenograft recession after exposure to different drugs.

Identifying optimal conditions for precise knock-in of exogenous DNA into the zebrafish genome

Poster Number: 86

Theme: Emerging Technologies

Presenting Author: **Sarah Oikemus, phd** - UMASS Chan Medical School

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Abstract: CRISPR nucleases can be used to insert exogenous DNA into the zebrafish genome via homology-dependent repair (HDR), although germline transmission rates for precise edits remain quite low. Comparative studies to optimize HDR parameters for introducing base pair changes using short-read deep sequencing have been successful, but similar analysis for insertions is challenging due to read length constraints. Here, we quantified editing outcomes using long-read sequencing to identify optimal template and CRISPR parameters for precise targeted insertion in zebrafish. Through side-by-side

comparisons, we found that chemically modified templates out-perform those released in vivo from a plasmid, while Cas9 and Cas12a nucleases performed similarly for targeted insertion. Consistent with previous studies, precise editing rates were dependent on the distance between a double-strand break and the inserted sequence. We further found that non-homologous base pairs in homology templates significantly reduced precise editing rates. Using optimized parameters, we consistently achieved germline founder rates of greater than twenty percent for precise insertions across four loci. Together, our quantitative analyses identified optimal conditions for precise insertion of exogenous DNA into the zebrafish genome

An optogenetic toolkit to dynamically manipulate FGF, BMP, & Nodal signaling in vivo

Poster Number: 87

Theme: Emerging Technologies

Presenting Author: **Katherine Rogers, PhD** - National Institutes of Health (NICHD)

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Abstract: Signaling regulates many biological processes including development, homeostasis, and disease. Molecular optogenetic technologies that use light to manipulate signaling can provide precise experimental control and have applications in both basic and applied research. We systematically characterized a zebrafish-optimized optogenetic toolkit to activate FGF, BMP, and Nodal signaling and are using it to study developmental patterning. Pathway-specific receptor kinase domains fused to blue light-homodimerizing LOV domains ectopically activate signaling in response to ~455 nm light exposure, but not in the dark or at wavelengths above 495 nm. Using immunofluorescence staining for phosphorylated signaling effectors, we found that signaling on/off kinetics are rapid (minutes) and tool-specific, with FGF signaling activation occurring faster than Nodal activation. Signaling strength depends on light irradiance and is also tool-specific: The FGF activator requires lower light dosages for activation than the BMP or Nodal activators. We further demonstrate spatially localized FGF signaling activation in gastrulation-stage zebrafish with the FGF activator. We also developed a transgenic zebrafish line containing the FGF activator driven by a ubiquitous promoter, and demonstrate blue light-mediated signaling activation up to 72 hours post-fertilization. For tissue-specific experiments, we additionally generated a UAS line. We are using this optimized toolkit to investigate 1) how patterning information is encoded in spatial signaling gradients, and 2) how genes convert signaling levels, durations, and combinations into diverse expression profiles during development. Funding: NIH Intramural ZIAHD009002-01 to KWR.

A simple but effective immobilization method for Zebrafish Larvae, Agarose Stamped Device with Customizable 3D Printed Molds for Danio rerio (Zebrafish)

Poster Number: 88

Theme: Emerging Technologies

Presenting Author: **Hossein Mehrabi, PhD** - University of Illinois Chicago

Co-Author(s): Erica Jung – University of Illinois Chicago; John Jutoy – University of Illinois Chicago

Abstract: Precise immobilization of zebrafish larvae is critical for high-resolution neural imaging and behavioral studies, yet conventional methods are often labor-intensive, low-throughput, and can induce stress. To address these challenges, we introduce the Agarose Stamped Device (ASD), a novel platform enabling efficient and gentle immobilization of zebrafish larvae for high-resolution imaging and behavioral assays. By employing a stamping approach to mold an array of consistent agarose wells, the ASD enables rapid, parallel, and reproducible positioning of larvae in a standardized orientation while minimizing stress and preserving viability. We validated ASD performance by comparing larval survival, heart rate, and imaging stability to conventional agarose embedding, finding no adverse effects on larval physiology and significantly enhanced imaging throughput and data consistency. Demonstrations in neuroscience and behavioral experiments underscore the device's versatility for diverse in vivo studies. The ASD offers a simple, cost-effective, and powerful tool for advancing zebrafish-based neuroscience and behavioral research.

An overexpression screen to identify genes involved in alternative-end joining and single-strand annealing

Poster Number: 89

Theme: Emerging Technologies

Presenting Author: **Tessa Bierbaum** - Iowa State University

Co-Author(s): TJ Cofer – Genetics Development & Cell Biology – Iowa State University; Wes Wierson – Genetics Development & Cell Biology – Iowa State University; Maura McGrail – Genetics Development & Cell Biology – Iowa State University; Jeff Essner – Genetics Development & Cell Biology – Iowa State University

Abstract: Double-strand DNA (ds-DNA) breaks can be repaired by various mechanisms, which are utilized for precision genome editing techniques such as CRISPR-Cas9. Previous research has shown that ds-DNA break repair can create gene knockouts by non-homologous end joining (NHEJ). However, NHEJ is error-prone compared to other forms of ds-DNA break repair by causing nucleotide insertions and deletions independent of a template. Homology-directed repair (HDR) is a less error-prone DNA repair mechanism that uses a DNA template for more precise repair. Two ds-DNA break repair mechanisms are alternative end joining (alt-EJ) and single-strand annealing (SSA), which share a variety of proteins for repairing DNA. While alt-EJ is not as well characterized as other DNA repair methods, it is known that this mechanism is the primary form of DNA repair during early embryogenesis in zebrafish and other organisms. Our lab previously generated a broken RFP reporter line at the noto locus that repairs RFP expression via direct repeats using alt-EJ/SSA HDR at a CRISPR/Cas9 cut site. Following the generation of a DNA double-strand break at this site, repair can occur via NHEJ/alt-EJ and HDR mechanisms. However, only repair utilizing the direct repeats via HDR will generate an RFP signal in the notochord. With this line, we conducted an overexpression screen with genes encoding DNA repair enzymes to identify those influencing alt-EJ/SSA repair at a DNA double-strand break. Preliminary results show that increased

expression of MRE11, a nuclease that detects double-strand breaks as part of the MRN complex, decreased DNA repair by 54.1 percent. This screen aims to identify genes that significantly impact DNA repair when overexpressed to improve our understanding of DNA repair mechanisms in early embryos. Additionally, this screen aims to identify factors that would increase the efficiency of precision genome editing.

Optimized donor plasmids for efficient recovery of cell type-specific transgenic driver lines via CRISPR/Cas9 integration

Poster Number: 90

Theme: Emerging Technologies

Presenting Author: **Krishan Ariyasiri** - Dartmouth College

Co-Author(s): Jung-Hwa Choi – Center for Genome Engineering, Institute for Basic Science, Daejeon, Republic of Korea; Ji Cheng – UMass Chan Medical School, Worcester, MA; Cuixin Lai – 1. Geisel School of Medicine at Dartmouth, Hanover, NH; Emma Spikol – Chan Zuckerberg Biohub Network, San Francisco, CA; Marnie Halpern – 1. Geisel School of Medicine at Dartmouth, Hanover, NH

Abstract: CRISPR/Cas9 technology has revolutionized genome editing, enabling precise and efficient genetic alterations. Importantly, targeted integration into specific genes capitalizes on their cis-regulatory elements, bypassing the need for isolating promoters to generate transgenic lines for cells of interest. Binary transcriptional regulatory systems, such as Gal4/UAS and QF/QUAS, increase the versatility of transgenic lines. We have generated cell type-specific driver lines with presumably less toxic Gal4 or QF variants (Gal4ff or QF2, respectively) using CRISPR/Cas9-mediated integration by non-homologous end joining (NHEJ) or microhomology-mediated end joining (MMEJ) methods. These approaches can have drawbacks, such as unwanted inclusion of plasmid backbone into transgenes, reduced expression from constructs integrated with 2A peptide sequences, and lack of an early marker to indicate successful integration. We therefore introduced several modifications to Gal4ff and QF2 donor plasmids to increase integration efficiency and ease of screening for transgenic founders (F0). For the NHEJ donor plasmid, a second bait sequence was added to eliminate vector DNA upon Cas9-mediated cleavage, as well as a secondary marker consisting of a promoter derived from the hatching enzyme 1, tandem duplicate 1 (he1.1) gene driving a fluorescent reporter gene. We replaced the 2A peptide sequence on the MMEJ donor plasmid with the basal promoter of heat shock cognate 70-kd protein, like (hsp70l), and also incorporated a reporter that labels hatching gland cells. The he1.1 secondary reporter enables reliable detection of integration events and identification of transgenic embryos as early as 24 hours post-fertilization (hpf), facilitating screening for potential founders in the F1 generation and maintaining transgenic lines. Moreover, since labeling from the he1.1 promoter declines after 72 hpf, it does not interfere with confocal imaging. Our overall goals are to minimize variation in transgene expression and streamline the generation of cell type-specific driver lines.

Improvement of the versatile QF/QUAS binary expression system and their applications in zebrafish

Poster Number: 91

Theme: Emerging Technologies

Presenting Author: **Jae-Geun Lee** - Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea

Co-Author(s): Jeongkwan Hong – Department of Biological Sciences, College of Bioscience and Biotechnology, Chungnam National University, Korea; Jeong-Soo Lee – Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Korea; Hyunju Ro – Department of Biological Sciences, College of Bioscience and Biotechnology, Chungnam National University, Korea

Abstract: The QF/QUAS binary system, which originated from a quinic acid-sensing system of Neurospora crassa, is composed of a QF transcriptional activator and QF-responsive element (QUAS). Though the QF binary system has been shown to function in various organisms for basic and biomedical research, several intrinsic limitations of the system including high toxicity of the QF protein have hampered its direct application in vertebrates. We newly developed a more intuitive and reliable QF variant(QFDBD-2xAD*-VP16*, hereafter EQ-On) having lower cellular toxicity. We also modified EQ-On by fusing it with a hormone-binding domain of the insect ecdysone receptor(EcR)(QFDBD-2xAD*-VP16*-EcR, hereafter IQ-Switch). IQ-Switch can be regulated by a safe chemical inducer tebufenozide, allowing precise control of transgene expression in a spatiotemporal manner. We used EQ-On and IQ-Switch to generate two proof-of-concept models in zebrafish: a tissue-specific cell ablation/regeneration model and a RASopathy disease model. In both cases, we were able to achieve precise control of transgene expression using the EQ-On and IQ-Switch systems. Our results suggest that EQ-On and IQ-Switch are versatile tools for controlling transgene expression in zebrafish. These systems could be used to study a variety of biological processes in zebrafish, including development, disease, and regeneration.

An Updated LipoGlo Protocol for the Characterization of ApoB-Containing Lipoproteins in Zebrafish

Poster Number: 92

Theme: Emerging Technologies

Presenting Author: **Monica Hensley** - JHU

Co-Author(s): Steven Farber – Biology – JHU

Abstract: Apolipoprotein B-containing lipoproteins (ApoB-LPs) transport lipids throughout the circulation and are closely associated with cardiovascular disease in humans. While cardiovascular disease is the leading cause of death worldwide, many aspects of ApoB-LP cell biology remain elusive. To characterize ApoB-LP quantity, size and in vivo localization from a single zebrafish larvae we generated the LipoGlo reporter system utilizing a luciferase enzyme fused to the C-terminus of the zebrafish ApoBb.1 protein (a key structural element of ApoB-LP). Each lipoprotein contains one ApoB molecule, making the luminescence emitted proportional to the total number of ApoB-LPs. In our original publication, we observed that homozygous LipoGlo zebrafish emitted twice as much luminescence as heterozygous animals, but this 2-fold increase was not consistently observed over the years. We tested whether the attenuated assay performance was due to premature depletion of LipoGlo substrate, furimazine, during the assay. We found that using 10x less tissue homogenate restores assay performance. ApoB-LPs come

in a variety of sizes, with chylomicrons being the largest and low-density lipoproteins (LDL) being the smallest. The LipoGlo system can differentiate and quantify the different ApoB-LP size subclasses by running tissue homogenates on a 3% native polyacrylamide gel electrophoresis (PAGE). Previously, we used a commercially available DiI-labeled human LDL as a migration marker, yet found variation in the migration of the DiI-LDL, perhaps due to batch variation. To address this, we used tissue homogenates from ldlrasd52 mutants that are enriched in small LDL particles, and apoC2sd38 mutants that have high levels of large VLDL particles, in an orthogonal density gradient ultra-centrifugation (DGUC) assay to differentiate small and large particle fractions. Importantly, the DiI-LDL marker and ldlrasd52 ApoB-LPs consistently appear in the same DGUC fraction. In sum, these LipoGlo protocol improvements provide a powerful platform for studying ApoB-LPs in zebrafish.

Suppression of Base Excision Repair Boosts HDR-Dependent Knock-In Efficiency Mediated by CRISPR-Cas9n in Medaka

Poster Number: 93

Theme: Emerging Technologies

Presenting Author: **Takashi Yamanaka** - Graduate School of Agriculture, Kyoto University

Co-Author(s): Satoshi Ansai – Ushimado Marine Institute, Okayama University; Masato Kinoshita – Graduate School of Agriculture, Kyoto University

Abstract: Medaka is a small teleost fish with external fertilization and transparent embryos, making it a useful model for developmental and disease studies. Moreover, the availability of genomic data from various wild-derived strains enables a wide range of studies, including those on genetic diversity, gene function, and comparative genomics . While gene knockouts via genome editing have become routine, efficient and precise knock-ins (KIs) using homology-directed repair (HDR) remain challenging in medaka. Previous studies have demonstrated that using CRISPR-Cas9 nickase (Cas9n) improves KI efficiency in medaka with reduced embryonic damage compared to conventional Cas9 methods (Murakami et al., 2020, DGD). However, few approaches have aimed to enhance HDR activity itself. In this study, we proposed a novel strategy to suppress base excision repair (BER), a DNA repair pathway that competes with HDR, and assessed its impact on KI efficiency. Using CRISPR-Cas13d, we knocked down parp1, a key BER component, at the mRNA level in early embryos. This led to a significant reduction in parp1 transcript levels at 5.5 and 12 hours post-fertilization. However, no significant difference was detected at 24 and 48 hours, suggesting that the knockdown effect was transient. Subsequently, we performed GFP knock-in at the acta1 and pdia6 loci. The parp1 knockdown significantly increased the proportion of GFP-positive embryos (acta1: 46.2% → 66.3%, pdia6: 1.3% → 29.9%). Furthermore, in the SK2 strain lacking melanophores due to a mutation in splicing donor site of slc45a2, a two-base insertion to revive pigmentation was more efficiently performed with parp1 knockdown than without (9.1% → 31.4%). Our results demonstrate that transient suppression of BER can substantially enhance HDR-mediated KI efficiency. This approach holds promise for advancing genome editing technologies in fish and can be applicable to aquaculture species and other vertebrates.

Zebrafish Embedding Mold (ZEM) for high-quality imaging of zebrafish embryo and larvae

Poster Number: 94

Theme: Emerging Technologies

Presenting Author: **Jinyoung Jeong, PhD** - Korea Research Institute of Bioscience and Biotechnology

Co-Author(s): Yugyeong Sim – KRIBB

Abstract: Biological imaging is an essential tool for observing and analyzing biological phenomena, offering valuable insights into various biological processes. In this context, zebrafish, due to their transparency, are widely used as model organisms for observing phenotypes and biological responses. However, maintaining a consistent orientation for multiple specimens during imaging is challenging and requires significant time and labor. In this study, we designed an embedding mold for zebrafish and developed a standardized fixation system using agarose gel to facilitate high-quality and reproducible imaging of zebrafish embryos and larvae. For embryo mold, we designed a half-sphere shape with 36 chambers, each capable of holding a single embryo. This embryo mold enables the observation of developmental stages up to 48 hours post-fertilization. The larval mold supports imaging of specimens aged 3-7 days post-fertilization in both lateral and dorsal orientation. This imaging tool allows for systematic and precise analysis of developmental and phenotypic changes over time. Furthermore, we exposed zebrafish to 50 nm polystyrene nanoplastics and visualized fluorescence signals across different concentrations from embryos and larvae, demonstrating the potential of this imaging platform for high-throughput screening of target substances. This system can be used into pharmacological and toxicological studies involving environmental stressors, such as chemical compounds, heavy metals, nanoparticles, and microplastics thereby providing effective experiment conditions for obtaining reliable and reproducible datasets. Additionally, the standardized imaging bed manufacturing system developed in this study enables continuous high-throughput experimental workflows. This imaging tools enhances imaging accuracy, experimental efficiency, and reproducibility, making it applicable to zebrafish-based biomedical and toxicological research on zebrafish.

3D Whole-organism Connectomics Using Submicron, Centimeter-scale Histotomography

Poster Number: 95

Theme: Emerging Technologies

Presenting Author: **Keith Cheng, MD, PhD** - Pennsylvania State University

Co-Author(s): Mahmut Kandemir – Professor, Computer Science and Engineering, Pennsylvania State University; Khai Chung Ang – Assistant Professor, Pathology, Penn State College of Medicine; Alex Lin – Assistant Professor, Pathology, Penn State College of Medicine; David Northover – Bioinformatics and Genomics PhD candidate, Pathology, Penn State College of Medicine; Andrew Sugarman – MD/PhD candidate, Pathology, PSU Information Sciences and Technology, Penn State College of Medicine; Dan Vanselow – Visualization Specialist and Image Informatician, Pathology, Pennsylvania State University; Sharon Huang – Professor, Information Sciences and Technology, Pennsylvania State University; Roger Hanlon – Senior Scientist, Bell Center, Marine Biological Laboratory; Patrick La Riviere – Professor, Radiology, University of Chicago

Abstract: The idea of making whole-organism phenotyping computational and inclusive of all organ systems and cell types is driven by the fact that all living things are made of cells, that all pathological process are reflected in micron-scale change, and by recent and coming advances in computational and imaging technology. More complete understanding of gene function, and knowledge of the full range of potentially harmful and beneficial effects of chemicals will both benefit from quantitative whole-organism phenotyping. We used the zebrafish develop a 3D form of histology, x-ray histotomography that has isotropic submicron voxel resolutions for centimeter-wide organisms. This form of imaging, x-ray histotomography, was based on tomographic projections of fixed and metal-stained, and is now possible for unstained, paraffin-embedded organisms. Whole-organism histotomography addresses phenotyping challenges including undersampling, subjectivity, lack of measurability, and lack of ground truth for histopathological analysis. 3D whole organism images were created using 5 and 10 mm field-of-view (FOV) systems with submicron, isotropic resolution, in collaboration with investigators at U Chicago, Lawrence Berkeley National Laboratory, Argonne National Laboratory, and Marine Biological Laboratory. A Computational Phenomics Consortium is creating and segmenting whole zebrafish, Daphnia, and a centimeter-wide octopus for distribution through atlas resources and the creation of new state-of-the-art synchrotron and local instrumentation. From one 2.6 TB reconstruction of a centimeter-wide Octopus bimaculoides, every organ system was segmented, with full web-based access to histology-like multiplanar images at any plane of “section”, 3D segmentations, and combined visualizations inclusive of the whole organism “zoomable” to any region of interest. The whole-organism nervous connectome includes newly discovered pathways. Democratization of this technology will enable large-scale 3D genetic and chemical phenome projects to advance our understanding of cells and tissues in organismal context, and how genes, environment, and disease determine phenotype.

A Chemical-Genetic Screening Platform in Zebrafish Identifies GPRC6A-Mediated Non-Genomic Androgen Signaling

Poster Number: 96

Theme: Emerging Technologies

Presenting Author: **Vahid Zadmajid** - Baylor College of Medicine

Co-Author(s): Shayan Shahriar – Baylor College of Medicine; Daniel Gorelick – Baylor College of Medicine

Abstract: Chemical-genetic screening is a powerful approach for dissecting complex signaling pathways in vivo. Here, we present a zebrafish-based screening platform that integrates CRISPR-Cas9 mutagenesis, small-molecule exposure, and transcriptomic profiling to uncover non-canonical androgen signaling mechanisms during early embryonic development. Exposure of wild-type zebrafish embryos to 5α-Androstane-3,17-dione (androstanedione), dihydrotestosterone (DHT), and testosterone induced distinct cardiac morphogenesis defects, including atrial elongation and incomplete heart looping. Remarkably, androgen receptor (ar) mutant embryos displayed similar cardiac phenotypes following exposure to these androgens, suggesting the involvement of ar-independent pathways. To identify the causative receptor, we used the CRISPR F0 screening pipeline and targeted genes encoding integral membrane proteins known to bind androgens in vitro, but for which there was little or no evidence of androgen receptor activity in vivo. We exposed gprc6a, hcar1-4, or zip9 (slc39a9) mosaic mutant embryos (crispants) to androstanedione, DHT or testosterone and found that hcar1-4 and zip9

crispants developed cardiac morphogenesis defects following exposure. In contrast, we observed a significant reduction in cardiac morphogenesis defects in the gprc6a crispants compared to the wild-type embryos following testosterone treatment. Co-treatment with GPRC6A antagonists similarly suppressed cardiac defects in wild-type embryos, confirming receptor specificity. These results suggest that testosterone causes cardiac phenotypes in zebrafish embryos by acting via the integral membrane protein GPRC6A, independently of nuclear androgen receptors. To identify downstream effectors, we conducted RNA-seq and functional rescue experiments. Transcriptomic analysis revealed that GPRC6A-mediated testosterone signaling downregulates pak1, a kinase critical for heart development. Overexpression of pak1 mRNA partially rescued the cardiac phenotype, supporting a mechanistic link between GPRC6A signaling and cardiac morphogenesis. Our study provides insights into non-genomic androgen signaling during embryonic development and identifies GPRC6A as a key receptor mediating androgen action.

Optimizing prime editing to engineer zebrafish harboring human disease variants

Poster Number: 97

Theme: Emerging Technologies

Presenting Author: **Gina Rizzo** - Stony Brook University

Co-Author(s): Rehman Basharat – Stony Brook University; Josiah Zoodsma – Stony Brook University; Lonnie Wollmuth – Stony Brook University; Howard Sirotkin – Stony Brook University

Abstract: Creating zebrafish lines harboring strong loss of function mutations with CRISPR has become routine. These lines have proven to be valuable tools to investigate the biological functions of many genes. However, such mutations often fail to capture the complexity of the most common human disease associate variants which are missense mutations. Recently, a novel method called prime editing has been developed to enable precise genome modifications. Prime editing combines a prime editor (PE), consisting of a Cas9 nickase fused to a reverse transcriptase (RT) domain, and a prime editing guide RNA (pegRNA) that includes the spacer sequence that recruits the complex to the target site, a primer binding site (PBS) that allows binding of the pegRNA to the nicked strand, and a reverse transcriptase template (RTT) that encodes the intended edit. Because of broad interest in the approach, improved designs are rapidly emerging. Two of the earliest PEs -- PE2 and PE3 -- have been tested in zebrafish using ribonucleoprotein microinjection. Unfortunately, PE protein is not readily available, and protein purification presents a challenge for many research groups. We have optimized mRNA-based approach that enables rapid testing of the innovative designs are continually expanding the repertoire of PE proteins. Here, we compared the efficiency of five different PEs – PE2, PE6B, PE6C, PEmax, and PE7 – to install five point mutations in the zebrafish genome. Notably, PEmax was the most effective, with precise editing rates as high as 15%. In addition, we found that subjecting injected embryos to hypoxic conditions further increased editing frequencies. These findings demonstrate that mRNA-based PEs – an accessible and cost-effective approach – can be used to efficiently install missense mutations in zebrafish.

Triple Fin-esse! A novel tripartite system confers an enhanced ability to restrict exogenous gene expression in zebrafish

Poster Number: 98

Theme: Emerging Technologies

Presenting Author: **Annalie Martin** - Brigham Young University

Co-Author(s): Allie Dietz – Undergraduate Researcher, Neuroscience, Brigham Young University; Arminda Suli, PhD – Associate Professor, Cell Biology and Physiology, Brigham Young University

Abstract: Expression of exogenous genes in selective tissues can be challenging as it greatly relies on enhancer/promoter availability. We developed a novel system that restricts gene expression to overlapping expression domains of any two distinct enhancer/promoters. We fused the tryptophan receptor DNA binding domain (TrpR) to the bacteriophage λ M protein (λM), and the Gal4 activation domain (Gal4AD) to the bacteriophage λ Fi protein (λFi), which binds to λM. We placed both fusion proteins (TrpR-λM and Gal4AD-λFi) under the control of the neuroD promoter, which is expressed in the CNS and spinal cord, and injected them into one-cell stage zebrafish embryos, along with tUAS:nlsEos. The fluorescent protein Eos should express only when TrpR binds to its operon binding sequence (tUAS), subsequently recruiting Gal4AD through the interaction between TrpR-λM and Gal4AD-λFi. Injection of all three plasmids showed consistent Eos expression in spinal cords of 1dpf zebrafish embryos. To demonstrate gene expression is restricted to overlapping domains, we placed TrpR-λM under the control of the neuroD promoter, and λFi-Gal4AD under the control of the ubiquitin (ubi) promoter, which is expressed in nearly all cells. When both constructs are co-expressed with the tUAS:Eos reporter, Eos is expressed only in the CNS/spinal cord where ubi and neuroD expression domains overlap. Similarly, we see expression is restricted to overlapping areas when TrpR-λM and λFi-Gal4AD are driven by the neuroD and RibA promoters, respectively. This demonstrates the efficacy of our system with distinct promoters and confirms the ability to restrict gene expression based on unique domains, mitigating the need for specific promoters for each area of interest. As the tUAS sequence lacks CpG islands, it has also been shown to resist silencing via methylation over subsequent generations, increasing the long-term utility of this system.

A pipeline to analyze untargeted metabolomics data

Poster Number: 99

Theme: Emerging Technologies

Presenting Author: **Carl Berggren** - University of Rochester

Co-Author(s): Carl Berggren – Department of Biomedical Genetics – University of Rochester Medical Center; Salome Ghvinephadze – Department of Biomedical Genetics – University of Rochester Medical Center; Carter Long – Center for Advanced Research Technology – University of Rochester Medical Center; Xiaolu Wei – Center for Advanced Research Technology – University of Rochester Medical Center; Julie Reisz – Department of Biochemistry and Molecular Genetics – University of Colorado; Angelo D'Alessandro – Department of Biochemistry and Molecular Genetics – University of Colorado;

Phillip Seitzer – Calico Life Sciences, LLC; Paul Brookes – 5Department of Anesthesiology and Perioperative Medicine – University of Rochester Medical Center; Marlies Rossmann – Department of Biomedical Genetics – University of Rochester Medical Center

Abstract: Understanding metabolic controls of hematopoiesis can suggest novel approaches for treating blood cancers. To establish the metabolic profile of the hematopoietic system in zebrafish, we optimized a protocol for untargeted metabolomics on one- and two-day post fertilization embryos using an orbitrap liquid chromatography coupled mass spectrometer (LC-MS) with the ability to resolve peaks separated by 0.0017 Da at a mass to charge ratio of 200. We tested different methods to extract small-molecule metabolites from whole zebrafish embryos, comparing numbers of embryos per sample, different homogenizing beads, and different extraction solvents. In addition, due to the scarcity of simple open-access analysis tools that go from raw data to fold-change metabolite values, we optimized a pipeline for the analysis of untargeted LC-MS data, including MS/MS spectra. Our pipeline combines freely accessible spectral processing software, for which we developed peak-calling parameter optimization tools, with an R based Shiny app for statistical analysis. The Shiny app offers several normalization methods, determines differences between sample groups using Huber weighting and Benjamini-Hochberg false discovery correction, and provides multiple data visualization options. At the same time, we have emphasized making the analysis pipeline transparent and approachable to biologists without specialization in bioinformatics or metabolomics. To test our pipeline, we treated zebrafish embryos with rotenone, an inhibitor of complex I of the electron transport chain, which, among other metabolites, showed lactate and NADH to be upregulated in a dose-dependent manner. We have also applied our pipeline to define the metabolomes of zebrafish larval and adult red blood cells using 100,000 cells. This work provides the community with protocols that can be used in experiments elucidating the metabolic regulation of lineage differentiation processes that could lead to the discovery of new therapeutic targets for various diseases.

Lipid-based transfection for delivery of nucleic acids to zebrafish embryos

Poster Number: 100

Theme: Emerging Technologies

Presenting Author: **Jacqueline Fernandez** - CZ Biohub SF

Co-Author(s): Aslihan Terzi, PhD – CZ Biohub SF; Emma Spikol, PhD – CZ Biohub SF; Tiger Lao – CZ Biohub SF; Adrian Jacobo, PhD – CZ Biohub SF

Abstract: Microinjection, the traditional method for introducing exogenous genetic material into zebrafish embryos, is highly efficient but poses significant technical challenges and limits throughput. To address this, we developed a novel approach to deliver nucleic acids into zebrafish embryos, using Lipofectamine (LTX) for lipid-based transfection. Our protocol is straightforward, easy to follow, and does not require special equipment or technical expertise. We demonstrate efficient and consistent delivery of mRNA into zebrafish embryos with our transfection protocol and we have also tested co-delivery of Tol2 plasmid with Tol2 mRNA for generating stable transgenic zebrafish lines. We observed germline transmission from transfected fish, albeit with low efficiency, and are currently optimizing our protocol to increase the efficiency of DNA/RNA co-delivery. This method provides a new strategy for

nucleic acid delivery in zebrafish and shows great potential for enhancing the throughput and scope of genetic studies.

Building fluorescent sensors to detect cell-cell signaling

Poster Number: 101

Theme: Emerging Technologies

Presenting Author: **Emma Spikol, PhD** - CZ Biohub SF

Co-Author(s): Akilandeswari Balasubramanian, PhD – CZ Biohub SF; Tiger Lao – CZ Biohub SF; Jacqueline Fernandez – CZ Biohub SF; Allyson Quinn Ryan, PhD – CZ Biohub SF; Keir Balla, PhD – CZ Biohub SF; Adrian Jacobo, PhD – CZ Biohub SF

Abstract: To self-organize into tissues and organs, cells coordinate their behaviors by sending and receiving information through signaling pathways. Biosensors that report signaling activity in real time can provide key insights into this process. Unfortunately, traditional transcriptional reporters of signaling activity suffer from a myriad of shortcomings, such as poor kinetics, positional effects, and lack of specificity. A system to overcome such limitations has been reported in C. elegans and consists of an effector protease fused to the endogenous Notch protein and a nuclear-localized protease sensor (1). When Notch signaling is active, the effector localizes to the nucleus and cleaves the sensor, resulting in a quantifiable change in the sensor's subcellular localization. To implement this system in zebrafish, we first tested sensor and effector constructs and found that they function as expected when expressed transiently in zebrafish embryos. However, detection of physiologic signaling activity requires endogenous genomic modification of the corresponding transcription factors, such as Notch. To achieve this, and to adapt the system to other signaling pathways such as Wnt, we are exploring strategies to overcome low CRISPR/Cas9 integration efficiency at multiple genomic loci. Overcoming this technical challenge will enable the development of more accurate and temporally resolved sensors to report intracellular signaling in a developing vertebrate in real time. 1. Shaffer, J. M. & Greenwald, I. SALSA, a genetically encoded biosensor for spatiotemporal quantification of Notch signal transduction in vivo. Dev. Cell 57, 930-944.e6 (2022).

Poster Session II

Themes: Cardiovascular, Disease Models, Organ Formation & Function, Physiology& Metabolism, Stem Cells, and Toxicology, Environmental Biology and Sustainability

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Dissecting the Regulation and Function of microRNA-223 in Early Vascular and Blood Development

Poster Number: 1

Theme: Cardiovascular

Presenting Author: **Dionna Kasper, PhD** - Dartmouth Geisel School of Medicine

Co-Author(s): Elizabeth Jones – Dartmouth Geisel School of Medicine; Carolyn Winston – Dartmouth Geisel School of Medicine

Abstract: The hematopoietic and vascular (i.e., hematovascular) systems are vital to the health of an organism, working seamlessly together to supply all tissues with oxygen, provide routes for gas exchange, and establish circulating cells of the immune system. As the first organ systems to form, understanding the mechanisms that regulate their architecture and functional integration with each other will provide critical insight into the key principles of organ formation. We have identified microRNA(miR)-223 as an ideal candidate to coordinate the development and differentiation of hematovascular tissues. We find that miR-223 is expressed earlier than previously described– during the emergence of all major hematovascular cell types, including primitive and definitive blood cell populations, migrating angioblasts, arterial and venous endothelial cells, and lymphatic progenitors. Analysis of the genomic region upstream of miR-223 across different species revealed several conserved sequence motifs. Among these motifs were binding sites for several key hematovascular transcription factors, suggesting that distinct modes of regulation drive its expression in diverse tissues. To determine whether the predicted transcription factors regulate miR-223 expression, we are conducting a knockdown screen. Thus far, we have identified Foxk1 as novel candidate transcriptional regulator for this miRNA. Phenotypic analysis of miR-223 mutants reveal defects in several hematovascular cell populations that express the miRNA, including progenitors in the lateral plate mesoderm from which blood and vascular cell types arise. From these data, we propose that miR-223 acts as a master regulator to coordinate the formation of hematovascular tissues.

SH2 domain protein E and ABL signaling regulate blood vessel size

Poster Number: 2

Theme: Cardiovascular

Presenting Author: **Surendra Kumar Anand, PhD** - University of South Florida

Co-Author(s): Jennifer Schumacher – Miami University; Zoe Wright – University of South Florida; Diandra Florat – University of South Florida; George Davis – University of South Florida; Saulius Sumanas – University of South Florida

Abstract: Blood vessels in different vascular beds vary in size, which is essential for their function and fluid flow along the vascular networks. Molecular mechanisms involved in the formation of a vascular lumen of appropriate size, or tubulogenesis, are still only partially understood. Src homology 2 domain containing E (She) protein was previously identified in a screen for proteins that interact with Abelson (Abl)-kinase. However, its biological role has remained unknown. Here we demonstrate that She and Abl signaling regulate vessel size in zebrafish embryos and human endothelial cell culture. Zebrafish she mutants displayed increased endothelial cell number and enlarged lumen size of the dorsal aorta (DA) and defects in blood flow, eventually leading to the DA collapse. Vascular endothelial specific overexpression of she resulted in a reduced diameter of the DA, which correlated with the reduced arterial cell number and lower endothelial cell proliferation. Chemical inhibition of Abl signaling in zebrafish embryos caused a similar reduction in the DA diameter and alleviated the she mutant phenotype, suggesting that She acts as a negative regulator of Abl signaling. The DA collapse and blood circulation failure in she mutants is preceded by reduced DA elasticity. ScRNA-seq analysis identified a

set of novel differentially expressed genes in she mutants at a stage prior to DA collapse. SHE knockdown in human endothelial umbilical vein cells resulted in a similar increase in the diameter of vascular tubes, and also increased phosphorylation of a known ABL downstream effector CRKL. These results argue that SHE functions as an evolutionarily conserved inhibitor of ABL signaling and regulates vessel and lumen size during vascular tubulogenesis.

A Novel Zebrafish Model for Studying Galectin-3's Role in Arrhythmogenic Cardiomyopathy and Pathway-based Therapy

Poster Number: 3

Theme: Cardiovascular

Presenting Author: **Giovanni Risato, PhD** - University of Padua

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Abstract: Galectin-3 (LGALS3/GAL3) dysregulation has been identified as a key driver of inflammatory responses in Arrhythmogenic Cardiomyopathy (AC), impacting both desmosomal complex stability and Wnt/β-catenin signaling. AC is a rare inherited heart disease, primarily caused by desmosomal gene mutations, leading to fibro-fatty replacement of the ventricular myocardium. This condition significantly increases the risk of life-threatening arrhythmias and sudden cardiac death, particularly in young individuals and athletes. Disease progression happens in episodic "hot phases," often triggered by physical exercise. To investigate Gal-3's role in AC pathogenesis, we developed and characterized a stable Gal-3 knock-out (KO) zebrafish model. KO larvae exhibited severe cardiac dysfunction, including reduced ventricular contractility, lower ejection fraction, and arrhythmic episodes. Additionally, increased inflammatory cell infiltration and elevated cell death were observed. Ultrastructural analysis of adult zebrafish revealed desmosome disorganization, directly linking Gal-3 deficiency to desmosomal instability. Histological examinations further showed ventricular dilation and myocardial fat substitution. RNA sequencing and immunofluorescence analysis confirmed the upregulation of pro-inflammatory and necroptotic genes in the hearts of adult mutant zebrafish. Notably, genes associated with macrophage motility and inflammatory responses (e.g., MMP12, CCL38, and IL16) were significantly overexpressed. Pharmacological activation of the Wnt/β-catenin pathway, which was downregulated in mutant larvae and adult hearts, improved cardiac function in larvae but had minimal impact on structural recovery in adults, where desmosomal defects persisted at advanced disease stages. Meanwhile, Jak/Stat3 modulation led to slight improvements in ventricular contractility but did not prevent disease progression. In conclusion, these findings highlight Gal-3's crucial role in intercellular adhesion and

inflammatory signaling in AC. Gal-3 depletion alone is sufficient to cause desmosomal disorganization, recapitulating the cardiac abnormalities and disrupted cell signaling seen in human AC. This zebrafish model serves as a valuable platform for studying disease mechanisms and evaluating potential therapeutic strategies to mitigate AC progression.

Early retinoic acid signaling is necessary to produce Wnt signaling in cardiomyocytes that drive atrioventricular valve development

Poster Number: 4

Theme: Cardiovascular

Presenting Author: **Andrew Fernandes** - Cincinnati Children's Hospital Medical Center

Co-Author(s): Joshua Waxman, PhD – Associate Professor, Molecular Cardiovascular Biology, Cincinnati Children's Hospital Medical Center

Abstract: Perturbations of retinoic acid (RA) signaling have been associated with congenital valve defects. Although the earliest role of RA signaling is to restrict cardiomyocyte specification, the requirements for early RA signaling in valve development are poorly understood. Here, we investigated a requirement of early RA signaling on the development of the zebrafish atrioventricular valve (AVV), the mammalian mitral valve equivalent. Inhibition of RA production with DEAB beginning at 3 hours post-fertilization (hpf) and zebrafish aldh1a2 mutants indicated that RA-deficient embryos lack AVVs at 72 hpf, despite having enlarged hearts with intact endocardial lining. Examination of atrioventricular canal (AVC) cardiomyocyte markers (AMHC/VMHC); including bmp4 and tbx2b, showed that the myocardial AVC was expanded in RA-deficient embryos. Despite this expansion in AVC cardiomyocyte markers, endocardial valve markers, including klf2a and notch1b, were lost in RA-deficient embryos, implying that early RA signaling loss affects a factor(s) that signals from the AVC myocardium to the endocardium to promote AVV induction. Canonical Wnt signaling within AVC cardiomyocytes promotes proper AVV development. In contrast to other AVC cardiomyocyte markers, we found that RA-deficient zebrafish embryos lack myocardial wnt2bb expression. Consistent with the loss of wnt2bb expression, we found that RA-deficient zebrafish embryos lack 7XTCF-Siam:GFPWnt reporter expression within AVC cardiomyocytes by 36 hpf. Furthermore, inhibition of canonical Wnt signaling via XAV939 treatment from 32-40 hpf was sufficient to reduce valve size and endocardial cushion cell number. CRISPR-mediated knockdown of wnt2bb recapitulated the loss of Wnt reporter expression within AVC cardiomyocytes and the reduction of endocardial cushion cells by 72 hpf. Altogether, our results suggest that early RA signaling is necessary to establish a population of wnt2bb+ AVC cardiomyocytes that promote appropriate AVV development, which may provide insights into fundamental mechanisms underlying vertebrate congenital valve defects.

Selective Estrogen Receptor Degraders (SERDs) Induce Bradycardia by Modulating Nuclear Estrogen Signaling

Poster Number: 5

Theme: Cardiovascular

Presenting Author: **Sandeep Basu, PhD** - BIDMC-Harvard Medical School

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Abstract: Introduction: Selective estrogen receptor degraders (SERDs) are increasingly used to inhibit ER signaling in patients with ER+ breast tumors. Preliminary safety data from phase-1 trials for oral SERDs in development (giredestrant and camizestrant) have highlighted sinus bradycardia as a common side effect in patients treated with these agents. Whether this bradycardia occurs due to on-target effects on nuclear estrogen receptor (ERα) signaling, the G-coupled estrogen receptor (GPER), or other off-target pathways remains unknown. Aims: We aimed to investigate the molecular mechanisms of SERD-induced bradycardia in an embryonic zebrafish model. Methods: We used a chemical biology approach, coupled with genetic mutation of Gper and the nuclear estrogen receptors Esr1 and Esr2a/Esr2b, to investigate the mechanisms contributing to bradycardia induced by SERDs. Results: Consistent with safety data in patients, wild-type zebrafish embryos exposed to giredestrant and camizestrant exhibited a decrease in heart rate compared to controls, whereas those exposed to SERDs that do not cause bradycardia in patients (fulvestrant and amcenestrant) maintained a normal heart rate. We began by evaluating known regulators of heart rate and found no alteration in thyroid (T3) levels or expression of cardiac conduction genes. Treatment with ER activators protected against SERD-induced bradycardia in zebrafish, suggesting an on-target/off-tissue effect. However, treatment with SERDs did not affect GPER signaling in zebrafish, as assessed by ERK phosphorylation. Concordant with this observation, giredestrant-induced bradycardia was intact in zebrafish with Gper mutation, as well as in zebrafish with mutant Esr2a and Esr2b. However, zebrafish carrying Esr1 mutation were resistant to giredestrant-induced bradycardia, indicating that Esr1 signaling is necessary for the development of this cardiac phenotype. Conclusions: Chemical and genetic studies in zebrafish model indicate that SERD-associated bradycardia is mediated by the Esr1 nuclear estrogen receptor. Future studies in iPSC and mice models will further investigate the mechanisms underlying this effect.

Regulation of Capillary Flow by Sphincter Endothelial Cells

Poster Number: 6

Theme: Cardiovascular

Presenting Author: **Arndt Siekmann** - University of Pennsylvania

Co-Author(s): Jia Kang – University of Pennsylvania

Abstract: Blood vessels distribute oxygen and nutrients to all organs of the body. They are lined by endothelial cells (ECs) that ensure proper vessel size. In the human disease condition hereditary hemorrhagic telangiectasia (HHT) blood vessel sizes and flow are impaired. Some blood vessels become enlarged, while other shrink, leading to an imbalance of blood flow distribution. HHT is caused by mutations in components of the transforming growth factor beta (TGFb) pathway, such as Alk1 (acvrl1) or Endoglin (eng). We discovered a new EC population that required Alk1/Endoglin signaling for their formation. These cells are located at the entry points of capillary side branches emanating from the

dorsal aorta. We therefore named them sphincter ECs. Sphincter ECs mutant for either acvrl1 or eng enlarge, causing capillaries to widen and carry excess flow. When we analyzed sphincter EC morphogenesis during normal development, we found that they contracted more when compared to neighboring ECs, restricting capillary flow. They also expressed a distinct set of genes not found in neighboring ECs. In eng mutants, expression of these genes is lost, suggesting that Alk1/Endoglin signaling is important for the proper differentiation of these specialized ECs. We are currently investigating the mechanisms through which sphincter ECs become distinct from their neighboring ECs and study how they might regulate capillary blood flow distribution. Ultimately, these findings will help us to better understand how Alk1/Endoglin signaling differentially affects distinct EC populations and thereby precipitates the formation of vascular lesions. This might direct therapies towards targeting sphincter ECs to ameliorate symptoms in HHT patients.

Role of Collagen Col22a1 in Intracranial Aneurysms and Vascular Stability

Poster Number: 7

Theme: Cardiovascular

Presenting Author: **Vishal Mardhekar, M Pharm** - University of South Florida

Co-Author(s): Diandra Florat – University of South Florida; Suman Gurung – University of South Florida; Saulius Sumanas – University of South Florida

Abstract: Intracranial aneurysms (IAs) pose a significant health risk due to their potential for rupture, leading to substantial morbidity and mortality worldwide. Despite known environmental risk factors, genetic predisposition plays a substantial role in IA pathogenesis. Whole exome sequencing studies of affected individuals with IA have identified mutant variants in collagen XXII (COL22A1), a fibril-associated collagen subtype. expressed in perivascular fibroblasts. Our previous study has demonstrated that col22a1 is expressed in perivascular fibroblasts and is required to maintain cranial vascular integrity in zebrafish. However, the functional impact of these IA-associated mutant variants remains unclear. To investigate this, we developed a zebrafish model with inducible expression of human COL22A1 variants, identified in individuals with IA. Our findings reveal that overexpression of two different variants increases the incidence of cranial hemorrhages, disrupts cranial vessel structure, and leads to reduced pericyte number, which are important in supporting vascular stability. Additionally, we demonstrate that zebrafish col22a1 is required to maintain stability of intersegmental vessels in the zebrafish trunk vasculature. Notably, col22a1 is co-expressed with type V collagen col5a1 in perivascular fibroblasts in the zebrafish trunk. Simultaneous deletion of both genes significantly increased trunk hemorrhages and vascular abnormalities, especially under heightened physical stress. These findings underscore the critical role of Col22a1 in vascular stability and argue that mutations in human COL22A1 may increase the incidence of hemorrhages and IAs.

Deciphering the Role of Ehd2b in Cardiac Regeneration: Insights from Zebrafish

Poster Number: 8

Theme: Cardiovascular

Presenting Author: **Ayisha Marwa Mangattu Parambil** - Institute of Anatomy, University of Bern

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Abstract: In vertebrates, the heart is derived from two sets of progenitors, resulting in first and second heart field. The role of the second heart field is not yet understood clearly. Previous work by the Mercader lab (Sanchez Iranzo et al., 2018, Nat Commun) revealed an upregulation of the ATPase Ehd2b in the second heart field, inspiring our investigation into how Ehd2b levels affect cardiac function and regeneration in zebrafish. Using an ehd2b knockout model and cryoinjury-induced myocardial infarction, we evaluated the regenerative response over time. Three days post-injury (dpi), ehd2bKO hearts exhibited heightened macrophage infiltration, indicating an amplified inflammatory phase. At 7 dpi, BrdU incorporation revealed increased cell proliferation within the compact myocardium and a defined 100μm border zone from the injury. Two weeks later, cardiomyocyte proliferation in the border region remained elevated, concomitant with extracellular matrix remodeling characterized by increased fibrin and collagen deposition. Although scar sizes at 30 dpi were comparable between genotypes, ehd2bKO hearts displayed more extensive myocardial closure, reflecting accelerated regeneration. Echocardiography revealed that ehd2bKO hearts outperformed their wild-type siblings, exhibiting significantly higher stroke volume, fractional shortening, and ejection fraction at 14 dpi. These data suggest that Ehd2b inhibition enhances cardiac repair, positioning Ehd2b as a potential therapeutic target. Ongoing transcriptomic analyses aim to delineate the molecular mechanisms underlying Ehd2b-mediated regeneration in zebrafish heart.

Decoding regulatory logic underlying cardiac regeneration gene regulatory networks in zebrafish

Poster Number: 9

Theme: Cardiovascular

Presenting Author: **Megan Guyer** - University of Wisconsin-Madison

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Abstract: Zebrafish have remarkable regenerative potential in the heart, which relies on the activation of gene regulatory networks (GRNs) in response to cardiac injury. This activation is orchestrated by diverse classes of tissue regeneration enhancer elements (TREEs), and dissecting how these TREEs are precisely activated during regeneration will elucidate GRNs that control heart regeneration. Previous studies on a cardiac leptin b regeneration enhancer (cLEN) in zebrafish identified that its activity is tightly regulated by maturation-associated repression and injury-induced activation, dictating its spatiotemporal dynamics in uninjured and regenerating hearts. I hypothesize that essential motifs and their binding partners within cardiac regeneration enhancers constitute regeneration-dedicated GRNs, ensuring precise gene expression during heart regeneration. To identify a group of cardiac regeneration

enhancers like cLEN, we performed a genome-wide search for the cLEN enhancer code in zebrafish and filtered for candidate enhancers with epigenomic and transcriptomic profiles. For selected candidates, we are performing transgenic assays to identify functional cardiac regeneration enhancers. Additionally, we are developing biochemical methods to identify the binding factors of cLEN to further elucidate enhancer binding partners within the cardiac regeneration GRN. This work will expand our understanding of how cardiac regeneration enhancers are precisely regulated, shedding light on the molecular mechanisms driving heart regeneration program.

Connecting the Head and the Heart: How Does Tbx1 Build the Template of Pharyngeal Development?

Poster Number: 10

Theme: Cardiovascular

Presenting Author: **Gwynneth Mcleod** - University of Toronto

Co-Author(s): Ian Scott – Professor, Molecular Genetics, University of Toronto; Simon Monis – Graduate Student, Molecular Genetics, University of Toronto; Michael Wilson – Professor, Molecular Genetics, University of Toronto

Abstract: Congenital heart defects (CHDs) and orofacial clefting (OFC) co-occur in DiGeorge Syndrome, a condition caused by hemizygous deletion of TBX1, a gene encoding a transcription factor (TF) important for the development of cardiac and facial muscle progenitors. These progenitors arise from a conserved cell lineage, called the cardiopharyngeal mesoderm (CPM). Previously, we demonstrated at 13 hours post-fertilization (hpf) that knockdown (KD) of essential cardiac TFs Gata5/6 causes a loss of cardiac progenitors, an expansion of tbx1+ pharyngeal progenitors, and global alterations to chromatin accessibility in zebrafish. However, the genetic mechanisms guiding decisions between cardiac and pharyngeal fates, and how Gata5/6 interact with Tbx1 in this process, are not clear. Pseudotime analysis of scRNA-seq elucidated the phasic nature of gata5 and tbx1 expression in pharyngeal progenitors, with tbx1 upregulation occurring after selective downregulation of gata5. RNA Fluorescent in situ Hybridization (RNA FISH) expression analysis revealed mostly distinct gata5 and tbx1 expression domains by 13 hpf, supporting a model in which tbx1 performs an early function after gata5/6 in pharyngeal progenitors. To interrogate the role of Tbx1 in pharyngeal fate at early stages, we developed a Tbx1 KD model in zebrafish. This was used for accessible chromatin profiling (ATAC-seq) at 13 hpf to identify changes to open chromatin in the absence of Tbx1. Despite minimal changes in global accessibility, we detected an increase in predicted binding at Gata5/6 motifs, supporting an early role for Tbx1 in maintaining pharyngeal fate against the alternative cardiac fate. Future experiments will use sequencing to characterize transcriptional and epigenetic changes in Tbx1 KD to elucidate the target genes and regulatory elements involved in pharyngeal fate specification. An improved model for CPM fate decisions and the relevant genetic factors will expand our understanding of why some forms of CHD and OFC co-occur.

The role of centriolar protein WDR90 in endothelial cells and cardiac tissue

Poster Number: 11

Theme: Cardiovascular

Presenting Author: **Amber Stratman** - Washington University in St. Louis, School of Medicine

Co-Author(s): Sarah Colijn – Washington University in St. Louis

Abstract: Congenital heart disease (CHD) is the most common type of birth defect and the leading cause of birth defect-associated infant mortality. Interestingly, genetic studies from patient cohorts with CHD reveal an unexpectedly high association with cilia-related gene variants. Through collaborative access to whole-exome sequencing, whole-genome sequencing, and RNA-sequencing datasets from parent-offspring trios enrolled in the Pediatric Cardiac Genomics Consortium, we have been able to identify de novo and recessive cilia-related variants that associate with diagnosed, symptomatic CHD. From this, we have uncovered WDR90 as a novel driver of heart malformations. WDR90 has been previously identified as a centriolar wall protein, but no connection has been made between WDR90 function and vascular defects. We have found that while wdr90 mutant zebrafish embryos survive through the embryonic stages, adult wdr90 mutant zebrafish experience partial lethality, stenosis, and an appreciably decreased level of fitness, consistent with CHD defects. Using cell culture, we find that WDR90 is highly expressed in endothelial cells and co-localizes with VE-cadherin at adherens junctions, suggesting a role for WDR90 in the function or stability of EC junctions. Furthermore, WDR90 can be found at the tip of microtubules, which may indicate a role for WDR90 in nucleating microtubule structures. Consequently, uncovering the mechanisms behind novel cilia-related CHD driver genes will have a meaningful impact on understanding and treating CHD.

Characterizing the role of RHOA in regulating blood vessel tip cell filopodia

Poster Number: 12

Theme: Cardiovascular

Presenting Author: **Ekaterina Monakhova** - Western Washington University

Co-Author(s): Laura Pillay, PhD – Assistant Professor, Biology, Western Washington University

Abstract: The GTPase RHOA is a molecular switch that transduces signals by activating effector proteins to generate a biological response. Overactive RHOA has been found in human patients with blood vessel patterning defects and cerebral hemorrhage, making it and its effectors attractive targets for treating vascular disease. However, it is currently not clear how RHOA regulates blood vessel development and integrity in vivo at the molecular level. Zebrafish are an excellent model for studying the role of RHOA in blood vessels; either increased or decreased RHOA activity in zebrafish embryos causes impaired blood vessel growth and hemorrhage. New blood vessels sprout from existing blood vessels in a process called angiogenesis. During angiogenesis, blood vessel tip cells extend actin-rich filopodia, slender projections that detect environmental signals and help guide and shape the direction of new vessel growth. We and others have shown that RHOA modulates actin-based cytoskeletal structures in in vitro cultured blood vessel endothelial cells. We therefore hypothesize that RHOA may be influencing in vivo blood vessel growth and integrity by impacting blood vessel tip cell filopodia formation. To test this hypothesis, we are using confocal microscopy to live-image blood vessel tip cell filopodia in the intersegmental vessels of Tg(fli1a:LIFEACT-eGFP); Tg(egfl7:GAL4ff); Tg(UAS:Tomato-2A-rhoaaV14 or N19) triple transgenic zebrafish embryos with fluorescently labeled filamentous actin, and blood vessel endothelial cell-specific expression of either constitutively active RHOA (rhoaaV14), or dominant-negative RHOA (rhoaaN19) at

26 hpf. Our research findings will help us to understand the molecular mechanisms by which RHOA regulates blood vessel development and integrity.

Epicardial activation of Fabp7 is an aging-associated pathological event in cardiomyopathies that can be inhibited for cardioprotective effects

Poster Number: 13

Theme: Cardiovascular

Presenting Author: **Baul Yoon, PhD** - Mayo Clinic

Co-Author(s): Yonghe Ding, PhD – Biochemistry and Molecular Biology, Cardiovascular Medicine – Mayo Clinic; Maryam Moossavi, PhD – Biochemistry and Molecular Biology – Mayo Clinic; Xiaolei Xu, PhD – Principal Investigator, Biochemistry and Molecular Biology, Cardiovascular Medicine, Mayo Clinic

Abstract: Epicardial remodeling refers to the structural and functional changes in the epicardium caused by both genetic and environmental factors. While its crucial role in myocardial injury and regeneration in the adult heart has been reported, the functions of the epicardium during the pathogenesis of cardiomyopathies and cardiac aging remain unknown. From our recent cardiac transcriptomic studies of BCL2-associated athanogene 3 (bag3) dilated cardiomyopathy (DCM) adult zebrafish model, we identified fatty acid binding protein 7a (fabp7a) as a candidate gene activated during epicardial remodeling. This remodeling is characterized by a pronounced gap between the myocardium and epicardium along with disorganized collagen fibers within these layers. We also observed similar fabp7a activation and epicardial remodeling in anthracycline-induced cardiotoxicity (AIC) zebrafish, an accelerated cardiac aging model, suggesting that epicardial activation of fabp7a could be a common pathological event in cardiomyopathies. To interrogate functions of fabp7a in epicardial remodeling, we generated fabp7a mutants and epicardial-specific fabp7a-overexpressing zebrafish models. Inhibition of fabp7a ameliorates cardiac dysfunction and rescues epicardial remodeling whereas overexpression of fabp7a in the epicardium is sufficient to drive epicardial remodeling. Finally, we found that normally aged killifish and mouse models exhibit strong activation of fabp7a in the epicardium, suggesting that epicardial remodeling is a conserved event during normative cardiac aging. Collectively, our findings identify epicardial remodeling, represented by fabp7a activation, as a previously unrecognized hallmark of cardiomyopathies and normative cardiac aging. Potentially, targeting fabp7a represents a novel rejuvenating therapy for cardiac aging and an anti-aging-based therapeutic strategy for cardiomyopathies.

Lineage tracing with endocardial specific zebrafish lines shows hemogenic potential of the endocardium

Poster Number: 14

Theme: Cardiovascular

Presenting Author: **Luiza Loges, B.A.** - University of South Florida

Co-Author(s): Nicole Restrepo – University of South Florida; Ivana Zlatanova – University of California, San Francisco; Brian Black, PhD – University of California, San Francisco; Saulius Sumanas, PhD – Principle Investigator, Pathology and Cell Biology, University of South Florida

Abstract: Recent studies by our lab and others have suggested that the endocardium, which forms the endothelial lining of the heart tube, can function as a hematopoietic site in zebrafish. However, genetic evidence for endocardial derived hematopoiesis is still missing and its full lineage potential is unknown. Here we have implemented a recently identified endocardial specific enhancer, endo83, to generate transgenic zebrafish lines expressing GFP, Cre, CreERT2, and Gal4. We then performed characterization and functional validation of these lines. We show that endo83:GFP expression is largely restricted to the endocardium during 24-72 hpf stages, although some expression in the adjacent cranial vasculature is also apparent. When endo83:Cre or endo83:CreERT2 lines were crossed to the ubiquitous EF1a:loxP-dsRed:loxP-GFP switch line, switched cells were largely restricted to the endocardium, demonstrating the relative specificity of these lines at timepoints from 1-3 dpf. To confirm if endocardial cells contribute to hematopoiesis, the endo83:Cre line was mated to the EF1a:loxP-dsRed:loxP-GFP switch line and analyzed for GFP labeling in hematopoietic cells at approximately 52 hpf. GFP expression was observed in multiple neutrophils and some macrophages, suggesting that these cells were derived from the endocardium. These experiments validate our previous findings and show, using a genetic lineage tracing approach, that the endocardium contributes to hematopoiesis. Additional experiments to elucidate the timeline of endocardial hematopoiesis and hematopoietic lineage potential of the endocardium are currently ongoing. These results will be valuable in understanding alternative mechanisms of hematopoietic regulation. In addition, newly generated endocardial specific zebrafish lines will be a useful tool for many researchers interested in cardiovascular and hematopoietic development.

The effect of blood flow on lymphangiogenesis in zebrafish

Poster Number: 15

Theme: Cardiovascular

Presenting Author: **Krishna Rentachintala, M.S.** - Department of Pathology and Cell Biology, University of South Florida

Co-Author(s): Surendra Anand, Ph.D. – Postdoctoral Research Scholar, Department of Pathology and Cell Biology, University of South Florida; Saulius Sumanas, Ph.D. – Department of Pathology and Cell Biology – University of South Florida

Abstract: The lymphatic system primarily functions to maintain fluid homeostasis, aids in absorbing lipids in the digestive system, and is a part of the immune system. Molecular and physiological mechanisms regulating the formation of the lymphatic system, or lymphangiogenesis, are not fully understood. In this study, we are investigating the impact of blood flow on lymphangiogenesis in zebrafish embryos. To test this, we used morpholino (MO) injections to block ttnt2a, a cardiac isoform of troponin T, which resulted in the absence of blood flow. Tnnt2 MO injected embryos showed absence of parachordal lymphangioblasts at the horizontal myoseptum and the absence of thoracic duct, labeled by the lymphatic specific prox1 reporter line. In addition, venous sprouting of intersegmental vessels was greatly reduced in tnnt2 MO injected embryos. These results demonstrate that blood flow is required

for the development of lymphatic vasculature. Further studies to investigate the molecular mechanism of how blood flow affects lymphangiogenesis are currently ongoing. These results will be important to understand the mechanisms regulating lymphangiogenesis during normal development and in lymphatic diseases.

Hedgehog signaling and Bmp signaling play opposing roles during the establishment of the cardiac inflow tract in zebrafish

Poster Number: 16

Theme: Cardiovascular

Presenting Author: **Hailey Edwards, PhD** - UC San Diego

Co-Author(s): Rhea-Comfort Robertson – UC San Diego; Deborah Yelon – School of Biological Sciences – UC San Diego

Abstract: Cardiac pacemaking activity is confined to a specialized population of cardiomyocytes in the cardiac inflow tract (IFT), but the patterning processes that establish IFT dimensions remain unknown. Our data indicate that Hedgehog (Hh) signaling has a potent effect on limiting the number of IFT cells in the embryonic zebrafish heart. Using either genetic or pharmacological manipulation of the Hh pathway, loss of Hh signaling results in a significantly expanded population of IFT cardiomyocytes. Conversely, we find that Bmp signaling plays a dose-dependent role in promoting the formation of IFT cardiomyocytes: reduction of Bmp signaling diminishes the number of IFT cells, and increased Bmp signaling enhances the number of IFT cells. Temporal inhibition of each pathway demonstrates that Hh and Bmp signaling are both required in the same timeframe, prior to myocardial differentiation, to establish a normal number of IFT cardiomyocytes. Furthermore, simultaneous reduction of both Hh and Bmp signaling restores the IFT population to a relatively normal size, suggesting that these pathways act in opposition during IFT patterning. Lastly, epistasis analysis demonstrates that Bmp signaling acts upstream of canonical Wnt signaling to promote IFT cell formation, whereas Hh signaling appears to restrict IFT cell formation in a Wnt-independent manner. Together, our results support a model in which Hh signaling limits the size of the IFT progenitor pool, while BMP signaling promotes IFT progenitor specification, upstream of Wnt-directed IFT differentiation, to generate an appropriate number of pacemaker cells in the IFT.

Investigating the roles of meningeal perivascular macrophages in vascular development

Poster Number: 17

Theme: Cardiovascular

Presenting Author: **Melanie Holmgren** - University of Utah, Department of Human Genetics

Co-Author(s): Marina Venero Galanternik – University of Utah

Abstract: Perivascular macrophages (PVMs) are specialized macrophage populations that associate with blood vessels in various tissues throughout the body. PVMs have been shown to have many functions, including promoting and regulating vascular development, as well as maintaining vascular integrity and

permeability. Fluorescent granular perithelial cells (FGPs) are a population of PVMs tightly associated with the vasculature of the zebrafish meninges, a set of tissue layers surrounding the central nervous system. Previous studies have hinted at FGPs' pro-angiogenic capacities, however, the mechanisms underlying FGP-mediated regulation of vascular development, or vascular maintenance and integrity, are not completely understood. Because of their optical transparency and genetic tractability, zebrafish present an ideal model system in which to study these PVM-mediated vascular mechanisms in vivo. To this end, we have developed genetic models in which to manipulate FGP numbers and assess effects on the meningeal vasculature. We identified a mutant zebrafish line that shows increased FGP numbers compared to their siblings, and we predicted these mutants would show enhanced vascular development. Indeed, mutant larvae with higher FGP numbers showed an increased cephalic vascular density. Interestingly, this increased vascular density was not observed in the trunk of the body, which is devoid of FGPs, supporting that FGPs may promote meningeal vascular development. In order to genetically target FGPs for cell-specific ablation, we have identified a candidate molecular marker that is sufficient to drive transgene expression in FGPs. Future work will seek to dissect mechanisms of FGP-mediated vascular development in these models by examining distinct developmental events such as blood endothelial cell proliferation and survival as well as changes in vessel integrity when FGP numbers are manipulated. The results from this study support a role for FGPs in meningeal vascular development and have established models in which to study the underlying mechanisms in vivo.

Characterization of novel lymphatic capillary mural cells (LCMCs)

Poster Number: 18

Theme: Cardiovascular

Presenting Author: **Vishakha Vishwakarma** - National Institues of Health

Co-Author(s): Daniel Castranova – National Institues of Health; Madeleine Kenton – National Institues of Health; Kiyohito Taimatsu – National Institues of Health; Brant Weinstein – National Institues of Health

Abstract: Lymphatic vessels are essential for fluid homeostasis, immune responses, and tumor metastasis. Unlike blood vessels and capillaries, which are supported by mural cells like smooth muscle cells and pericytes, lymphatic capillaries have long been thought to be devoid of these important supporting cells. Using a novel transgenic line, we recently fortuitously discovered a previously undescribed population of mural cells tightly associated with zebrafish lymphatic capillaries which we have named 'Lymphatic Capillary Mural Cells' (LCMCs). We have been working to uncover the structure and functions of LCMCs and the narrow lymphatic capillaries they surround, revealing new insights into lymphatic biology. We have characterized their morphology using high-resolution microscopy and further plan to analyze their ultrastructure using volume electron microscopy. To determine their molecular identity, we performed single-cell RNA sequencing (scRNA-seq), preliminarily revealing a pericyte-like signature for LCMCs. To obtain a more in-depth transcriptional profile of these cells, we are conducting bulk RNA sequencing on LCMCs. To uncover their function, we will selectively ablate LCMCs and assess their role in lymphatic vessel growth and stability. This first description of lymphatic capillary mural cells in any vertebrate opens exciting new avenues for understanding lymphatic biology.

Dual Regulation of Vascular Patterning by FNDC5/Irisin Paralogues During Zebrafish Development

Poster Number: 19

Theme: Cardiovascular

Presenting Author: **Yi-Shan Huang** - National Sun Yat-Sen University

Co-Author(s): Ming-Hong Tai – National Sun Yat-Sen University; Chang-Yi Wu – National Sun Yat-Sen University

Abstract: Vascular development is a tightly orchestrated process critical for vertebrate embryogenesis. While the role of fndc5a, a paralogue of the irisin-encoding gene FNDC5 (Fibronectin type III domain containing protein 5), has been implicated in zebrafish vasculature formation via VEGF and BMP signaling on our previous study, the specific function of fndc5b remains unclear. In this study, we identify fndc5b as a novel and essential regulator of intersegmental vessel (ISV) and caudal vein plexus (CVP) development. In situ hybridization revealed that fndc5b is dynamically expressed in vascular structures from the 16-somite stage through 48 hpf. Morpholino-mediated knockdown of fndc5b led to marked angiogenic defects, including disrupted ISV elongation and impaired CVP sprouting, which were accompanied by reduced endothelial cell proliferation and migration in Tg(kdrl:mCherry; fli:nEGFP)y7 embryos. These phenotypes were associated with dysregulation of vascular marker genes such as ephrinb2, flt4, mrc1, flk, and stabilin. Notably, vascular abnormalities in fndc5b morphants were significantly rescued by overexpression of fndc5b or exogenous irisin. Mechanistically, fndc5b acts upstream of VEGF and BMP pathways, as its loss attenuated expression of their key signaling components. Moreover, we test the interaction between fndc5a and fndc5b, we found dual knockdown of fndc5a and fndc5b enhance the growth defects of ISV and CVP. We also showed knockdown of fndc5a and fndc5b down-regulated VEGF and BMP signal molecules dramatically. Together, our findings identify fndc5b as a critical modulator of angiogenesis and highlight its cooperative role with fndc5a in regulating zebrafish vascular development.

The pendulum swing: genetic regulators of cardiopharyngeal development

Poster Number: 20

Theme: Cardiovascular

Presenting Author: **Benjamin Akande** - Miami University

Co-Author(s): Griffin Chadwick – Graduate student, Biology, Miami University; Sylvie Earlewine – Undergraduate student, Biology, Miami University; Lily Hofman – Undergraduate student, Biology, Miami University; Grace Leonard – Undergraduate student, Biology, Miami University; John Postlethwait – University of Oregon; Jennifer Schumacher – Principal Investigator, Biology, Miami University; Saulius Sumanas – University of South Florida

Abstract: Congenital heart defects (CHD) occur in about 1 in 100 live babies annually, underscoring the urgent need to elucidate the cellular and molecular processes driving heart development. Proper cardiac morphogenesis relies on coordinated contributions from multiple progenitor lineages. Early differentiating first heart field (FHF) cardiomyocytes form the primitive heart tube, which is subsequently extended by late differentiating second heart field (SHF) cells that give rise to the inflow tract of the atrium and outflow tract (OFT) of the ventricle. Additionally, neural crest cell (NCC)

progenitors also populate the pharyngeal arches and arterial pole of the OFT. Thus, the OFT is derived from both SHF and NCC lineages, while the adjacent bulboventricular (BV) valve is primarily SHF-derived. Despite this understanding, the molecular and cellular basis of OFT development remains poorly understood. Here, we characterized a novel zebrafish mutant, pendulum (pen), which exhibits severe cardiopharyngeal defects. OFT integrity in pen embryos is markedly disrupted with reduced or absent smooth muscle layers and a notable increase in endocardial cell number. Transcriptomic profiling on 72 hours post-fertilization (hpf) pen embryos reveals elevated TGF-β signaling alongside downregulation of genes associated with adrenergic signaling in cardiomyocytes, cardiac contractility, and vascular smooth muscle contraction. At 96 hpf, pen mutants also demonstrate craniofacial abnormalities—including shortened cartilage length and widened cranial angles—reminiscent of microcephaly and micrognathia, along with reduced bone mineral density in their otoliths, suggesting defective SHF-NCC interactions. These phenotypes are likely attributed to their lethality at 120 hpf. Altogether, these findings establish the pen mutant as a valuable genetic model for investigating cardiopharyngeal development. Our results also identify dysregulated TGF-β signaling as a potential mechanistic driver for OFT and craniofacial abnormalities. This work advances understanding of the genetic and developmental basis of OFT formation, while offering new perspectives on the etiology of CHD.

Regionalized regulation of actomyosin organization influences cardiomyocyte cell shape changes during chamber curvature formation

Poster Number: 21

Theme: Cardiovascular

Presenting Author: **Deborah Yelon** - University of California, San Diego

Co-Author(s): Dena Leerberg – University of California, San Diego; Gabriel Avillion – University of California, San Diego; Rashmi Priya – MPI Bad Nauheim; Didier Stainier – MPI Bad Nauheim

Abstract: Cardiac chambers emerge from a heart tube that balloons and bends to create expanded ventricular and atrial structures, each containing a convex outer curvature (OC) and a recessed inner curvature (IC). The cellular and molecular mechanisms underlying the formation of these characteristic curvatures remain poorly understood. Here, we demonstrate in zebrafish that the initially similar populations of OC and IC ventricular cardiomyocytes diverge in the organization of their actomyosin cytoskeleton and subsequently acquire distinct OC and IC cell shapes. Altering actomyosin dynamics hinders cell shape changes in the OC, and mosaic analyses indicate that actomyosin regulates cardiomyocyte shape in a cell-autonomous manner. Additionally, both biomechanical cues and the transcription factor Tbx5a influence the basal enrichment of actomyosin and squamous cell morphologies in the OC. Together, our findings demonstrate that intrinsic and extrinsic factors intersect to control actomyosin organization in OC cardiomyocytes, which in turn promotes the cell shape changes that accompany curvature morphogenesis.

Remodeling of ColIV during Development and Regeneration of the Cardiovascular System in Zebrafish

Poster Number: 22

Theme: Cardiovascular

Presenting Author: **Astha Tuladhar** - Iowa State University

Co-Author(s): Astha Tuladhar – Iowa State University; Fang Liu – Iowa State University; Zhitao Ming – Iowa State University; Maura McGrail – Iowa State University; Pascal Lafontant – Grinnell Collage/ Iowa State University; Jeffrey Essner – Iowa State University

Abstract: One of the major players involved in the development and regeneration of the cardiovascular system is the extracellular matrix (ECM). Besides providing support and structures to cells and tissues, the spatiotemporal distribution and remodeling of different ECM components are known to regulate signaling pathways that control cell proliferation, migration, and differentiation. This project aims to understand how the remodeling of an ECM component, ColIV, regulates the development and regeneration of the cardiovascular system using zebrafish model. Previous work done in ColIV, and my preliminary data, indicated that mutation in ColIV leads to cardiovascular defects during development. Additionally, proteomics data have also identified changes in ColIV during heart regeneration. However, its remodeling dynamics and tissue-specific role during the development and regeneration of the cardiovascular system remain largely unexplored. To address this gap, a conditional, targeted foxed allele is being developed that allows the expression of colIVa1 to be followed from gal integration. Initial analysis using transient expression of this conditional allele in live zebrafish embryos has identified multiple cell types, including vasculature, cardiac cells, and muscle cells, to express ColIVa1 during development. Furthermore, to monitor the remodeling of ColIV in vivo, a fluorescent tag was integrated into ColIVa2, enabling live confocal imaging of its expression dynamics. Our preliminary data reveal that ColIVa2 is expressed within the cardiovascular system, including the dorsal aorta, intersegmental vessels, and heart, as well as in the myoseptum during development. These findings provide novel insights into the cellular sources and deposition patterns of ColIV during zebrafish development. Using this Cre/Lox conditional allele system, we will further analyze the tissue-specific role of ColIV in cardiac and vasculature using our Cre and CreERT2 knock-in alleles in hand2 and fli1b. Overall, this research provides novel insight into ColIV dynamics and its tissue-specific role, ultimately helping advance regenerative medicine.

CaMPARI2 Enables Stimulus-Locked Whole-Brain Activity Mapping at Cellular Resolution in Unrestrained Larval Zebrafish

Poster Number: 24

Theme: Circuits and Behavior

Presenting Author: **Kathryn Robbins** - Haverford College

Co-Author(s): Amelia Bredbenner – Haverford College; Viktor Merkulov – Haverford College; Cristina Campos – Haverford College; Roshan Jain – Haverford College

Abstract: Visualizing active neurons and circuits in vivo is critical for investigating the neural activity that underlies behaviors of interest. While several established methodologies are available to achieve this end in larval zebrafish, they are limited by the scale of tissue visualization, temporal resolution, need to restrain larvae, and/or accessibility of necessary instruments. Here, we establish a pipeline for the visualization and quantification of spatiotemporally precise whole-brain neural activity in larval zebrafish

using CaMPARI2, a genetically encoded calcium indicator. Because CaMPARI2 irreversibly photoconverts from a green to a red fluorescent form upon concurrent calcium binding and UV light exposure, a temporally precise whole-brain “snapshot” of neural activity can be obtained at cellular resolution during behaviors of interest. We outline the variables that must be considered and optimized to use the CaMPARI2 system to visualize neural activity associated with temporally fixed behaviors of interest in larval zebrafish. We then demonstrate the utility of our pipeline for comparing patterns of neural activity between discrete behavioral and learning states in larvae, focusing on acoustically-evoked behavior and learning.

The rhythmic lncRNA cu693494.2 and the rhythmic micropeptide Cu693494.2-ORF3 regulate the zebrafish circadian transcription through the activity of Rorαa-Per2-mediated-RRE element

Poster Number: 26

Theme: Physiology & Metabolism

Presenting Author: **Lianxin Wu, Graduate student** - Soochow University

Co-Author(s): Han Wang – Professor/Director, Center for Circadian Clocks, Soochow University

LncRNAs, as non-coding RNAs, directly regulate or indirectly regulate the circadian clock, and their encoded micropeptides participate in various biological functions. However, whether lncRNAs and the micropeptides regulate the rhythmic transcription by affecting the activity of transcription factors has not yet been studied. Firstly, rhythmic and encoding lncRNA cu693494.2, from the time-series whole-transcriptome and Ribo-seq, was regulated by the zebrafish circadian clock via E-box and D-box, and mainly expressed in the eyes and microglia in the meninges. And the rhythmic micropeptide Cu693494.2-ORF3 encoded by lncRNA cu693494.2 located in the eyes and whole brain pan-express in zebrafish, indicating the biological functions between cu693494.2 and Cu693494.2-ORF3 have both similarities and differences. In addition, knockout of cu693494.2 or cu693494.2-ORF3 delayed the phase of locomotion and disrupted the rhythmicity of the circadian clock genes. Finally, the mechanism of the lncRNA cu693494.2 in the cytoplasm participated in the regulation of the zebrafish circadian clock was that overexpression of Per2-Rorαa by cu693494.2-Rbm4.3-per2 and cu693494.2-rorαa activated the Ror/Rev-erb response elements (RRE), thereby upregulating bmal2 expression, promoting neuron development and autophagy, and inhibiting lipid peroxidiation-mediated ferroptosis; while the micropeptide complex Cu693494.2-ORF3-Ddx3a decreases the activity of the RRE elements by binding to Per2-Rorαa, thereby downregulating bmal2 expression, inhibiting nervous system development and promoting Fenton reaction-mediated ferroptosis. Our findings provide new directions for the study of the circadian clock regulatory network and have potential clinical implications.

Myosin-Binding Protein C: Defining Function in Larval Zebrafish Muscles, One Isoform at a Time

Poster Number: 27

Theme: Physiology & Metabolism

Presenting Author: **Andrew Mead, PhD** - Larner College of Medicine, University of Vermont

Co-Author(s): Shane Nelson – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Samantha Beck Previs – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Angela Ploysangngam – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Bradley Palmer – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Guy Kennedy – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Marcus Zimmermannn – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Michael Previs – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont; Maura McGrail – Department of Genetics, Development and Cell Biology – Iowa State University; David Warshaw – Molecular Physiology and Biophysics – Larner College of Medicine, University of Vermont

Abstract: The mechanical output of vertebrate muscle is tuned by the myosin-binding protein C (MyBP-C) family of proteins. MyBP-C is found in the 'C-zone', a distinct region of the muscle sarcomere, where it binds to the myosin molecular motors and actin filaments, thus modulating muscle power generation. Four MyBP-C gene paralogs (MYBPC1, MYBPC2, MYBPC3, and MYBPH) encode multiple isoforms that have varying degrees of contractile modulation, based on in vitro measurements. In mammalian skeletal muscle fibers, isoforms are co-expressed and occupy the C-zone simultaneously. Therefore, understanding how each individual isoform contributes to contractile modulation within intact muscle is challenging. Although zebrafish possess orthologs for all mammalian MyBP-C family genes, the well-characterized myotomal muscles of 5-day-old larvae express predominantly a single isoform from the MYBPH ortholog mybphb, based on proteomic analysis. With new genetic tools available to zebrafish, this observation makes them a promising model system to create "designer" muscles that express recombinant MyBP-C, one isoform at a time. Using the CRISPR GeneWeld technique for precision targeted integration, we generate insertion alleles in exon one of mybphb that simultaneously interrupt endogenous gene expression, while preserving 5' gene regulatory domains to drive the expression of DNA cassettes encoding recombinant, MyBP-C family variants. To test this approach, we injected embryos with a GeneWeld construct encoding a FLAG-tagged, N-terminal truncation mutant Mybphb and a soluble eGFP marker. At five days post-fertilization, mosaic eGFP fluorescence was restricted to muscle cells where endogenous mybphb is normally expressed at high levels, while confocal imaging of immunolabeled FLAG fusion peptide showed transgenic Mybphb-FLAG properly accumulates in the sarcomere C-zone. Thus, stable zebrafish lines generated by this approach will allow us to define the impact of individual MyBP-C isoforms, domains, and disease-causing mutations, on contractile modulation from molecule to intact larval tail muscle mechanics.

A role for the de novo pyrimidine synthesis enzyme dihydroorotate dehydrogenase as an inhibitor of ferroptosis in erythroid differentiation

Poster Number: 28

Theme: Physiology & Metabolism

Presenting Author: **Marlies Rossmann, MD, PhD** - University of Rochester Medical Center

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Medical Center; Paul Kingsley, PhD – Department of Pediatrics – University of Rochester Medical Center; Kathleen McGrath, PhD – Department of Pediatrics – University of Rochester Medical Center; James Palis, MD – Department of Pediatrics – University of Rochester Medical Center

Abstract: Understanding the metabolic requirements for erythroid differentiation is critical to develop novel therapeutic approaches for anemias of various etiologies. Although inhibitors of dihydroorotate dehydrogenase (DHODH), an essential enzyme in de novo pyrimidine synthesis, have shown promise in pre-clinical models of acute myeloid leukemia (AML), anemia has emerged as an early adverse side-effect, preventing their success in clinical trials. Through a chemical screen in zebrafish, we have previously identified several structurally diverse DHODH inhibitors that cause an erythroid differentiation block as observed by a marked reduction of embryonic globin expression and hemoglobin production. We have now found that treatment of zebrafish embryos with DHODH inhibitors results in a phenotype of ferroptosis, a type of programmed cell death due to iron-dependent lipid peroxidation. DHODH inhibition by brequinar causes an accumulation of lipid peroxides in zebrafish embryos. In addition, we observed that several ferroptosis inhibitors with distinct mechanisms of action rescue the anemia caused by the inhibition of DHODH. Morphological examination of sorted fluorescent erythroid precursors revealed that the presence of DHODH inhibitors led to a delay of erythroid differentiation, which was partially reversed by co-treatment with the ferroptosis inhibitor liproxstatin-1. To identify the mechanism responsible for the rescue of erythropoiesis by ferroptosis inhibitors in the presence of DHODH inhibition, we performed untargeted metabolomic analyses. While we did not find evidence for rescue of de novo pyrimidine synthesis, several metabolites of the pyrimidine salvage pathway were among the top 25 upregulated metabolites suggesting re-wiring from lineage-essential de novo to salvage pyrimidine synthesis under ferroptosis inhibition. Our results demonstrate a novel tight link between pyrimidine metabolism and ferroptosis defense in erythropoiesis and uncover distinct adaptive plasticity of de novo and salvage nucleotide synthesis during erythroid differentiation, with implications for using DHODH inhibitors as a treatment for AML and other cancers.

Zebrafish as a model animal to study the metabolic effects of prolonged fasting

Poster Number: 29

Theme: Physiology & Metabolism

Presenting Author: **Mun-Gu Song** - Washington University in St.Louis

Co-Author(s): Madelyn Jackstadt – Washington University in St.Louis; Kevin Cho – Washington University in St.Louis; Triston Groff – Washington University in St.Louis; Leah Shriver – Washington University in St.Louis; Gary Patti – Washington University in St.Louis

Abstract: Prolonged fasting induces profound changes in physiology and has been proposed as a lifestyle intervention for metabolic conditions such as obesity and diabetes. However, the lack of an optimal model system to study long-term fasting has limited our understanding of its systemic effects. We demonstrate that zebrafish can serve as a suitable model for studying the broad metabolic impacts of long-term fasting on the body. Zebrafish possess all the organs that maintain metabolic homeostasis in humans, making it an excellent model system to investigate physiological responses to fasting. To investigate the adult zebrafish as a model for fasting studies, we withheld food from fish for a period of 14 days. As expected, we found alterations to hepatic glycogen and ketone bodies within zebrafish after

14-days fasting. Additionally, metabolomics analyses showed changes to systemic physiology that mimic known human metabolism. Our study highlights the utility of zebrafish as a model system to study metabolic homeostasis in long-term fasting.

Mapping interactions between gut bacteria and hepatic metabolism

Poster Number: 30

Theme: Physiology & Metabolism

Presenting Author: **Madelyn Jackstadt** -

Co-Author(s): Ronald Fowle-Grider – Washington University in St. Louis; Mun-Gu Song – Washington University in St. Louis; Matthew Ward – Washington University in St. Louis; Madison Barr – Washington University in St. Louis; Kevin Cho – Washington University in St. Louis; Hector Palacios – Washington University in St. Louis; Sam Klein – Washington University in St. Louis; Leah Shriver – Washington University in St. Louis; Gary Patti – Washington University in St. Louis

Abstract: The gut microbiome provides many health benefits to animals, however, the metabolic interactions between gut bacteria and host metabolism remain incompletely understood. To probe the small molecule communication between intestinal bacteria and the animal host, we developed an antimicrobial-based model for gut bacterial depletion in adult zebrafish. By using a workflow we developed to analyze serum and organs from a single zebrafish, we observed global and tissue-specific differences in metabolite levels. Specifically, we found increased polyol pathway metabolites in the intestine and liver of animals lacking gut bacteria. We investigated the use of these metabolites by using isotope tracers, which allowed us to track nutrient use in the intestine and the liver. Dysregulated metabolism of polyol pathway compounds from the intestine to the liver in antimicrobial-treated animals led to the development of hepatic steatosis. These results provide another link between gut microbial metabolism and hepatic health.

Tissue failure in response to pressure-induced strain for inner ear homeostasis

Poster Number: 33

Theme: Organ Formation & Function

Presenting Author: **Ian Swinburne, PhD** - University of California, Berkeley

Co-Author(s): Ibrahim Abuzahriyeh – Lab Assistant, UC Berkeley; Samara Williams – Lab Assistant, University of Oregon

Abstract: Sensory and neurological organs like the ear, eye, and brain contain pressurized, fluid-filled cavities essential for structure, protection, and function. Imbalances in fluid pressure underlie widespread diseases, including glaucoma (~70 million cases), traumatic brain injuries (~70 million annually, often with hydrocephalus), and hearing/balance disorders (~17 million cases). Despite this burden, fluid pressure regulation remains poorly understood due to its interdisciplinary nature. I previously discovered a tissue-scale pressure relief valve in the epithelial tissue of the ear's endolymphatic sac whose behavior is consistent with long observed physiologies that lacked explanations. Using a combination of advanced live imaging, genetics, and cell and molecular biological

technologies like genome editing, we study these systemic processes in zebrafish embryos and larvae whose inner ears are optically accessible in the living animal instead of buried within the temporal bone as in mice and humans. With a zebrafish with the endogenous Ctnnb1 tagged with the fluorescent protein mNeonGreen, we uncovered a broad distribution in the amount of nuclear Ctnnb1 in the cells of the endolymphatic sac prior to initiation of its activity as a tissue scale-pressure relief valve. Imaging through development, we have characterized heterogeneity in accumulation of Ctnnb1, Cldn7a/b, and Cdh2 in lysosomes. One of the earliest gross morphological phenotypes of a zygotic zebrafish mutant of wls is a distended endolymphatic sac—a phenotype I previously characterized in a lmx1bb mutant. Analysis of developmental differences in the mutants versus wild-type ears indicate that Wnt signaling is necessary for both patterning of a endolymphatic sac tissue and then heterogeneity in signaling within sac tissue leads to opening of the epithelial tissue in response to pressure induced strain. These findings align with Wolfgang Knauss's material failure theory, where adhesive property heterogeneity dictates failure at the weakest point under strain.

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Epigenetic regulation of cilia stability and kidney development by the chromatin remodeling SWI/SNF complexes

Poster Number: 34

Theme: Organ Formation & Function

Presenting Author: **Ying Cao** - Tongji University

Co-Author(s):

Abstract: Cilia are important subcellular organelles and are regulated by master regulator transcription factors including Foxj1 and Rfx proteins. Whether and how cilia are regulated at epigenetic level remains unknown. We addressed this question by knocking down or knocking out of chromatin remodeling genes. Interestingly, depletion of multiple components of the switch/sucrose non-fermentable (SWI/SNF) complexes lead to ciliopathy-like phenotypes in zebrafish embryos. Specifically, depletion of Actl6a, one of the essential components of the SWI/SNF complexes, leads to cilia disassembly and cystic kidney, but does not affect cilia motility. Omics studies show that in Actl6a-depleted pronephros or embryos, a set of cilia genes, including master regulators foxj1a and rfx2, were down-regulated at transcriptional level, chromatin accessibility level and the SWI/SNF complexes binding level. Depletion of foxj1a or rfx2 in zebrafish also leads to cilia disassembly and cystic kidney. Furthermore, overexpression of foxj1a or rfx2 mRNA partially rescued cystic kidney and cilia disassembly defects in actl6a mutants. Thus, our study reveals that the SWI/SNF complexes regulate cilia stability and kidney development by direct modulating the expression of foxj1a or rfx2.

Severe Intestinal Defects Resulting from aip Loss of Function in Humans and Zebrafish

Poster Number: 35

Theme: Organ Formation & Function

Presenting Author: **Xian Wang** - Queen Mary University of London

Co-Author(s): Oliver Haworth – Lecturer, School of Life Sciences, University of Westminster; Sayka Barry – Centre for Endocrinology, William Harvey Research Institute – Queen Mary University of London; Adele Leggieri – School of Biological and Behavioural Sciences – Queen Mary University of London; Charlotte Hall – Centre for Endocrinology, William Harvey Research Institute – Queen Mary University of London; Joanne Martin – Centre for Genomics and Child Health, Barts and the London School of Medicine and Dentistry – Queen Mary University of London; Márta Korbonits – Centre for Endocrinology, William Harvey Research Institute – Queen Mary University of London; Caroline Brennan – School of Biological and Behavioural Sciences – Queen Mary University of London

Abstract: Background: Heterozygous mutations in the chaperone Aryl Hydrocarbon Receptor Interacting Protein (AIP) are associated with pituitary adenomas, whereas complete AIP loss results in embryonic lethality in animal models. To date, five individuals with homozygous AIP mutations have been identified, all presenting with severe failure to thrive and life-threatening diarrhoea requiring parenteral nutrition. While AIP has been implicated in maintaining cellular proteostasis under nutrient-deprived conditions, its role in intestinal development remains largely unknown. Methods: aip loss-of-function zebrafish model using CRISPR-Cas9. Survival was monitored daily, and intestinal morphology was assessed using haematoxylin and eosin staining. Cellular proliferation and stem cell populations were analysed using PCNA immunohistochemistry and in situ hybridisation, respectively. Cell morphology was visualised using DAPI and Alcian Blue staining. Wnt signalling components were examined through RNA sequencing and RT-qPCR. Food ingestion capacity was assessed using a fluorescence-labelled feeding assay. Results: aip mutant zebrafish exhibited growth retardation from 6 days post-fertilisation (dpf), followed by a sharp increase in mortality between 8 and 11 dpf. Although they retained the ability to capture food, a severe loss of epithelial folds and a reduction in Goblet cells were observed from 5 dpf. By 4 dpf, WNT signalling dysregulation and increased proliferation were evident, accompanied by a significant depletion of intestinal stem cells and disrupted epithelial elongation. By 7 dpf, mutants displayed an accumulation of bi-nucleated intestinal epithelial cells and cell jamming. Similarly, small intestine biopsies from an AIP-deficient human patient revealed villous integrity defects, epithelial cyst formation, oedema, and loss of longitudinal muscles. Conclusion: Our findings suggest that aip loss disrupts intestinal development, potentially due to WNT signalling dysregulation and stem cell depletion. The aip mutant zebrafish serves as a valuable model for this novel paediatric disease.

Loss of mechanotransduction activity impacts hair cell development and lateral line mediated behavior

Poster Number: 36

Theme: Organ Formation & Function

Presenting Author: **Keziah Khue Nguyen** - Washington University School of Medicine in St. Louis

Co-Author(s): Sophie Cohen-Bodénès – Postdoctoral Associate, Washington University School of Medicine in St. Louis; Jacob Rudin-Luria – Undergraduate Associate, Washington University School of Medicine in St. Louis; Lavinia Sheets – Associate Professor, Washington University School of Medicine in St. Louis

LHFPL Tetraspan Subfamily Member 5 (Lhfpl5) is a gene required for sensory hair cell function with mutations linked to sensorineural hearing loss. When hair cells are stimulated, Lhfpl5 conducts the force necessary to open mechanotransduction channels by tensioning the connection between the channels and tip-link proteins. Loss-of-function of proteins similarly implicated in hair cell function impair hair cell development, but the role of Lhpl5 in development has not been defined. We leveraged the differential expression of Lhfpl5 zebrafish gene ohnologs and assessed hair cell development and lateral line function in lhfpl5b mutants, which have functional inner ear hair cells but dysfunctional lateral line hair cells. We evaluated lateral line hair cells at three developmental stages—prior to functional maturation (3 dpf), immediately following maturation (5 dpf), and well into functional maturation (7dpf). At 3 dpf, mutants showed a modest but significant reduction in hair cells per neuromast. By 5 and 7 dpf, mutants exhibited markedly fewer hair cells, despite showing increased hair cell proliferation compared to wildtype siblings. To examine the function of mature lateral line organs in lhfpl5b-/- larvae, we assessed rheotaxis—a behavior mediated by the lateral line. Mutant fish showed an inability to station hold, decreased mean and total duration of rheotaxis events, and decreased proportion of fish performing rheotaxis. Overall, loss of lhfpl5b impaired rheotaxis behavior and resulted in fewer hair cells per neuromast. Since hair cell proliferation appears unimpaired, but rather upregulated, in lhfpl5b -/- larvae, our results suggest that the mutants primarily exhibit defects in hair cell maintenance. Future studies will focus on investigating the mechanisms underlying impaired hair cell maintenance and loss.

Identification of a biphasic RA-Notch regulatory cascade governing islet development and differentiation

Poster Number: 37

Theme: Organ Formation & Function

Presenting Author: **Christopher Krueger** - Peking University

Co-Author(s): Mengxuan Wang – Peking University; Qijing Chen – Peking University; Gaofan Meng – Peking University; Xiangjun Tong – Peking University; Zuoyan Zhu – Peking University; Hongkui Deng – Peking University; Bo Zhang – Peking University

Abstract: Pancreatic islet cells play a vital role in blood glucose homeostasis, and their dysfunction leads to diabetes. Understanding pancreatic islet development is crucial for advancing diabetes diagnosis and treatment. Zebrafish (Danio rerio) are an effective model for study of pancreatic development due to their transparent embryos and rapid growth. The zebrafish pancreas consists of primary and secondary islets, and development is regulated by key signaling pathways including retinoic acid (RA) and Notch. RA promotes ectopic islet formation during early development (8–10 hpf), while RA inhibition is required for secondary islet differentiation (3–5 dpf). Similarly, Notch signaling maintains pancreatic

duct cells but must be inhibited for their differentiation into secondary islets. However, the precise crosstalk between RA and Notch pathways remains unclear.

Study of zebrafish mutants to investigate RA/Notch interaction indicated that Notch target gene expression was downregulated in RA-deficient mutants, suggesting that Notch signaling functions downstream of RA. Further, transient activation of Notch during early pancreatic development induced ectopic islet formation, mimicking RA treatment. Furthermore, inhibition of either RA or Notch at later stages enhanced this effect, demonstrating the biphasic nature of their regulation.

Through analysis of transcriptomic data, 48 RA-regulated genes associated with Notch signaling were identified, and analyzed by knockout mutation. Mutant line pku2024 showed reduced primary islet size, increased secondary islet numbers, and decreased number of duct cells. RA was found to regulate pku2024 via conserved enhancer elements, while pku2024 modulated Notch signaling by interacting with GSK-3β. Chemical genetics confirmed the RA-pku2024/GSK-3β-Notch cascade's role in islet formation is conserved across zebrafish and human pancreas. These findings provide novel insights into pancreatic islet development and insights for novel diabetes therapies.

Identification of Notch Receiving Secretory Cells within the zebrafish intestinal epithelium

Poster Number: 38

Theme: Organ Formation & Function

Presenting Author: **Pijush Sutradhar** - Clarkson University

Co-Author(s): Kenneth Wallace – Professor, Biology, Clarkson University

Abstract: The zebrafish intestine develops into a functional organ by 5 dpf that can sustain the post embryonic fish with continual energy after the embryonic yolk runs out. During the third day of embryogenesis, the intestinal epithelium expresses markers indicating specific cell types are differentiating. Previously, we identified a time between 68 to 74 hours where epithelial cells make a choice between enterocyte and secretory cell lineages using Notch lateral inhibition. After 74 hpf (when the epithelial cells are differentiating) we find Notch signaling is used again to specify a subset of secretory cells (identified with a Notch responsive CreERT2 coupled with a nuclear mCherry responder). We originally referred to these cells as Notch Receiving Secretory Cells (NRSCs). As they receive Notch signaling in their differentiation, we disrupted Notch signaling to determine the function of the cells. Upon Notch disruption, we find an increase in epithelial proliferation. Recently, with single cell sequencing, a homologue to the human Best4 cells has been identified in the zebrafish epithelium. As Best4 cells receive Notch signaling, we hypothesized that NRSCs are Best4 cells. We find that the NRSCs are Best4 cells and a single Notch receptor is responsible for their differentiation.

The effects of glypican 4 and wnt5b mutations on hyomandibular cartilage development inlarval zebrafish

Poster Number: 39

Theme: Organ Formation & Function

Presenting Author: **Barbara Sisson** - Ripon College

Co-Author(s): Benjamin Rahlf – Ripon College

Abstract: Craniofacial disorders are among the most common birth defects, many of which are caused by perturbed cartilage development. One signaling pathway that plays an important role in cartilage formation is the Wnt/Planar Cell Polarity (PCP) pathway. This study aims to increase our understanding of craniofacial cartilage development by investigating the effects of PCP mutations glypican 4 (gpc4) and wnt5b on chondrogenesis in zebrafish. Prior research shows that these mutations cause abnormal chondrocyte organization in areas of stacked cartilage, such as the symplectic jaw element. Here we examine the impact of these same mutations on a region of unstacked cartilage adjacent to the symplectic called the hyomandibular. We used MorphoJ software to conduct a morphometric analysis comparing hyomandibular elements of wildtype zebrafish with gpc4m818 and wnt5bta98 mutants. Procrustes ANOVA was run to analyze variations in shape, producing p-values of < 0.001 between each phenotypic group. These results suggest that PCP pathway mutations not only impact stacked cartilage development, but unstacked chondrocyte structures in larval zebrafish as well.

3D modeling of choroid fissure closure in zebrafish

Poster Number: 40

Theme: Organ Formation & Function

Presenting Author: **Parker Coffelt** - University of Northern Colorado

Co-Author(s): Andrea James, PhD – associate professor, Biology, University of Northern Colorado

Abstract: Throughout development there are multiple examples of when two apposed sides of tissues migrate into proximity and engage in cell adhesion; palate formation, neural tube formation, and choroid fissure closure. With multiple organisms and processes contributing to our understanding of the processes there is little information on how force distribution contributes within these processes, specifically in a 3-dimensional model. We are currently creating a 3D model of tissue adhesion accounting for both intra and extracellular forces in addition to adhesive forces upon tissue closure. This study aims to create a 3D representation of retinal choroid fissure closure based on wildtype embryo development. We have attempted confocal imaging in both in vivo and fixed samples to fully define the space measurements between retinal choroid fissure edges at the beginning (44hpf), middle (47 hpf), and completion (52hpf) of choroid fissure closure (CFC). These measurements have created a rubric for testing different intracellular force inhibitors and their effects on CFC. Specifically, we have inhibited tissue migration with the use of both Cyto D, an actin treadmilling inhibitor, and NSC-23766, a Rac-GEF inhibitor. As predicted with our overall eye studies the amount of space between apposed sides of the closing choroid fissure was not reduced in the presence of actin or Rac-GEF inhibition. The data and data that we will further collect from mutant backgrounds of extracellular matrix mutants will be later utilized to refine the computational side of a 3D tissue adhesion model.

THE ROLE OF CALCIUM SIGNALING DURING ANGIOGENESIS

Poster Number: 41

Theme: Organ Formation & Function

Presenting Author: **Megan Detels** - Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH

Co-Author(s): Miranda Marvel – Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH; Dan Castranova – Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH; Van Pham – Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH; Brant Weinstein – Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH

Abstract: Calcium signaling is a critical regulator of cellular responses in both excitable and non-excitable cells. The frequency, length, and intensity of calcium oscillations can differentially regulate a variety of different cellular responses. Endothelial cells, a non-excitable cell type, display calcium oscillations both in culture and in vivo, however the biological relevance of calcium signaling in vascular development and patterning has been largely unexplored. To address this gap, we are developing new transgenic lines to visualize and manipulate calcium signaling specifically in endothelial cells in vivo. By driving endothelial specific expression of the ratiometric calcium indicator GCaMP7s we have been able to observe calcium dynamics in the vascular endothelial cells of living, intact developing zebrafish embryos and larvae. We have also developed novel transgenic lines to drive expression of the far-red light activated channelrhodopsin ChrimsonR in endothelial cells in vivo. Preliminary results using this transgenic line have shown endothelial calcium signaling is increased upon activation, suggesting this will be a valuable tool for testing the functional role of calcium oscillations in the vasculature. In parallel studies, we are also testing the functional role of several calcium transporters expressed in endothelial cells during vascular development. Together, this research will yield important new insights into the role calcium signaling plays in regulating angiogenesis, vessel patterning, and vascular homeostasis, with potentially important implications for future therapeutic approaches to vascular pathologies.

Optogenetic activation of hypothalamic AgRP neurons in transgenic zebrafish larvae increased food intake

Poster Number: 42

Theme: Organ Formation & Function

Presenting Author: **Hossein Mehrabi, PhD** - University of Illinois Chicago

Co-Author(s): Pushkar Bansal – The University of Utah; John Jutoy – University of Illinois Chicago; Erica Jung – University of Illinois Chicago

Abstract: Agouti Related Peptide (AgRP) neurons are located in the hypothalamus, and upon stimulation, these neurons regulate hunger and hunger-mediated behaviors, especially food-seeking and compulsive eating. AgRP neurons are naturally activated by ghrelin binding onto the ghrelin receptors on the neuron surface during starvation or fasting state to evoke the aforementioned behaviors. In this study, we used channelrhodopsin (ChR2), an optogenetic actuator, to control AgRP neuronal activity. For the first time, we observed food-intake behavior in zebrafish larvae by optogenetically triggering AgRP1 neurons. We created a transgenic line, Tg(AgRP1:ChR2-Kaede), where ChR2-Kaede is expressed in AgRP1 neurons. Transgenic zebrafish Tg(AgRP1:ChR2-Kaede) larvae at 6 days post fertilization and wild-type (ABWT) larvae were used to compare the suction behavior. We found

that AgRP1 neuron activation in transgenic larvae led to a significantly higher food-consumption behavior than wildtype larvae when analyzed using Particle Image Velocimetry (PIV) to calculate the food particle velocity initiated by larval suction behavior. These findings in this novel transgenic zebrafish model would be useful in studying various hunger-related behaviors, their underlying neural circuits, and substrates subjected to different chemical stimuli, including drugs of abuse.

GPR37 paralogs mediate diverse roles in the developing nervous system of zebrafish

Poster Number: 43

Theme: Organ Formation & Function

Presenting Author: **Narendra Pathak, Ph.D** - Smith College

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Abstract: Gpr37 or the “Parkin -associated endothelin receptor” is an orphan G-protein coupled receptor that is implicated in negative regulation of the neural stem/progenitor cell dynamics induced by ischemic brain injury in rats. Gpr37 expression is enriched in oligodendrocytes. Seeking to decipher the roles of Gpr37 during injury repair, we sought to characterize its homolog(s) in the highly regenerative zebrafish model. We discovered two gpr37 paralogs (gpr37a and gpr37b) in the zebrafish genome, and here we describe interesting divergence in their expression patterns within the eye and central nervous system along first 6 days of development. Whereas gpr37a appears to be almost exclusively expressed in Gfap+ Müller glia within the inner nuclear layer (INL) of the retina, gpr37b expression is elevated in distinct layers of the retina as well as in the forebrain and olfactory bulb. Not much is known about the functions of gpr37 during eye development. Considering that Gpr37 likely plays a neuroprotective role and regulates gliosis during CNS injury in murine models, we sought to determine whether gpr37 in zebrafish may play a role in Müller glia-mediated injury repair. Interestingly, Müller glia are a unique type of glial cell found in the eye that mediate diverse physiological functions and may also function as multipotent progenitors. We describe here our findings that test the hypothesis that gpr37 is essential for both the development and regeneration of the retina in zebrafish.

The Axillary Lymphoid Organ - an External, Experimentally Accessible Immune Organ in the Zebrafish

Poster Number: 44

Theme: Organ Formation & Function

Presenting Author: **Daniel Castranova** - NICHD, NIH

Co-Author(s): Madeleine Kenton – NICHD, NIH; Aurora Kraus – NICHD, NIH; Christopher Dell – NCI, NIH; Jong Park – NICHD, NIH; Marina Venero Galanternik – NICHD, NIH; Gilseung Park – NICHD, NIH; Daniel

Lumbantobing – National Museum of Natural History, Smithsonian Institution; Luis Dye – NICHD, NIH; Brant Weinstein – NICHD, NIH

Abstract: Lymph nodes and other secondary lymphoid organs play critical roles in immune surveillance and immune activation in mammals, but the deep internal locations of these organs make it challenging to image and study them in living animals. Zebrafish have a previously uncharacterized external immune organ located near the base of the pectoral fins that is ideally suited for studying immune cell dynamics in vivo, the axillary lymphoid organ (ALO). This small, translucent organ has an outer cortex teeming with immune cells, an inner medulla with a mesh-like network of fibroblastic reticular cells along which immune cells migrate, and a network of lymphatic vessels draining to a large adjacent lymph sac. Noninvasive high-resolution imaging of transgenically marked immune cells can be carried out in ALOs of living animals, which are readily accessible to external treatment. We have thoroughly characterized the ALO using confocal microscopy, transmission electron microscopy, array tomography, and single-cell RNA-seq. This newly discovered tissue provides a superb model for dynamic live imaging of immune cells and their interaction with pathogens and surrounding tissues, including blood and lymphatic vessels.

Sclerotome marker, pax9, gene expression in zebrafish development

Poster Number: 45

Theme: Organ Formation & Function

Presenting Author: **Ziyu Dong** - Purdue University

Co-Author(s): GuangJun Zhang – Purdue University

Abstract: Pax (Paired domain) genes play critical roles in embryonic development. Group I pax genes, pax1 and pax9, were known to be essential for pharyngeal and axial skeletal formation. However, the detailed pax9 gene expression in zebrafish ontology remains mainly unexplored, partially due to technical limitations of in situ hybridizations and immunohistochemistry. To overcome this hurdle and examine the pax9-expressing cells under physiological conditions, we employed CRISPR-Cas9-mediated knock-in and inserted fluorescent proteins (mNeongreen and tdTomato) into the endogenous pax9 locus. We found that pax9 is initially expressed in the sclerotome, the ventrolateral regions of the somite during early embryogenesis, in addition to pharyngeal expression. At larval stages, pax9 expression is detected in the pharyngeal region, myosepta, and fin folds. In post-metamorphic juveniles, pax9-positive cells are observed in subsets of fin rays of both median and paired fins. Additionally, pax9 is expressed in craniofacial skeletal elements and the axial skeleton. Our findings suggest that pax9 may play essential roles in skeletal development in zebrafish.

Single-cell analysis of zebrafish reveals process of tooth development

Poster Number: 46

Theme: Organ Formation & Function

Presenting Author: **Kanako Inoue** - National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development

Co-Author(s): Zoe Zwick – National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development; Kiyohito Taimatsu – National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development; Madeleine Kenton – National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development; Gennady Margolin – National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development; Brant Weinstein – PI, National Institute of Health/ Eunice Kennedy Shriver National Institute of Child Health and Human Development

Abstract: Once human teeth are lost, they do not regenerate, and their loss substantially reduces quality of life. Understanding how teeth develop is a critical step toward future tooth regeneration. Although the process of tooth development is highly conserved from fish to mammals, the mechanisms are still not well understood. The zebrafish is an efficient and powerful model for studying tooth development. Zebrafish have 22 pharyngeal teeth that are constantly replaced every 10 days throughout life, allowing observation of multiple stages of tooth development within the same animal. Fish are also highly accessible to high-resolution optical imaging, and an enormous number of transgenic lines are already available marking different cell populations. I performed single-cell RNA-sequencing to uncover the cell types present in developing zebrafish teeth and their gene expression profiles. I used transgenic lines labeling specific cell types, hybridization chain reaction (HCR) RNA fluorescent in situ hybridization targeting cell type-specific genes, and a novel clearing technique enabling optical imaging through bone to analyze the 3D morphology and cellular dynamics of dental and surrounding cell populations, identifying distinct cell types corresponding to the major dental cell types found in mammalian teeth. Many of the genes expressed in zebrafish teeth are also found in mammalian teeth, suggesting their differentiation processes are evolutionarily conserved. Further characterization of these genes will provide important clues into the developmental origins of teeth. Together, our work has provided a comprehensive framework for anatomical, cellular, and molecular analysis of tooth development, identified potential regulators of tooth development that we are following up on, and underscored the utility of the zebrafish as a powerful model for analyzing tooth development.

Active NFkB holds hematopoietic precursors quiescent during specification

Poster Number: 47

Theme: Stem Cells

Presenting Author: **Rodolfo Calderon** - Iowa State University

Co-Author(s): Clyde Campbell – Iowa State University; Raquel Espin Palazon – Iowa State University

Abstract: Hematopoietic stem cells (HSCs) are responsible for maintaining the entirety of the blood network in mature systems. With maintaining this population, HSCs also must self-renew their own pool because they are produced only during embryogenesis. Disruptions in the healthy maintenance and population of hematopoietic cells can have. Fatal effects, begging a solution by medical intervention. A large goal in hematopoietic research is to fully understand the signals required for HSC development to engraft cells that have been reprogrammed to a hematopoietic state. This full set of signals has yet to be described. In this developmental process, the earliest cells expressing hematopoietic identifying markers arise from endothelial tissue. The activity of transcription factor NFkB has been identified to play a key role in the specification and establishment of the hematopoietic population from this endothelial

population by specific targeting of genes. Recently, our team found the role of NFkB is also temporally important and has specific importance during each phase of its dynamic activity. Here, we show that an active NFkB molecule will hold cells in a quiescent state, halting proliferation and allowing for specification towards a hematopoietic fate. To accurately investigate the temporal activity of NFkB, we utilized a fluorescent destabilized reporter line. Through antibody staining, we analyzed cell cycle and mitotic state by both confocal imaging and flow cytometry. In this effort, we aim to provide more detail to what is required for natural hematopoietic development and the potential for amplifying developing hematopoietic cells for engraftment.

Extracellular Matrix-Associated Lysyl Oxidases as Novel Regulators of Hematopoietic Stem Cell Specification

Poster Number: 48

Theme: Stem Cells

Presenting Author: **Alissa Jackson** - St. Jude Children's Research Hospital

Co-Author(s): Wilson Clements – PI, Hematology, St. Jude Children's Research Hospital; Elizabeth Coffey – Postdoctoral researcher, Hematology, St. Jude Children's Research Hospital

Abstract: Hematopoietic Stem Cells (HSCs) give rise to all mature and immature blood cells throughout adult life. HSCs are an example of a tissue-specific stem cell population with multipotency and self-renewal capacities, and they are clinically used to treat hematological malignancies and immunodeficiencies. HSCs are specified from hemogenic endothelial cells in the developing aorta in an evolutionarily conserved process termed endothelial to hematopoietic transition. HSC specification is coordinated by intrinsic cell processes within hemogenic endothelium and extrinsic signals from neighboring cell populations in the microenvironment such as Wnt, Notch, and TGFβ, among others. Investigation of the native microenvironment in which HSCs emerge during development could inform what signals are necessary for HSCs to be specified in vitro. In addition to heterogeneous cell populations, a major component of the local microenvironment is the extracellular matrix (ECM). ECM gene expression is a feature of both embryonic specification and adult homeostatic HSC niches. Although cellular signaling regulating HSC specification has been extensively examined, little is known about the direct contribution of ECM organization and ECM-directed signaling to HSC specification. Our preliminary data implicate regulators of ECM maturation—specifically members of the lysyl oxidase (LOX) enzyme family—as required factors for HSC specification, suggesting that proper ECM organization is necessary for HSC emergence. Knockdown of Loxl3b, a LOX family member in zebrafish, results in diminished expression of HSC marker genes at multiple stages of hematopoietic development. This project aims to investigate the role of ECM enzymatic remodeling in the microenvironment proximal to the dorsal aorta during specification of HSCs. We aim to uncover connections between ECM architecture and HSC specification by elaborating normal physiological ECM organization and using genetic loss of function (LOF) models in LOX family enzymes to define the requirement for proper ECM development in forming a productive embryonic specification HSC niche.

Investigating Hdac1 mechanisms integrating forebrain FGF signaling with neural progenitor cell cycle control.

Poster Number: 49

Theme: Stem Cells

Presenting Author: **James Preston** - Iowa State University

Co-Author(s): Fang Liu – Iowa State University; Alec Gardner – Iowa State University; Maura McGrail – Iowa State University

Abstract: During development of the forebrain primordium the FGF signaling pathway ensures proper brain growth by regulating progenitor cell cycle dynamics and cell fate. Using Cre/lox conditional knockout we found epigenetic regulator Histone deacetylase 1 (Hdac1) is required for forebrain development and neural progenitor proliferation. Loss of Hdac1 in Ascl1b progenitors results in a significant decrease in the number of pH3+ mitotic cells in the forebrain. This correlates with a reduction of post-mitotic Elavl3+ neurons at 2 dpf and of Th+ mature neurons at 5 dpf. These results indicate Hdac1 is required to maintain Ascl1b progenitors in the cell cycle and prevent premature cell cycle exit. To determine if Hdac1 is a component of FGF signaling in forebrain development, we first tested whether Hdac1 activity is regulated by an FGF downstream effector, the serine-threonine kinase Casein Kinase 2 (CK2). CRISPant deletion of the CK2 regulatory subunit Cskn2b in wild type embryos led to reduced forebrain at 2 dpf, consistent with a role for CK2 signaling in forebrain development. Mutagenesis of conserved CK2 target Serine residues disrupted Hdac1 function and localization activity in transient injections. We next isolated a stable hdac1-C-terminal-V5 knock-in line using CRISPR/Cas9 HDR with a single strand oligonucleotide template. Preliminary RNASeq analysis of Ascl1b-Hdac1 KO 2dpf embryos indicates altered expression of Notch, Wnt and cellular senescence pathways. Experiments are in progress with our Hdac1-V5 line to test whether Hdac1-S421/423-phosphorylation and nuclear localization are dependent on FGF/Csnk2 signaling, and to identify FGF/CK2 pathway cell genomic targets regulated epigenetically by Hdac1 to control neural progenitor proliferation. Together, these results point to a central role for Hdac1 in FGF signaling driving neural progenitor proliferation. These studies will lead to increased understanding of mechanisms linking forebrain growth factor signaling with Hdac1 epigenetic cell cycle control for forebrain development.

NF-kB Signaling Dynamics Govern Hematopoietic Stem Cell Development

Poster Number: 50

Theme: Stem Cells

Presenting Author: **Jonathan Praseutsack** - Iowa State University

Co-Author(s): Clyde Campbell – Assistant Professor, Genetics, Development, Cell Biology, Iowa State University; Raquel Espin Palazon – Assistant Professor, Genetics, Development, Cell Biology, Iowa State University

Abstract: Hematopoietic stem and progenitor cells (HSPCs) are multipotent and self-renewing cells that aid an organism in maintaining its blood system throughout its life; this process is conserved among all vertebrates. Our group has identified inflammatory signaling as a critical part of HSPC specification. However, the spatial-temporal requirements of these pathways have remained ambiguous. To address this knowledge gap, we investigated NF-kB signaling dynamics since it is the hub for which many pro-

inflammatory signals converge during HSPC specification. Here, we generated novel zebrafish NF-kB reporter lines that drive destabilized versions of eGFP, shortening the half-life to two hours and allowing us to pinpoint the precise developmental window these signals are required. This enabled us to identify two distinct waves of NF-kB activity within the blood-forming regions of the dorsal aorta. Utilizing confocal microscopy, live imaging, and flow cytometry, we identify that NF-kB is activated in the pre-hemogenic endothelium between 18-24 hpf. We then disrupt the window of NF-kB activity via chemical inhibition with CAPE; the HSPCs fail to specify. In addition, we demonstrated that this early NF-kB requirement is conserved in the generation of human iPSC-derived HSPCs in vitro. The second wave of NF-kB activity occurred between 31-44 hpf, which coincides with the time HSPCs complete endothelial to mesenchymal transition. Surprisingly, upon stage-specific disruption of the second wave, we identified a significant proliferative expansion of the HSPCs, resulting in large intra-aortic cd41:GFP+ clusters and the absence of cd41+ circulating cells; this consequently led to a decrease in colonization of the caudal hematopoietic tissue (CHT). Our data identify for the first time the oscillatory dynamics of NF-kB activity during HSPC development and could be applied in vitro to improve protocols of hematopoietic differentiation from HPSCs.

Evolutionary trajectories of hematopoietic stem cells: From conserved regulatory circuits to clade-specific diversification

Poster Number: 51

Theme: Stem Cells

Presenting Author: **Wang Chunling** - Shandong university

Co-Author(s): Xuejing Zhang – Shandong university

Abstract: Hematopoietic stem cells (HSCs) are pivotal for lifelong blood production, yet their evolutionary origins and developmental trajectory across species remain incompletely understood. Here, we conducted a cross-species analysis of HSC emergence and differentiation, spanning diverse organisms from invertebrates, basal chordates, and vertebrates to higher mammals. Our study reveals both evolutionarily conserved and species-specific features of hematopoietic development. Notably, through single-cell RNA sequencing (scRNA-seq) and cross-species analysis, we identified shared molecular signatures and regulatory pathways governing HSC specification and homeostasis , while also uncovering adaptations unique to certain lineages. These findings provide a comprehensive view of hematopoietic evolution, and underscore the importance of cross-species analyses in unraveling the fundamental principles of hematopoiesis, offering insights into the ancient origins of HSCs and the adaptive mechanisms that have shaped their functional diversity.

iRhom1a regulates vascular and hematopoietic development via ADAM17b-dependent signaling

Poster Number: 52

Theme: Stem Cells

Presenting Author: **Taylor Schoen, PhD** - University of California - San Diego / HHMI

Co-Author(s): Lin Grimm – Cedars-Sinai Medical Center; Sonya Neal – University of California - San Diego / HHMI; David Traver – Cedars-Sinai Medical Center

Abstract: During embryogenesis, the concurrent development of blood vessels and blood cells relies on proper endothelial cell differentiation and signaling. Endothelial cells integrate cues from growth factors, inflammatory cytokines, and chemotactic signals to guide vascular and hematopoietic development. A key regulator of these signals is the metalloprotease ADAM17, which requires the catalytically inactive rhomboid protein iRhom1 for its trafficking and maturation. While ADAM17 has a well-established role in cleaving vascular surface molecules, no direct role for ADAM17 in hematopoiesis has been described. Furthermore, the specific contribution of iRhom1 as an upstream regulator in these developmental processes remains poorly understood. Here, we show that irhom1a, a zebrafish ortholog of iRhom1, is highly expressed in the central arteries of the hindbrain, and that morpholino-mediated knockdown of irhom1a leads to impaired vascular development in both the trunk and brain. irhom1a morphants also exhibit spontaneous hemorrhaging, indicating compromised vascular integrity. Given the overlap in signaling pathways that regulate angiogenesis and hematopoiesis, we hypothesized that iRhom1a may also contribute to hematopoietic stem and progenitor cell (HSPC) development. Indeed, we observed a significant reduction in HSPC emergence from the dorsal aorta at 48 hours post-fertilization in irhom1a morphants. To determine whether these effects are mediated through ADAM17, we performed targeted knockdowns of adam17a and adam17b. While adam17a knockdown had no significant impact on HSPC numbers, adam17b knockdown phenocopied irhom1a loss, reducing HSPC production to similar levels. These findings uncover an unrecognized role for the iRhom1a–ADAM17b sheddase complex in HSPC emergence and warrant further investigation into the endothelial-specific role of this complex during vascular and hematopoietic development.

Leptin-induced fatty acid β-oxidation is critical for hematopoietic stem cell generation

Poster Number: 53

Theme: Stem Cells

Presenting Author: **Lina Sun** - Chinese Academy of Medical Sciences and Peking Union Medical College

Co-Author(s): Mengyao Liu – Chinese Academy of Medical Sciences and Peking Union Medical College; Feng Liu – Institute of Zoology, Chinese Academy of Sciences; Lu Wang – Chinese Academy of Medical Sciences and Peking Union Medical College

Abstract: Hematopoietic stem and progenitor cells (HSPCs) arise via endothelial-to-hematopoietic transition (EHT) during embryogenesis, a process precisely regulated by cell metabolism. Leptin is a peptide hormone involved in metabolic regulation, yet its role in HSPC development remains elusive. Here, we identify leptin signaling as a key regulator of HSPC generation in zebrafish. Spatiotemporal profiling reveals that leptin signaling is highly enriched in muscle cells at 36 hours post-fertilization (hpf), concurrent with HSPC generation in zebrafish embryos. Genetic ablation of leptin or its receptor disrupts myofiber organization and severely impairs HSPC production. Mechanistically, leptin signaling enhances fatty acid oxidation (FAO) through AMPK activation in muscle cells, inducing the expression of the myokine to orchestrate HSPC generation. Our findings establish leptin as a metabolic orchestrator

linking muscle FAO to HSPC generation, providing new insights for enhancing HSPC production in regenerative medicine.

Contribution of NOD-Like Receptors to Hematopoietic Stem Cell Development

Poster Number: 54

Theme: Stem Cells

Presenting Author: **Shuvra Dutta** - Iowa State University

Co-Author(s): Xiaoyi Cheng – Columbia University; Masuma Khatun Usha – Iowa state University; Rodolfo Calderon – Iowa state University; Jonathan Praseutsack – Iowa State University; Clyde Campbell – Iowa state University; Raquel Espin Palazon – Iowa State University

Abstract: In the presence of infection, immune cells recognize pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs), leading to NF-kB activation and pro-inflammatory cytokines expression and triggering inflammation. However, pro-inflammatory signals are also required to specify hematopoietic stem and progenitor cells (HSPCs) in the absence of infection, a phenomenon called developmental inflammation. There is still a gap in understanding the mechanism involved in driving HSPC specification via developmental inflammation, which needs to be uncovered to generate efficiently functional human induced pluripotent stem cell (hiPSC)- derived HSPCs and their derivatives. We have been exploring the NOD-like receptors (NLRs), which is one of the major sub-families of PRRs, in the context of HSPC development. Two NLR family members, Nod1 and Nod2, drive MAPK and NF-kB activation via Ripk2 phosphorylation during classical inflammation. We revealed that during embryonic development, the Nod1-Ripk2-NF-kB inflammatory pathway in endothelial cells (ECs) primes them to switch fate towards definitive hemogenic endothelium, which is a pre-requisite to specify HSPCs. Since Nod1 drives the expression of pro-inflammatory cytokines and crosstalk with Nod2, we are now exploring the role of Nod2 in HSPC specification. Using transgenic, knockout, and chemically treated zebrafish embryos, we discovered that Nod2 regulates HSPC specification in vivo in a Ripk2-dependent manner. However, Nod2-mediated HSPC specification did not involve NF-kB activation. Unexpectedly, we found contradictory phenotypes for Nod1 and Nod2. While HSPC specification increased following Nod1 overexpression, HSPCs failed to specify after Nod2 overexpression. Interestingly, Nod2 knockouts had significantly reduced HSPCs. These data indicate that precise levels of Nod2 control HSPC development. Understanding the interplay between these NLRs and their manipulation could assist in improving the yield of hiPSC-derived HSPCs and their derivatives for blood disorder treatment.

Embryonic Origins of Hematopoietic Stem Cell Diversity

Poster Number: 55

Theme: Stem Cells

Presenting Author: **Joey Ghersi** - CHU Sainte Justine, University of Montreal

Co-Author(s):

Abstract: In a process conserved across vertebrates, definitive hematopoietic stem/progenitor cells (HSPCs) are produced during embryonic development from an endothelial-to-hematopoietic transition

of a specialized population of endothelial cells. Recently, HSPCs have been shown to be heterogeneous, with distinct cell division, differentiation, and transplantation capacities. Here, we demonstrate that embryonic endothelial cells control the formation of HSPC heterogeneity, reshaping our understanding of hematopoiesis.

In a genetic screening for cardiovascular phenotype, we identified a miRNA loss of function mutant, miR-128 (128-/-), with increased EHT resulting in supernumerary HSPCs. Transcriptome analysis of ECs from wild-type and 128-/- zebrafish embryos revealed miR-128 target genes are involved in the inhibition of the EHT signaling pathway, Wnt and Notch. The direct genetic manipulation of miR-128-target genes supported the functional role of miR-128 in controlling this HSPC heterogeneity. Altogether, we showed that embryonic endothelial cells are at the top of the hierarchy of hematopoiesis, shafting our previous understanding.

The new questions arising from these advances focus on defining the consequences and significance of the tight regulation of HSPC heterogeneity established early in development. In this context, we have identified a novel regulator of lineage potential associated with an autoimmune disorder. Notably, our regulator, one of the most frequently mutated genes predisposing to type 1 diabetes, plays a key role in regulating embryonic HSPC diversity. These preliminary findings suggest that HSPC heterogeneity is, at least in part, established during embryonic development, and that its disruption may lead to an imbalanced blood cell composition, contributing to the onset of type 1 diabetes.

Blood flow directs Yap/Taz-mediated transcriptional regulation of self-renewal programs to control developmental HSPC expansion by mechanical stimulation of Piezo1

Poster Number: 56

Theme: Stem Cells

Presenting Author: **Wade Sugden** - Versiti Blood Research Institute

Co-Author(s): Zachary LeBlanc – Boston Children's Hospital; Stephan George – Boston Children's Hospital; Morgan Walcheck – Boston Children's Hospital; Wandi Zhu – Brigham and Women's Hospital; Rubul Mout – Boston Children's Hospital; Caroline Schuster-Kubaczka – Boston Children's Hospital; Calum MacRae – Brigham and Women's Hospital; George Daley – Boston Children's Hospital; Trista North – Boston Children's Hospital

Abstract: Hematopoietic stem and progenitor cells (HSPCs) emerge from artery-derived hemogenic endothelium (HE) in vertebrate embryos, driven by the Runx1 transcription factor (TF). Physical forces of wall shear stress (WSS) and cyclic stretch (CS) produced by blood flow are required to generate HSPCs from HE, but mechanisms by which these forces are sensed and converted into a “stemness” regulatory module remain incompletely understood. Using scRNA-sequencing of zebrafish trunk endothelial cells, we find via gain- and loss-of-function that the Hippo pathway TF YAP drives a glycolysis-to-oxidative phosphorylation shift, cell cycling and propagation of a hematopoietic gene regulatory network in the earliest specified HE cells. By employing a heat shock-inducible dominant negative YAP zebrafish line, we reveal an unanticipated role for the YAP paralogue TAZ in hematopoiesis, which can promote CD41+ and Flk+/Myb+ HSPC production upon reduced YAP function. YAP and TAZ initiate transcriptional responses

downstream of mechanical stimuli and require DNA binding cofactors to regulate target genes. Surprisingly, luciferase assays in HEK293 cells demonstrate a potent synergistic effect of TAZ/RUNX1, but not YAP/RUNX1, in transcriptional regulation at RUNX enhancers. In vivo allelic series experiments point to participation of canonical TEAD interactions in addition as necessary for hematopoiesis. Finally, by pharmacologic and genetic manipulation, we identify the stretch-gated membrane ion channel Piezo1 as a regulator of CS-induced YAP/TAZ mechanotransduction in HE. Stimulation of zebrafish embryos with the Piezo1 small molecule agonist Yoda1 increases HSPC number and YAP target gene expression in a YAP-dependent fashion. A similar modulation of blood and YAP target genes in human iPSC-derived CD34+ HE cells is seen with Yoda1, suggesting that this stretch-Piezo1-YAP axis can be chemically tuned in vitro to enhance HSPC differentiation. These results have broader implications for alternate regulatory effects of mechanically-stimulated Hippo TFs depending on the transcriptional milieu in cell-type specific contexts.

Loss of Stem Cells in the Extrahepatic Duct due to Biliary Atresia Compromises Liver Regeneration

Poster Number: 57

Theme: Stem Cells

Presenting Author: **Yu Fei Hao** - Sanford Burnham Prebys

Co-Author(s): Joseph Lancman, Ph.D. – Sanford Burnham Prebys; Chengjian Zhao, Ph.D. – Sanford Burnham Prebys; P. Duc Si Dong, Ph.D. – Associate Professor, Sanford Burnham Prebys

Abstract: Biliary Atresia (BA), the leading indication for pediatric liver transplantation, is characterized by the defect and blockage of the extrahepatic duct (EHD), causing cholestasis and consequently, liver damage and failure. However, despite a surgical bypass with the Kasai procedure to remove the EHD and directly connect the liver to the small intestine to redirect bile flow, most patients develop intrahepatic liver diseases later in life, suggesting BA to be a more profound liver disease beyond cholestasis. Using a zebrafish Alagille Syndrome cholestasis model, we uncovered a novel source of multipotent progenitors residing in the EHD that contribute to liver regeneration, suggesting a more critical role for the EHD as a source of liver stem cells. This discovery led us to hypothesize that EHD damage in BA may deplete these liver stem cells and compromise liver regeneration. To model BA-associated EHD damage, we are utilizing biliatresone, a plant toxin that induces BA phenotype with selective EHD damage in sheep, zebrafish, mice, and human EHD organoids. We confirmed that biliatresone treatment in zebrafish leads to damage in the EHD but not in the liver, indicating that this toxin does not directly affect the liver. Additionally, we find evidence of apoptosis and misdifferentiation in the EHD, suggesting that the stem cells in the EHD are impacted in BA. Consistently, we find impaired hepatocyte regeneration following BA exposure, providing the first functional evidence showing EHD damage compromises liver regeneration. Finding a loss of a critical stem cell source in BA will inform possible changes to the current Kasai procedure approach and new therapeutic strategies. Furthermore, with the EHD as a source of liver stem cells, targeting these cells may enhance liver gene therapy and transplantation strategies for other liver diseases.

The regulatory mechanism of small molecular ADM during hematopoietic stem and progenitor cell fate determination

Poster Number: 58

Theme: Stem Cells

Presenting Author: **Yanyan Ding** - Guangzhou Medical University

Co-Author(s): Jinzeng Wang – Shanghai Jiao Tong University School of Medicine; Yue ying Zhang – Guangzhou Medical University; Guihua Kang – Guangzhou Medical University; Nuona Lei – Guangzhou Medical University

Abstract: Protein kinases control numerous critical biological processes by mediation of protein phosphorylation, and become important drug targets. The hemato-vascular progenitor specification at the mesoderm stage is accompanied by dynamic changes in protein phosphorylation. However, which and how kinases regulate this process remain unclear. Using in vitro hematopoietic progenitor cell induction model, we aim to characterize the key regulatory kinases during hematopoietic specification at mesoderm stage through high-content and high-throughput screening of kinase inhibitors library. Systematical analysis identified that ADM is a previously unreported small molecule in vertebrate hematopoiesis, which obviously promotes hematopoietic progenitor cell generation both in vitro and in zebrafish embryos. Using mass spectrum analysis, we identified the direct target of ADM. Then, we will utilize in vitro induction system and zebrafish model, and perform high-throughput sequencing analysis, biochemical identification, genetic phenotypic detection, and in vivo imaging to validate the function of ADM and reveal the underlying mechanisms. Furthermore, we will optimize the strategies for hematopoietic cell induction based on ADM. This project will not only promote the understanding of the developmental origins of hematopoietic stem and progenitor cells but also provide insights for guiding hematopoietic cell induction in vitro.

Evolving paradigm of hematopoietic stem cell biology

Poster Number: 59

Theme: Stem Cells

Presenting Author: **Feng Liu** - Institute of Zoology, Chinese Academy of Sciences

Co-Author(s):

Abstract: In vertebrates, the earliest hematopoietic stem cells (HSCs) arise from the ventral wall of dorsal aorta during embryogenesis, which then migrate through successive organs, and finally lodge in the bone marrow (mammals) and the kidney marrow (teleost) to sustain hematopoiesis during adulthood. Although extensive efforts have been spent to explore the developmental path and underlying mechanisms of HSC generation, expansion and maturation, we are still unable to fully recapitulate the in vivo process through in vitro approaches. Clearly, our understanding of regulatory machinery of HSC development remains incompletely understood. In this talk, I will introduce our recent work in defining the genetic and epigenetic mechanisms of the roadmap of HSC formation and

expansion using zebrafish and mammalian systems, which may provide new insights for induction and expansion of HSCs in vitro.

Distinct Roles of Rpl22 and Rpl22-Like1 in Hematopoietic Stem Cell Emergence: Opposing Mechanisms Mediated by Their N-Terminal Domains

Poster Number: 60

Theme: Stem Cells

Presenting Author: **MANOJ SINGH** - Fox Chase Cancer Center

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Abstract: Ribosomal proteins (RP) play important roles in hematopoiesis, morphogenesis, and transformation; however, the mechanistic basis for their functions remains hotly debated. One model proposes that RP regulate these processes by altering the control of the ribosome, while the other competing model proposes that the regulatory roles of RP are mediated extra-ribosomally while physically separate from the ribosome. Distinguishing these mechanisms has been extremely challenging. Here, we present compelling data suggesting that the ribosomal protein L22 (RPL22) regulates hematopoietic stem cell (HSCs) emergence in zebrafish and leukemia progression in an extraribosomal manner. Rp22 and its paralog Rpl22-Like1 (Like1) regulate HSC emergence an antagonistic manner by binding the same collection of mRNA targets but having opposing effects on them. We find that Rpl22 is capable of performing its distinct, antagonistic function through binding a distinct set of co-factors through its unique N-terminal 15 amino acids. Specifically, we have determined that Rpl22 regulates stem cell emergence through association with splicing factors via its N-terminal 15 amino acids, including hnRNP-A1 and cwc22, which are required for the ability of Rpl22 to repress HSC emergence. Importantly, we found that hnRNP-A1 associates exclusively with the extra-ribosomal, free pool of Rpl22. Using HYPER-TRIBE target identification, we determined that one of the critical mRNA targets of RPL22 is Ypel3. We think Ypel3 is one of the targets through which Rpl22 regulates HSC function and the pathogenesis leukemias driven by the MLL-AF9 oncogenic fusion. Together, these finding provide evidence for extraribosomal function of RPL22 in regulating both the emergence and cancer predisposition of HSC through controlling the splicing of critical target genes.

Functional and molecular dissection of primitive and definitive myelopoiesis

Poster Number: 61

Theme: Stem Cells

Presenting Author: **Masuma Khatun Usha** - Iowa State University

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Abstract: Pre-natal immune cells such as macrophages not only sculpt tissues during development, but contribute to the long-standing homeostasis of adult tissues and their immune properties in health and disease. In recent years, it has become clear that layered hematopoiesis produces ontologically different subsets of immune cells. However, the temporal and spatial overlap of different embryonic myelopoietic waves and the lack of markers to separate each macrophage subset has complicated these analyses, leaving an important knowledge gap. In this study, we utilized the zebrafish temporal-spatial separation of the myelopoietic waves to study the role of granulin a (grna) on each wave. Through the generation of inducible grna zebrafish, in conjunction with knockout ablation models, FACS sorting, and transcriptomic profiling, we demonstrate that grna is essential to establish the myeloid program from embryonic transient erythromyeloid progenitors (EMPs), but dispensable for primitive myelopoiesis. In addition, in vivo lineage tracing experiments coupled with live imaging and tissue-specific ablations demonstrate that EMP-derived myeloid cells respond efficiently to injury insults, while primitive macrophages only recruited minimally to the wound. In addition, lymphatic development was greatly impaired when EMP-derived myeloid cells were disrupted. Together, these experiments demonstrate that primitive and EMP myeloid cells are functionally and molecularly distinct, and that EMP-derived macrophages are the main myeloid effectors of tissue regeneration and lymphatic development.

Advancing toxicology data integration through a community-led zebrafish atlas phenotype project ZAPP

Poster Number: 62

Theme: Toxicology & Environmental Assessment

Presenting Author: **Alexa Burger, PhD** - University of Colorado Anschutz Medical Campus

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Abstract: Zebrafish are a valuable model for toxicology and environmental health research due to their high fecundity, rapid development, and genetic similarity to mammals. However, the absence of universal standards for toxicology data limits data interoperability and poses a major barrier to scientific progress, with significant implications for human and environmental health. The Zebrafish Atlas Phenotype Project (ZAPP), a community-led effort, aims to address this gap by (1) standardizing zebrafish toxicological exposure experiments and their resulting phenotypic outcomes (toxicophenotypes), (2) the creation of a phenotype atlas (mostly, but not uniquely) focused on toxicophenotypes to serve as a visual definition for phenotypes. Central to this effort is the creation of

standards to report phenotypes and toxicological exposures, which will improve data integration and interoperability. The Atlas will allow users to explore annotated data, view example phenotypic images, and access comprehensive documentation of community standards. ZAPP relies on the community, including the zebrafish researchers and toxicology experts to submit images associated with standardized annotations. This project therefore requires community discussions to determine how toxicology data should be reported, which standards for phenotype and toxicology information should be used, etc. We conducted workshops to gather community requirements and input on conceptualizations of the atlas and best practices for reporting toxicological data and phenotypic outcomes. To date, a draft data model has been developed, capturing key experimental parameters, including exposure route and duration, toxicant identity and concentration, genotype and genetic manipulations, and observed phenotypic outcomes. Upcoming workshops will include discussions on existing standards and ontologies for reporting zebrafish toxicological data and phenotypes. Ultimately, this project is community-governed to engage diverse stakeholders from toxicology and environmental health sciences to ensure a fit-for-purpose design and long-term sustainability to enhance toxicological data integration. This work is supported by NIH/NIEHS grant 1R24ES036130-01.

Polypropylene nanoplastics enhances the intestinal inflammation induced by disinfectants in zebrafish larvae

Poster Number: 63

Theme: Toxicology & Environmental Assessment

Presenting Author: **Yugyeong Sim** - Korea Research Institute of Bioscience and Biotechnology

Co-Author(s): Jinyoung Jeong – Korea Research Institute of Bioscience and Biotechnology

Abstract: Microplastics are widely distributed environmental pollutants that enter the human body through various routes, including drinking water, food packaging, and food itself. Previous studies have reported that microplastic ingestion can induce oxidative stress, intestinal inflammation, and gut microbiota dysbiosis. However, most research has focused on healthy conditions, which do not accurately reflect real-life situations where individuals experience intestinal inflammation due to dietary habits, medications, or environmental pollutants. To better represent these conditions, we designed an experiment that simulates microplastic exposure in an inflamed intestinal environment. In this study, benzalkonium chloride (BAC), a commonly used disinfectant and environmental pollutant, was used to induce intestinal inflammation, while polypropylene nanoplastics (PPNPs) were selected as a representative microplastic. Using zebrafish (Danio rerio) larvae as an animal model, we first exposed the larvae to BAC at 3 days post-fertilization (dpf) for 48 hours to induce gut inflammation. Then, at 5 dpf, the larvae were exposed to PPNPs via ingestion for an additional 48 hours. Our findings revealed that PPNPs significantly enhanced intestinal toxicity in BAC-pre-exposed larvae. PPNPs accumulated 2.8 times more in the inflamed intestine compared to the intestine of larvae exposed only to PPNPs. Although PPNPs alone did not trigger immune cell migration, they markedly increased 3 times macrophage activity in the inflamed intestine and upregulated the expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-8. These results indicate that while PPNPs alone may not cause severe inflammation in the intestine, their presence exacerbates toxicity in an inflamed gut. This study highlights the potential health risks of microplastic ingestion, particularly in individuals with intestinal

inflammation, and underscores the importance of evaluating microplastic toxicity in more physiologically relevant conditions.

Using microinjection to test developmental toxicity of pharmaceuticals in zebrafish

Poster Number: 64

Theme: Toxicology & Environmental Assessment

Presenting Author: **Hannah Nonarath, PhD** - AbbVie

Co-Author(s): Kayla Frost – AbbVie; Rebecca Kohnken – AbbVie; Rita Ciurlionis – AbbVie

Abstract: Introduction/Objectives: Thalidomide is an established teratogen and causes severe developmental malformations in humans and other species. Replicating these effects in zebrafish is challenging because of its low solubility and stability in water. This study addresses these limitations by employing microinjection techniques to directly deliver thalidomide into zebrafish embryos. Experimental Design/Methods and Materials: Two methods were used to dose zebrafish embryos with thalidomide, bath application and microinjection. During bath application, adjustments were made to the chorion status and DMSO concentration to evaluate their impact on thalidomide absorption and the development of associated phenotypes (abnormal fin development). The second method involved microinjecting thalidomide into zebrafish embryos at various doses and developmental stages. Results: Bioanalysis results showed that the chorion status did not affect the levels of thalidomide in larvae 72 hours after treatment. Increasing DMSO levels to 1.5% during bath application allowed higher thalidomide dosing (600 μM) and improved aqueous solubility. Despite these modifications, the most significant developmental effect observed was a decrease in the length of the larvae. Abnormal fin development was present in less than 2% of animals following various bath application methods. However, by microinjecting thalidomide before 8 hours post-fertilization, we significantly increased the occurrence of altered fin development to over 30% of the animals. Conclusion: Microinjection effectively increases thalidomide exposure and the penetrance of altered fin development. Impact Statement: Microinjection opens new avenues for applying zebrafish models to study the developmental effects of compounds with low aqueous solubility.

Evaluation of the Nanotoxicological Properties of CYS-CuInS2/ZnS Quantum Dots using Larval Danio rerio

Poster Number: 65

Theme: Toxicology & Environmental Assessment

Presenting Author: **Camille Kate Palomares, MS** - Ateneo de Manila University

Co-Author(s): Olivia Erin Buenafe, Ph.D. – Assistant Professor / Chair, Chemistry, Ateneo de Manila University; Erwin Enriquez, Ph.D. – Professor, Chemistry, Ateneo de Manila University; David So – Ateneo de Manila University

The water-soluble L-cysteine-capped copper indium sulfide-zinc sulfide quantum dots (CYS-CuInS2/ZnS QDs) are fluorescent ternary semiconductor nanocrystals currently explored for bioimaging applications

due to their lower toxicity (versus traditional Cd- or Pb-based QDs) and tunable properties, as they can emit at wavelengths within the biological optical window (650-1350 nm). For such applications, their nanotoxicological properties need assessment, and using larval zebrafish (Danio rerio) as the first-tier model for nanotoxicological profiling provides advantages due to their small size, rapid development, and optical transparency, allowing in vivo observation of phenotypic changes related to the QD's possible nanotoxicological activities.

Utilizing an aqueous-based hot reflux setup, this study successfully synthesized CYS-CuInS2/ZnS QDs with a hydrodynamic diameter of 23.3 nm, stability at the physiological pH, and an emission wavelength of 663 nm. Without further QD modifications, healthy zebrafish embryos were semi-statically exposed to different QD concentrations from 24 to 96 hours post-fertilization (hpf) in a 96-well plate format. OECD TG 236 and ISO/TS 22802 were modified to account for the stability of QDs in the exposure medium.

Results show that hatching delays are often an overlooked indicator of nanotoxicity, as exposure of chorionated embryos to CYS-CuInS2/ZnS QDs induced hatching delays at lower QD concentrations, causing higher mortality rates and a high incidence of yolk deformation. We hypothesize that dissociated L-cysteine may have compromised the hatching enzymes, but confirmatory studies are needed. In contrast, dechorionation increased the embryo's sensitivity to the QDs, and higher mortality rates and higher incidence of morphological malformations were instead observed at higher QD concentrations. Lastly, altered heart rates were also observed among exposed embryos, indicating potential cardiotoxic effects.

While testing nanomaterial safety using zebrafish is still in its infancy, this study asserts that composition affects the nanotoxicological properties of QDs more than their size.

Neurotoxic aspects of dibutyl phthalate and its metabolite in zebrafish larvae model

Poster Number: 66

Theme: Toxicology & Environmental Assessment

Presenting Author: **Sangwoo Lee, PhD** - Korea Institute of Toxicology

Co-Author(s): Suyeon Lee – Korea Institute of Toxicology; Kojo Eghan – Korea Institute of Toxicology; Woo-Keun Kim – Korea Institute of Toxicology

Abstract: Though dibutyl phthalate (DBP), commonly used plasticizer, poses significant neurotoxic concerns, the effects of its metabolite, mono-n-butyl phthalate (MBP), remain unclear. This study examines the developmental and neurotoxic effects of DBP and MBP on zebrafish (Danio rerio) larvae. Wild-type and transgenic (tg(elavl3) and tg(mbp) zebrafish were exposed to DBP and MBP from 4 hpf to 120 hpf. Developmental toxicity was assessed using a variety of parameters, including survival, hatching rate, eye size, deformity, tail coiling, touch-evoked response, and locomotive activity. Neurotoxicity and oxidative stress markers and their associated gene transcription were evaluated by ELISA and qPCR. Fluorescence imaging revealed neurogenesis and demyelination. Studies have demonstrted that both DBP and MBP significantly altered the touch-evoked response at 72 hpf. Behavioral analysis revealed that DBP exposure affects various aspects, e.g., travel distance, velocity, and turning angle. Gene

transcriptional analysis indicated significant effects on neurodevelopmental genes (sox2, manf, gfap) and oxidative stress genes (gsta1, gr), providing further insight into the molecular mechanisms of DBP and MBP. Notably, MBP exerted a greater influence on molecular markers of neurodevelopment than DBP. These findings improve understanding of the neurotoxic potential of DBP and MBP in aquatic species, thus highlighting the importance of considering metabolites in toxicity and risk assessment. Disclaimer/Disclosure: This work was supported by the Korea Environmental Industry & Technology Institute (KEITI) through the Core Technology Development Project for Environmental Diseases Prevention and Management [grant number 2480000072 (RS-2021-KE001705)].

Differential neurotoxic profiles of naphthalene metabolites in zebrafish embryonic model

Poster Number: 67

Theme: Toxicology & Environmental Assessment

Presenting Author: **Donggon Yoo** - Korea Institute of Toxicology

Co-Author(s): Sangwoo Lee – Senior Researcher, Center for Predictive Model Research, Korea Institute of Toxicology; Woo-Keun Kim – Principal Researcher, Center for Predictive Model Research, Korea Institute of Toxicology

Abstract: Naphthalene (NAP) has been frequently detected in soils contaminated with polycyclic aromatic hydrocarbons (PAHs), and its residues may pose an ecotoxicological threat to soils and aquatic organisms. The toxic effects of NAP are closely related to the phenol and quinone metabolites produced by biological metabolism. However, current knowledge on the ecotoxicological effects of NAP metabolites at the animal level is lacking. This study was conducted to compare the toxic effects of naphthalene metabolites on the nervous system using a Zebrafish. Toxicity values were obtained using OECD TG 236 using zebrafish embryos. After exposing zebrafish embryos to various naphthalene metabolites, neurotoxicity was evaluated using transgenic models to confirm the development of the central nervous system, neural crest-derived cells, and myelin, as well as behavioral and biochemical indices. In addition, data were verified and utilization methods were sought using existing toxicity utilization databases. The results of the study showed that the intensity and pattern of neurotoxicity responses varied among metabolites, as evidenced by comparison of fluorescence intensities within the images and changes in motor abnormalities and biochemical toxicity markers. Through this, some metabolites were confirmed to have the potential to induce neurotoxicity and neurodevelopmental disorders. These results suggest that metabolites generated during the metabolism of naphthalene may have different neurotoxicity risks. This study is expected to contribute to a specific understanding of the neurotoxicity characteristics of naphthalene and its metabolites in environmental and human health risk assessment. This work was supported by Korea Environment Industry & Technology Institute through Core Technology Development Project for Environmental Disease Prevention and Management, funded by Korea Ministry of Environment (RE2021003310003), and the Korea Institute of Toxicology (KIT) Research Program (no. 2710008763, KK-2501-01).

Ractopamine disrupted mitochondrial energy synthesis and neurodevelopment in developing zebrafish: from transcriptomic alterations to phenotypic outcomes

Poster Number: 68

Theme: Toxicology & Environmental Assessment

Presenting Author: **Biing-Hui Liu, PhD** - National Taiwan University College of Medicine, Graduate Institute of Toxicology

Co-Author(s): Ying-Tzu Huang – National Taiwan University College of Medicine; Shih-Wei Wu – National Taiwan University College of Medicine; Hong-Tao Chen – National Taiwan University College of Medicine

Abstract: Ractopamine (RAC) is a β-adrenergic agonist widely used in livestock production to enhance lean muscle growth and feed conversion efficiency in many countries, including the United States. However, its use remains controversial and is banned in regions such as the European Union due to concerns over potential health risks. In this study, embryonic zebrafish were utilized as a model to assess the developmental toxicity of RAC, integrating transcriptomic analyses with phenotypic observations. Wild-type embryos were exposed to low (10 and 100 μg/L) and high (1000 μg/L) concentrations of RAC from 2 to 48 hours post-fertilization (hpf). Gene set enrichment analysis (GSEA) of transcriptome profiles from the 10 and 100 μg/L RAC groups revealed significant enrichment in biological processes related to mitochondrial energy production, purine nucleotide metabolism, neurodevelopment, and chromosome segregation. In the 1000 μg/L RAC group, both GSEA and directed acyclic graph (DAG) analysis showed a marked downregulation of ATP synthesis coupled proton transport and regulation of neurogenesis, echoing the disruptions observed at lower RAC concentrations. Subsequent ELISA analysis indicated that only 16% of the RAC in the exposure solution was absorbed into zebrafish tissues. Morphological assessments showed no significant abnormalities in embryos exposed to RAC ranging from 500 to 2000 μg/L. While fluorescent imaging and histological staining revealed no structural alterations in the heart, functional assays demonstrated a dose-dependent increase in heart rate and blood flow velocity. Furthermore, RAC-treated larvae exhibited enhanced locomotor activity, as reflected by increased swimming velocity. Collectively, RAC exposure at both low and high concentrations induced significant transcriptomic alterations, particularly in pathways related to mitochondrial energy metabolism and neurodevelopment. Moreover, high-dose RAC significantly modulated cardiac function and behavioral responses during zebrafish development.

Novel Tank Test: The Behavioral Profiles of Methylone and Photomethylone in Adult Casper Zebrafish

Poster Number: 69

Theme: Toxicology & Environmental Assessment

Presenting Author: **Logan Kountz** - University of Toledo

Co-Author(s): Gurmann Boparai – University of Toledo; Faith Latherow – University of Toledo; Briana Maktabi – University of Toledo; Alexander Wisner – University of Toledo; Frederick Williams – University of Toledo; Isaac Schiefer – University of Toledo; F. Scott Hall – University of Toledo

Abstract: Stimulants, particularly synthetic cathinones, have emerged as a major contributor to drug-related toxicity and overdose deaths in recent years. These compounds include a range of structurally diverse analogs. Among them, methylone has raised significant concern due to its potent psychostimulant properties, high abuse potential, and the lack of targeted pharmacological interventions. Methylone acts similarly to MDMA and can cause neurotoxicity, cardiovascular stress, and hyperthermia. Its widespread recreational use and associated adverse effects underscore the need for deeper investigation into its mechanisms of toxicity and the development of effective therapeutic strategies.

This study employed photoaffinity labeling (PAL) to investigate the structure-activity relationship of methylone and a novel PAL analogue of methylone, Photomethylone, which incorporates a photoreactive group for probing protein interactions – i.e. the set of proteins with which the probe physically interacts. The behavioral impacts of these drugs were assessed using Casper zebrafish, a transgenic model lacking pigmentation, which allows for effective UV light penetration to activate PAL probes. Mechanistically, methylone acts as an indirect monoaminergic agonist, primarily facilitating the release of serotonin while also affecting dopamine and norepinephrine systems.

The Novel Tank Test (NTT) was used to evaluate anxiety-related behaviors and locomotor activity. Key metrics include duration of time spent in different zones (top vs. bottom of the tank) and total distance moved (TDM). Zebrafish were exposed to various concentrations of methylone and Photomethylone. Visual heatmaps and tracking indicated a reduction in TDM for Photomethylone compared to methylone at equivalent concentrations, suggesting lower locomotor activity in the presence of the analogue. Quantitative analysis confirmed these findings, with Photomethylone-treated zebrafish showing reduced TDM compared to their methylone-exposed counterparts. Thus, although producing a similar behavioral affect the probe appeared to be more potent than methylone at reducing locomotor activity.

Utilizing single cell transcriptomics for toxicity testing of environmental pollutants relevant to human health

Poster Number: 70

Theme: Toxicology & Environmental Assessment

Presenting Author: **Yavor Hadzhiev** - University of Birmingham

Co-Author(s): Shaleen Glasgow – University of Birmingham; John Colbourne – University of Birmingham; Ferenc Mueller – University of Birmingham

Abstract: As part of the PrecisionTox consortium, our aim is to develop accurate methodologies to test and predict the adverse effects of environmental pollutants on human health using non-sentient animal models like zebrafish larvae, daphnia, and fruit flies. We aim to identify molecular toxicity pathways shared between animal phyla, including human. We are developing a single-cell transcriptomic approach for toxicity testing, allowing us to identify cell/tissue-specific responses for accurate cell origin identification, adverse pathway analysis, and potential biomarker identification. This approach can allow

dissection of pathways and response trajectories in distinct cells, which could be masked in bulk assays, and identifies responses to chemicals in organs/tissues where it is not known to be toxic. We compared the transcriptional responses by single-cell RNA-seq in zebrafish larvae exposed to fluoxetine and pirinixic acid. Fluoxetine, a widely used antidepressant drug and like another pharmaceutical is found in wastewater. It has been shown that can disrupt stress axis functions in fish and mammals and early life exposure at environmentally relevant concentrations can affect mating behaviour and aggression in fish. Transcriptomic analyses have shown disruption in neuroendocrine signalling, cholesterol metabolism, synaptogenesis, and nervous system development. Pirinixic acid, a peroxisome proliferator-activated receptor alpha (PPARα) agonist, is an experimental drug for modulating lipid metabolism in cardiac myocytes to treat lipid accumulation-induced cardiac dysfunction but has been shown to have adverse effects like liver toxicity. Here we use it as a benchmark compound for pollutants affecting the well conserved among the animal phyla PPARα signalling pathway, mainly regulating lipid metabolism but also neurodevelopment. Our data analyses revealed specific transcriptional responses of the two compounds in relevant tissues/cells, but we also identified a common response in epidermal cells suggesting a non-specific/general toxicity response to chemical exposure in this cell type.

The potential cardiotoxic effects of mycotoxin citrinin: from in vivo to in vitro

Poster Number: 71

Theme: Toxicology & Environmental Assessment

Presenting Author: **Feng-Yih Yu** - Chung Shan Medical University

Co-Author(s): Jui-Feng Tsai – Graduate Institute of Toxicology – National Taiwan University; Biing-Hui Liu – Professor, Graduate Institute of Toxicology, National Taiwan University

Abstract: Citrinin (CTN), a mycotoxin and secondary metabolite produced by fungi such as Penicillium, Aspergillus, and Monascus, is frequently detected in foods and Monascus-fermented dietary supplements, including red yeast rice and red yeast extract, raising significant public health concerns. Previous studies have demonstrated that CTN induces microtubule depolymerization and mitochondrial dysfunction in various cellular models. Due to the critical roles of microtubules and mitochondria in cardiac contraction, we aimed to assess the cardiotoxic potential of CTN both in vivo and in vitro. Zebrafish embryos were exposed to CTN from 15 hours post-fertilization (hpf), and cardiac function indices were assessed at 72 hpf. Embryos exposed to 25 and 50 μM CTN exhibited marked pericardial edema and venous blood stasis compared to the vehicle controls. Cardiac contraction analysis revealed a significant, dose-dependent reduction in ventricular systole and heart function parameters, including fractional shortening, ejection fraction, heart rate, and cardiac output. To further investigate the underlying cardiotoxic mechanisms, the rat H9c2 cardiac cell line was employed. RNA sequencing analysis indicated that CTN downregulated key biological pathways associated with cardiac contraction, sarcomere organization, and cardiomyocyte differentiation. Additionally, CTN (2–25 μM) significantly suppressed the expression of cardiac-specific Troponin T (Tnnt2) both during and after the cardiac differentiation process in H9c2 cells. In conclusion, CTN at food-relevant concentrations (2–50 μM) impairs cardiac contraction, diminishes heart function, disrupts cardiomyocyte differentiation, and downregulates cardiac-specific gene expression, highlighting its potential cardiotoxic effects.

Environmental Toxicity Study of Polysorbate20 and Ctrimonium bromide:Alternative Toxicity Test Using Zebrafish Embryo

Poster Number: 72

Theme: Toxicology & Environmental Assessment

Presenting Author: **Dong-Hyun Kim** -

Co-Author(s): Jung-hun Lee – Dept. of Safety & Environmental Technology Convergence; Ji-yoen Woo – Dept. of Safety & Environmental Technology Convergence; Chae-Sung Yim – H&H BIO

Abstract: This experiment was conducted according to the OECD Guide Line; Referring to TG 236 and the Regulations on Chemical Testing Methods, Chapter 3, Ecological Impact Test Section, Article 16, Fish Embryo Acute Toxicity Test, mix Tween20(Polysorbate20) and Cetrimonium bromide(CTAB) with zebrafish embryos in a 1:1 ratio. A toxicity test was conducted. The results are as follows 1. The toxicity value of Tween20 (Polysorbate20) wasLC50 = 397.898 mg/L as a result of observation for 96 hours, which could be considered non-toxic according to the OECD Guide Line. 2. The toxicity value of Cetrimonium bromide (CTAB) was observed for 96 hours, LC50 = 0.089 mg/L, which was very toxic. 3. An experiment was conducted by mixing Tween20 (Polysorbate20) and Cetrimonium bromide (CTAB) in a 1:1 ratio. As a result of observation for 96 hours, LC50 = 0.730mg/L and LC50 = 0.892 mg/L. 4. The results of the experiment showed that when Tween20 (Polysorbate20) and Cetrimonium bromide (CTAB) were mixed, the toxicity of Tween20 (Polysrobate20) became more than 65 times stronger. These results mean that in the process of mixing chemicals in sewage and wastewater, more toxic substances such as Tween20 (Polysorbate20) may exist. To prevent such situations, appropriate methods are needed for the amount of material used or treatment method, and continuous research and experimentation are necessary.

Uncovering developmental regulation of intestinal best4+ cells

Poster Number: 75

Theme: Disease Models

Presenting Author: **Abhinav Sur, PhD** - NICHD/NIH

Co-Author(s): Ella Segal – NICHD/NIH; Michael Nunneley – NICHD/NIH; Morgan Prochaska – NICHD/NIH; Jeffrey Farrell – NICHD/NIH

Abstract: Intestinal epithelial cells (IECs) perform specialized roles and are continuously regenerated throughout life to maintain intestinal function. While most IECs are well documented, recently described best4+/CFTR-high-expressing cells remain relatively uncharacterized. best4+ cells are absent from mouse but are conserved across several vertebrate species and their gene expression suggests they have potential functions in ion homeostasis, pH regulation, or infection. Given their recent identification, it remains unclear how best4+ cells develop. We previously captured best4+ cells in zebrafish from scRNAseq profiling and have since identified their evolutionarily conserved gene expression signature across 6 species. Using developmental trajectory analysis, we predicted the progenitors of best4+ cells (secretory), signals (Notch) and transcription factors (TFs) important for their

specification. Experimental validation via Notch inhibition led to best4+ cell loss and increased enteroendocrine cells (EECs), while Notch upregulation resulted in the opposite phenotype suggesting that best4+ cells share a common progenitor with EECs. We generated a secretory progenitor-specific knock-in line and followed these cells live to establish that best4+ cells arise from secretory progenitors. Live imaging of best4+ cells captured transient cellular projections in multiple directions, suggesting interactions with the gut lumen and other gut cells. To test the TF candidates, we generated stable whole gene deletions and profiled them via scRNAseq and staining, which identified TFs required for specification of best4+ cells and for regulating their regionalized gene expression. Complementary to mutants, we jointly profiled gene expression and chromatin accessibility of intestinal cells before, during, and after best4+ cell specification to begin more extensively predicting the developmental gene regulatory network of best4+ cells. Funding: ZIAHD008997 (JAF), 1K99HD115786 (AS).

Characterizing Ocular Manifestations of CLN1 Batten Disease in a Zebrafish Model System

Poster Number: 76

Theme: Disease Models

Presenting Author: **Morgan Barnes** - Collaborations Pharmaceuticals, Inc.

Co-Author(s): Seth Kullman – Professor, Biological Sciences, NCSU; Sean Ekins – CEO, Collaborations Pharmaceuticals, Inc.

Abstract: Batten disease is a group of rare neurodegenerative genetic disorders that affect young children. CLN1 disease is one form that has the earliest onset and fastest progression and is caused by mutations in the palmitoyl-protein thioesterase 1 (PPT1) gene leading to reduced PPT1 activity. PPT1 is a soluble lysosomal depalmitoylation enzyme that removes long-chain fatty acids from proteins. The homeostasis of palmitoylation and depalmitoylation is essential for normal physiological function including the autophagy-lysosome pathway, synaptic function, axonal outgrowth, neurite extension, dendritic spine morphogenesis and apoptosis. CLN1 disease is characterized by progressive intellectual and motor deterioration, seizures, loss of vision, and early death. Over 90 mutations in the PPT1/CLN1 gene in these patients have been described and while some genotype to phenotype correlations have been identified for the most severe mutations other genotype-phenotype relationships still need to be further investigated. Currently there are no treatment options available for this disorder. Our group is characterizing select zebrafish mutants to elucidate genotype to phenotype correlations in CLN1 disease with the end goal of identifying treatment strategies. Specifically, we are investigating treatment protocols to address vision loss in CLN1 patients as current research does not address this debilitating component of Batten disease. We generated a novel CLN1 transgenic zebrafish utilizing CRISPR-Cas9 that contains the most common CLN1 mutation, R151X. We characterized the effects of this mutation on the zebrafish development and behavior by assessing histology, PPT1 activity, optokinetic response, touch response and visual motor response. Lastly, we assessed the oculotoxicity of recombinant human PPT1 via intravitreal injection to identify whether enzyme replacement therapy is a viable option for vision loss in this model. Our findings are critical for furthering CLN1 research to better understand the wide variety of mutations and their effects as well as identify a treatment for this neurodegenerative disease.

Protective Role of Plant Extracts Against Cisplatin-Induced Hair Cell Loss in Zebrafish

Poster Number: 77

Theme: Disease Models

Presenting Author: **Jiann-Jou Yang** - Chung Shan Medical University

Co-Author(s): Hsinlin Cheng – Chung Shan Medical University; Yuxuan Wu – Chung Shan Medical University

Abstract: Iatrogenic hearing loss is defined as irreversible damage to cochlear hair cells caused by medical interventions, such as the administration of cisplatin, which leads to excessive production of reactive oxygen species (ROS). A medicinal plant known for its antioxidant properties, has recently demonstrated promising pharmacological potential. In this study, we utilized a transgenic zebrafish line (pvalb3b:TagGFP) as an in vivo platform to identify agents with otoprotective properties. Additionally, we employed behavioral assessments to evaluate functional outcomes. Exposure to 250 μM cisplatin for one hour resulted in severe injury to lateral-line hair cells, accompanied by significant cell death. To assess the protective effect of plant water extract (PWE) against cisplatin-induced ototoxicity, six readouts were analyzed: (1) radical-scavenging capacity, (2) hair cell viability, (3) mechanotransduction (MET) channel function, (4) apoptosis, (5) antioxidant defense gene expression, and (6) locomotor behavior. Our findings revealed that a one-hour pretreatment with non-toxic concentrations of PWE significantly enhanced hair cell survival. This protective effect is likely mediated by blocking cisplatin entry through the MET channel, thereby reducing subsequent apoptotic signaling. On a molecular level, PWE modulated the expression of antioxidant enzyme genes, which contributed to the restoration of swimming behavior impaired by cisplatin toxicity. Overall, this study provides compelling evidence that PWE can mitigate cisplatin-induced hair cell damage and functional impairment in zebrafish. These results support the potential of PWE as a candidate for further preclinical development as an otoprotective agent to prevent or reduce iatrogenic hearing loss.

Investigating Mitochondrial Dysfunction and Downstream Effects of stra6 Loss in Zebrafish RPE

Poster Number: 78

Theme: Disease Models

Presenting Author: **Allison Hall** - Medical College of Wisconsin

Co-Author(s): Ross Collery – Medical College of Wisconsin

11-cis retinol (vitamin A) is essential for ocular health, serving as the chromophore for opsins in photoreceptors and supporting other cellular signaling in the eye and RPE. STRA6 encodes a transmembrane protein responsible for bidirectional retinoid transport to the RPE. Mutations in human STRA6 cause Matthew-Wood Syndrome, a condition characterized by severe ocular, cardiovascular, and pulmonary defects. Despite its known role in retinoid trafficking and homeostasis, the mechanisms by which STRA6 loss affects retinal structure and function remain unclear. To investigate the effects of

STRA6 loss in the retina, stra6-/- zebrafish retinas were characterized at 7 days post-fertilization (dpf), 1 month post-fertilization (mpf), and 3 mpf using immunohistochemistry. Previously, RNA sequencing of stra6-/- RPE tissue revealed downregulation of expression of mitochondrial-associated genes, suggesting a potential link between STRA6 loss and mitochondrial dysfunction. The top four downregulated nuclear-encoded mitochondrial genes were selected for further investigation as potential mediators of STRA6-associated pathology (agk, got2a, prdx3, phb2a). To evaluate their roles, CRISPR mutant lines of each target are being generated for comparative histological and functional analysis. Additionally, transgenic RPE-specific overexpression lines of each candidate gene are being developed to assess whether overexpression rescues retinal defects in the stra6 knockout model. By elucidating the molecular consequences of STRA6 loss, this study aims to identify key pathways disrupted beyond retinoid transport deficiency in stra6-/- zebrafish. STRA6 has been implicated as a signaling hub, suggesting that its loss may trigger dysfunction through multiple mechanisms. By characterizing effects of candidate gene inactivation testing whether their overexpression rescues STRA6-associated retinal defects, we will determine whether mitochondrial dysregulation plays a central role in the pathology of STRA6 loss. These findings will advance understanding of the role of STRA6 in ocular health and disease and will reveal potential therapeutic targets for diseases where STRA6- or mitochondrial-associated pathways are disrupted.

Characterization of hypoxia-steroidogenesis interplay in zebrafish mutant lines

Poster Number: 79

Theme: Disease Models

Presenting Author: **Francesco Sernesi** - Università di Padova

Co-Author(s): Annachiara Tesoriere – Università di Padova; Angela Piersanti – Università di Padova; Luisa Dalla Valle – Università di Padova; Francesco Argenton – Full Professor, Università di Padova

The interplay between hypoxia and steroidogenesis, in the context of diseases like Von-Hippel–Lindau (VHL) syndrome, reveals how dysregulated HIF signaling can modulate key enzymes involved in steroid biosynthesis, leading to a pseudo-hypoxia condition that contributes to altered glucocorticoid (GC) production and to the pathophysiology of the disease. GC, like cortisol, are steroid hormones produced in the adrenal cortex from cholesterol through enzymatic steps that start with cholesterol, which is transported into the mitochondria by the Steroidogenic Acute Regulatory protein (StAR), where it is converted by CYP11A1 into pregnenolone. A series of enzymes then convert pregnenolone into adrenal and sex hormones. Cortisol is one of the main GCs and its synthesis is catalyzed by Cyp11b1 in humans and Cyp11c1 in zebrafish. They act via the glucocorticoid receptor (GR), a transcription factor that regulates gene expression by binding to glucocorticoid response elements (GREs). To model hypoxia-related GC suppression, zebrafish larvae were exposed to 5% oxygen. This led to reduced fluorescence in a GC-responsive transgenic line Tg(9xGCRE-Hsv.UI23:EGFP)ia20, indicating decreased endogenous GC activity. Gene expression analysis confirmed the downregulation of steroidogenesis-related genes. Further investigation using a cyp11c1 mutant zebrafish line, which lacks cortisol production, revealed compromised GC signaling, evident through diminished reporter fluorescence. Administering dexamethasone, a synthetic GC, restored this activity. These cortisol-deficient mutants exhibited increased HRE:GFP reporter activity, implying that GCs suppress hypoxia signaling. This was supported by widespread dysregulation of hypoxia- and angiogenesis-related genes across multiple tissues. RNA

sequencing of cortisol-treated larvae further demonstrated downregulation of genes associated with vascular development and endothelial migration. These findings suggest that cortisol exerts a negative regulatory effect on hypoxia pathways and angiogenesis, potentially offering a protective mechanism against tumor development in VHL syndrome. Zebrafish models offer a conserved platform to dissect the complex interplay between glucocorticoid signaling and hypoxia pathways, advancing potential novel therapautic approaches.

Oxidative stress induces intervertebral ECM remodeling, elevated tissue stiffness and idiopathic-like scoliosis

Poster Number: 80

Theme: Disease Models

Presenting Author: **Ran Xu** - Department of Molecular Genetics, University of Toronto; Developmental & Stem Cell Biology Program, The Hospital for Sick Children

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Abstract: Adolescent idiopathic scoliosis (AIS) is the most prevalent pediatric spine disorder, developing in the absence of obvious congenital or physiological defects. Patient genetic sequencing and mouse functional studies have demonstrated association of musculoskeletal collagen variants and cartilaginous extracellular matrix (ECM) defects in a subset of cases. However, the underlying biological causes of AIS are poorly understood, thus treatment options remain limited to physical bracing or invasive corrective surgery. Here we interrogate the biological causes of scoliosis in zebrafish preclinical models of AIS. We demonstrate that neuroinflammation-associated reduction-oxidation (redox) imbalance induces cell stress and collagen remodeling defects within intervertebral segments of the developing spine. Mutant spines are consequently stiffer, as measured by shear wave elastography, and exhibit deformations of intervertebral structures. Remarkably, both elevated spine stiffness and intervertebral ECM phenotypes are detectable prior to scoliosis onset in zebrafish models, suggesting a causal relationship, and can be suppressed by antioxidant treatment. Together, our studies implicate oxidative stress-induced intervertebral deformations in the pathogenesis of AIS and identify elevated spine stiffness and redox imbalance as plausible first-in-kind prognostic biomarkers and therapeutic targets.

Investigate Clrn1 functions in Müller glia using a zebrafish model of USH3A

Poster Number: 81

Theme: Disease Models

Presenting Author: **Hai Tran** - Medical College of Wisconsin

Co-Author(s): Ross Collery – Medical College of Wisconsin; Brian Link – Medical College of Wisconsin

Abstract: Usher syndrome is an autosomal recessive condition causing combined deaf-blindness, categorized into subclasses USH1, USH2, and USH3. USH3A, caused by mutations in the CLRN1 gene, leads to inner ear hair cell and retinal photoreceptor (PhR) degeneration. CLRN1 is mainly expressed in Müller glia (MG) in the retina, which supports retinal structure through apical processes and the outer limiting membrane (OLM). Previous studies indicate that USH proteins are crucial for PhR support. Our zebrafish model of USH3A (clrn1 -/-) shows that Clrn1 loss results in retinal dysfunction and degeneration starting at 4 months post-fertilization. Restoring Clrn1 in MG repairs the apical processes and OLM, highlighting its structural role. This study will investigate if Clrn1 loss renders PhRs sensitive to mechanical stress. The loss of low-density lipoprotein receptor-related protein 2 (lrp2) results in expanded ocular globe size which stretches the retina, inducing planar mechanical stress. This study used a CRISPR)/cas9 approach to mutate lrp2 in clrn1-/- fish. Spectral domain-optical coherence tomography (SD-OCT) was used to assess eye size via axial length. Immunofluorescence (IF) stained retinal flat-mounts and transverse sections were used to assess PhR number and morphology. Clrn1 loss with mechanical stress (lrp2-/- background) disrupts PhR mosaic organization. The double mutant fish also exhibited a reduction in all retinal layers, disruptions in PhR outer segments, and complete loss of (red/green) double cones compared to the wild-type, clrn1-/-, and lrp2-/- fish. There was also reduced N-cadherin localization at the OLM and fewer apical processes from MG. These results highlight Clarin1's key role in MG, supporting the structure and maintenance of PhR and its link to USH3A pathology.

Development of crispant, stable genetic knock-in, stable genetic knock-out, and chemical zebrafish models of seizure disorders for candidate therapeutic screening

Poster Number: 82

Theme: Disease Models

Presenting Author: **Melissa Hinman, PhD** - In Vivo Biosystems

Co-Author(s): Trisha Brock – In Vivo Biosystems; Joseph Bruckner – In Vivo Biosystems; Benjamin Jussila – In Vivo Biosystems; Anastasia Levichev – In Vivo Biosystems; Marnie Preston – In Vivo Biosystems; Amy Robbins – In Vivo Biosystems

Abstract: We have employed diverse methods to model several human seizure disorders in zebrafish. To model Dravet syndrome and related seizure disorders, we first used a rapid crispant approach to knock down zebrafish scn1lab and perform preliminary phenotyping, then created stable scn1lab genetic knockouts, and are generating a patient-specific point mutation in the scn1lab gene. To model STXBP1-related neurodevelopmental and seizure disorders, we used CRISPR-based generation of stable loss-of-function mutations in the zebrafish stxbp1a gene, including a clinically pathogenic point mutation. Zebrafish kcna1a crispants were generated to model human KCNA1 loss-of-function seizure disorder.

Finally, we used pentylenetetrazole (PTZ) to chemically induce seizures in larval zebrafish. Each of these zebrafish models displayed distinct seizure-like phenotypes, such as hyperactivity and exaggerated startle responses to light and/or acoustic stimuli. We have used these zebrafish seizure models to validate current therapeutic strategies and to test the efficacy of candidate therapeutics. In addition, the crispant, stable genetic knock-in, and stable genetic knock-out approaches that we optimized while generating these zebrafish seizure models have been successfully employed to develop zebrafish models of many other genetic disorders.

Visualizing immune cell-vessel interactions in a novel model of cerebrovascular injury in adult zebrafish.

Poster Number: 83

Theme: Disease Models

Presenting Author: **Aurora Kraus, PhD** - NIH/NICHD

Co-Author(s): Daniel Castranova – NICHD; Aleksandra Potapova – University of Maryland; John Prevedel – NICHD; Jean Sebastien Prosper Santiago – NICHD; Brant Weinstein – NICHD

Abstract: The CDC estimates that 5 million Americans live with disease caused by cerebrovascular head injuries (CVI). CVIs rupture blood vessels in the tissue surrounding the brain, known as the meninges, releasing blood and debris that elicit an acute immune response. For some patients, prolonged bleeding and inflammation from damaged vessels results in long-lasting sequelae. Although injured blood vessels regrow, the site of injury is full of inflammatory immune cells that may influence vascular function. Adult zebrafish have a thin, translucent skull and a mammalian-like meninges that is easily imaged in living animals. We have established a novel adult zebrafish model to investigate vessel-immune cell interactions after CVI. In our model, sonication ruptures major meningeal blood vessels without breaching the skull or causing significant damage to the underlying brain. By performing longitudinal live imaging of intubated adult fish, we observed vascular regrowth and immune responses to CVI with unprecedented resolution to define the time course of healing. Our imaging capabilities permit functional imaging analysis of blood flow, interactions between individual immune and vascular cells, and dynamics of vessel regrowth. Further, diving-tank anxiety tests indicate aberrant behavior after CVI compared to controls. Using this powerful new zebrafish model for live imaging of meningeal immune cell-vascular interactions after cerebrovascular injury will yield important insights into new approaches for treating chronic neuroinflammatory disease.

The fentanyl adulterant xylazine causes skin damage by acting directly on epithelial cells through both α2-AR and κOR dependent mechanisms

Poster Number: 84

Theme: Disease Models

Presenting Author: **Tanner Robertson, PhD** - University of Wisconsin-Madison

Co-Author(s): Adam Horn, Ph.D. – Postdoctoral Fellow, Medical Microbiology and Immunology, University of Wisconsin-Madison; Frances Smith – Graduate Student, Medical Microbiology and

Immunology, University of Wisconsin-Madison; Anna Huttenlocher, M.D. – Professor, Medical Microbiology and Immunology, University of Wisconsin-Madison

Abstract: Xylazine is an α2 adrenergic receptor (α2-AR) agonist and veterinary sedative that is increasingly found mixed into the illicit opioid supply in the United States. Known colloquially as “tranq”, xylazine causes severe, necrotic skin wounds in humans that ingest the drug either alone or in combination with fentanyl. The mechanism by which xylazine causes skin damage is unknown. Here, we have developed a new zebrafish model to study xylazine-induced skin damage. This larval zebrafish model mirrors the major effects seen in humans, including sedation, bradycardia, and severe skin damage. Xylazine is proposed to cause skin damage by altering cardiovascular function and driving peripheral vasoconstriction. However, we find that xylazine causes skin damage by acting directly on keratinocytes in both zebrafish and mammalian epithelial cells. This finding represents a shift in our understanding of xylazine-induced skin damage. Xylazine is a dual receptor agonist that acts on both α2-AR and κOR. By using receptor-specific agonists, we find that xylazine drives skin damage by simultaneously acting through both α2-AR and κOR. These data provide a framework for understanding how xylazine drives chronic skin wounds in humans and points to an underappreciated role for both adrenergic and opioid receptor signaling in regulating the wound healing response.

Zebrafish screens to identify novel opioid reversal agents

Poster Number: 85

Theme: Disease Models

Presenting Author: **Henry Stalnaker** - University of Toledo

Co-Author(s): Millicent Akere – University of Toledo; Alexander Wisner – University of Toledo; Jovana Duric – University of Toledo; Abigail Collins – University of Toledo; Briana Maktabi – University of Toledo; Frank Hall – University of Toledo; Frederick Williams – University of Toledo; William Messer – University of Toledo; Isaac Schiefer – University of Toledo

Abstract: With the ongoing opioid epidemic now in its third decade, there is a critical need for innovative countermeasures to combat fentanyl, a potent synthetic opioid responsible for the rising number of overdose deaths. Our approach utilizes a 7-days past fertilization larval zebrafish paradigm that functions as an in vivo, high-throughput, competitive screen for these new countermeasures. Previous studies have also shown that zebrafish, which have μ-opioid receptors similar to humans, exhibit fentanyl-induced overdose symptoms like humans. For the project, an 8,000+ compound library was curated for drug-like molecules that exhibit favorable physicochemical properties for potential use as CNS therapeutics. Then, these molecules were tested using a photomotor response assay to identify compounds capable of reversing fentanyl-induced effects on locomotor activity. This phenotypic screening strategy holds high potential for identifying new chemical classes that reverse opioid effects through μ-opioid and non-μopioid receptor (non-MOR) mechanisms. Unlike traditional target-based methods, our phenotypic approach offers the distinct advantage of uncovering novel pathways. In tandem, we have implemented an in vivo target identification strategy to elucidate the molecular targets of promising non-MOR compounds and to map a detailed binding interactome of synthetic opioids. Toward this goal, we have designed and synthesized photoaffinity labeling probes specifically

for use in competition assays with fentanyl. Initial studies using these probes are beginning to show the possibility of new molecular interactions that could deepen our understanding of fentanyl's mechanism of action. Lead compounds that successfully reverse fentanyl effects will be further validated in confirmatory assays assessing their ability to mitigate fentanyl-induced respiratory and cardiovascular depression. Ultimately, this campaign may yield novel small molecules that act as mu-opioid receptor allosteric modulators, orthosteric antagonists, or non-MOR agents with desirable CNS drug properties, making them strong candidates for future hit-to-lead optimization and development.

Investigating GPR68, OGM, and ferroptosis using Zebrafish as a model system

Poster Number: 86

Theme: Disease Models

Presenting Author: **Leif Neitzel, PhD** - Michigan State University

Co-Author(s): Charles Williams – Assistant Professor, Medicine, Michigan State University; Charles Hong – Chairperson, Medicine, Michigan State University

Abstract: Ogremorphin (OGM), a highly specific GPR68 inhibitor, was identified from an unbiased screen of ~30,000 compounds for zebrafish developmental defects. Treatment with OGM induced abnormal pigmentation, craniofacial defects, ventral curvature, shortened body axis, and a wavy notochord mimicking the known phenotypes of catastrophe/calamity. Acidification of the extracellular environment activates GPR68, a proton-sensing G-protein-coupled receptor, modulating cell function. Using OGM we identified a novel GPR68-ATF4 pro-survival pathway in glioblastoma (GBM). Furthermore, GPR68 inhibition by OGM or genetic knockdown induces ferroptosis, an iron-mediated cell death, in GBM as well as lung carcinoma and pancreatic ductal adenocarcinoma cells. OGM showed no non-specific toxicity in zebrafish larvae, sparing both neural and non-neural cells in Tg(neuroD1:EGFP) zebrafish larvae. In vivo, we established two orthotopic larval zebrafish xenograft models by grafting fluorescently labeled U87-MG and U138-MG human GBM cells, known to overexpress GPR68. In vitro, OGM and genetic knockdowns of GPR68 demonstrated potent cytotoxic effects against U87-MG and U138-MG cells with increased lipid peroxidation, indicating ferroptosis. In zebrafish, shRNA-mediated knockdown of GPR68 significantly reduced grafted GBM viability. Similarly, treatment with OGM reduced grafted GBM viability with minimal toxicity to zebrafish embryos. This study suggests that therapeutic targeting of GPR68 with small molecules like OGM represents a promising approach for the treatment of GBM. Lastly, we established a transgenic line, Tg(ubi:pHluorin2-GPI), to investigate pH changes in vivo. This line ubiquitously expresses pHluorin2, a pH-sensitive ratiometric green fluorescent protein, tethered to the extracellular face of the plasma membrane by a GPI anchor. Future experiments will use a modified orthotopic larval zebrafish xenograft model in the Tg(ubi:pHluorin2-GPI) line to investigate in vivo changes in the tumor microenvironment and the role of GPR68.

Danionella cerebrum is a novel adult vertebrate model for kidney and urological research.

Poster Number: 87

Theme: Disease Models

Presenting Author: **Steve Mangos, PhD** - RUSH UNIVERSITY

Co-Author(s): Pui Ying Lam, PhD – Assistant Professor, Cell Biology,Neurobiology and Anatomy, Medical College of Wisconsin

Abstract: Zebrafish larvae have been successfully used to study early kidney development and to model human kidney diseases. The larva's small size and optical transparency, combined with its relevant mammalian-like nephron structure, wide range of available techniques and resources for genome manipulation, and ease of transgenesis, have made this model attractive for research in nephrology. The use of adult zebrafish has been less attractive due to the loss of transparency and the position of the kidney deep within the body cavity making it optically inaccessible. Despite these drawbacks, some success has been achieved using adult zebrafish to uncover mechanisms leading to whole nephron regeneration. These studies are limited and challenging as they require technically difficult surgical skills, and/or sophisticated imaging equipment. In contrast, the recently described zebrafish relative, Danionella cerebrum, remains small and transparent into adulthood. We used different microscopy techniques to reveal that D. cerebrum's mesonephric kidney is simple and easily imaged. Taking advantage of these traits, we have created several fluorescent transgenic lines, including kidney cell-type specific lines, and established longitudinal live-imaging methods to develop Danionella cerebrum as a novel vertebrate model for mesonephric kidney-related research. Our initial characterization experiments show that adult D. cerebrum have a mesonephros with few nephrons that are size-discriminating and can be injured using standard approaches. With a focus on acute kidney injury (AKI) and by utilizing double transgenic fish, we are characterizing innate immune cell-kidney cell interactions in healthy and disease states. Interestingly, adult D. cerebrum display a clear sexual dimorphism in the architecture of the urogenital system with the males possessing a fully developed bladder. Our continued efforts promise to exploit the advantages offered by Danionella cerebrum and fill a research tool gap by providing a simple, yet relevant animal model possessing an easily imaged mesonephric kidney.

Zebrafish model of Cockayne Syndrome exhibits UV and metronidazole sensitivity, increased oxygen consumption, and impaired hair cell mechanoelectrical transduction

Poster Number: 88

Theme: Disease Models

Presenting Author: **Joseph Dugdale** - Mayo Clinic

Co-Author(s): Gabriel Hernandez Herrera – University of Puerto Rico School of Medicine; Jasmine Wallace – Mayo Clinic Alix School of Medicine; Ryan Cotter – University of Utah Health; Alyssa Jolliffe – Mayo Clinic; Karl Clark – Texas A&M University; Lisa Schimmenti – Mayo Clinic

Abstract: Cockayne Syndrome (CS) is an ultra-rare premature aging condition associated with UV sensitivity, neurocognitive decline, retinopathy, metronidazole-induced lethality, and sensorineural hearing loss (SNHL). In 70% of affected patients, bi-allelic pathogenic variants in ERCC6 are identified. Although the role of ERCC6 in DNA damage repair has been studied, little is known about the mechanism for defective ERCC6 function in clinical findings, particularly SNHL. To identify the

mechanism of disease caused by pathogenic variants in ERCC6, we developed a zebrafish (Danio rerio) ercc6 loss of function model. To validate this model, we assessed survival after UV and metronidazole exposure, measured basal respiration rates, and evaluated mechanoelectrical transduction (MET) channel function and morphology of lateral line hair cells. We found that UV exposure significantly reduced ercc6-/- larval viability. Metronidazole treatment of ercc6-/- larvae resulted in complete lethality, while wildtype controls showed near complete survival. Basal oxygen consumption rates in ercc6-/- embryos were significantly increased, measured by Oroboros Oxygraph 2K analysis, suggesting that mitochondrial function is abnormal. MET channel function after UV exposure, as measured by FM1-43 uptake, is reduced and yet lateral line hair cell morphology, visualized by phalloidin staining, remained grossly intact. Treatment with the superoxide dismutates mimetic, Mn(III)tetrakis(4-benzoic acid)porphyrin Chloride (MnTBAP), preserved MET channel function, prevented metronidazole lethality, and also restored oxygen consumption rates back to wildtype values. We propose that defective mitochondrial function and increased reactive oxygen species provide a mechanism for hair cell dysfunction in this zebrafish model of CS. These results provide a foundation for further experiments to explore disease mechanisms and treatment modalities for this premature aging condition.

Effects of mutations in epilepsy-associated gene grin2D on neurodevelopment and seizure susceptibility in larval zebrafish

Poster Number: 89

Theme: Disease Models

Presenting Author: **Carly Gomes, MD** - Stony Brook University

Co-Author(s): Julia Dovi – Stony Brook University; Katherine Otavalo-Barros – Stony Brook University; David Smith – Stony Brook University; Lonnie Wollmuth – Stony Brook University; Howard Sirotkin – Stony Brook University

Abstract: NMDA receptors (NMDARs) play a critical role in synaptic plasticity and neurodevelopment. They are comprised of two obligatory GluN1 subunits and some combination of GluN2 (A-D) subunits, each possessing distinct electrophysiological properties and precise temporal/anatomical expression patterns, suggesting unique, individual functions. Loss of function (LOF) mutations in GRIN2D in humans are associated with Developmental Epileptic Encephalopathy (DEE), a severe neurologic disease of infancy causing global cognitive/motor delays, intractable seizures, and shortened life-expectancy. The direct mechanisms underlying the neuropathology observed with GRIN2D mutations is unknown, resulting in a lack of effective treatment options and increased morbidity/mortality in affected children. Understanding how GRIN2D mutations contribute to neurodevelopmental pathology is critical for identifying therapeutic targets to improve medical management. Our objective is to develop a novel zebrafish model to characterize the effects of grin2D dysfunction on neurodevelopment and seizure susceptibility. We utilized CRISPR-Cas9 to generate zebrafish lacking both grin2D paralogues (grin2D-/-). Effects of grin2D-/- on complex larval motor and cognitive behaviors, including spontaneous/evoked movements (6dpf), response to photic stimuli (6dpf), and learning and memory (5-7 dpf), were assessed using locomotion and prey-capture behavioral assays. Seizure susceptibility (6dpf) was evaluated following exposure to pentylenetetrazol (PTZ), a GABA-A receptor antagonist. Immunohistochemistry was performed on cryotomy brain sections to compare populations of proliferative cells in mutants vs.

wild-type (WT) fish. Absence of GluN2D was associated with decreased spontaneous/evoked movement and lower threshold for seizure activity in larval fish. There were no differences in response to photic stimuli and no observed deficiencies in learning/memory with prey-capture assay. A significant decrease in proliferating neuroepithelial cells and radial glial cells was observed in the forebrain of grin2D-/- mutant fish. Our results suggest a critical role for GluN2D in seizure susceptibility and neural proliferation.

Characterization of Autism-Associated KMT2E in Zebrafish

Poster Number: 90

Theme: Disease Models

Presenting Author: **Emily Xu** - University of California, Davis

Co-Author(s): Megan Dennis – University of California, Davis; Nicholas Haghani – University of California, Davis

Abstract: Approximately 1 in 36 children are affected by autism spectrum disorder (ASD). Due to its genetic and phenotypic complexity, the causes of ASD are not completely elucidated. A severe subtype of autism with disproportionate megalencephaly (ASD-DM), characterized by larger brains proportional to body length, is associated with regressive autism and more severe cognitive defects. Recent sequencing of individuals with ASD-DM identified 153 candidate genes with loss of function (LOF) mutations. Although some of these are well-known, high-confidence genes, over 100 genes have not been functionally tested. We propose using zebrafish to efficiently test functions of candidate genes, starting with the high-confidence autism gene, KMT2E, implicated in cell-cycle regulation but with unclear effects on brain development. Modeling patient KMT2E LOF mutations, we generated kmt2e G0 mosaic knockout models (“crispants”) and measured morphometric features of larvae at 5 days post fertilization with an automated imaging system (VAST). We observed proportionally enlarged heads relative to body length in KMT2E zebrafish knockouts versus controls, driven by a reduced body length and seemingly unaffected head size. Preliminary data measuring brain size in km2te crispants with lightsheet microscopy in a HuC-GFP neuron-specific fluorescent zebrafish line verified no changes to brain area or volume. We also ectopically introduced KMT2E mRNA into single cell embryos to characterize putative gain-of-function effects. Although morphometric imaging of the body and head showed no difference in overexpression, preliminary results directly quantifying brain area revealed an increase in midbrain area versus controls. In combination with our data suggesting kmt2e crispant larvae having shorter body lengths, these findings are consistent with disproportionate megalencephaly implicating KMT2E with this subphenotype of autism. From these assays, we aim to identify concrete phenotypic association between KMT2E and DM to ultimately better understand ASD-DM etiology.

DEPDC5 loss of function mosaic zebrafish models demonstrate early death phenotype and neuronal hyperexcitability

Poster Number: 91

Theme: Disease Models

Presenting Author: **Sneham Tiwari, PhD** - Boston Children's Hospital

Co-Author(s): Christopher LaCoursiere – Neurology – Boston Children's Hospital; Annapurna Poduri, MD, MPH – Professor, Neurology, BCH

Abstract: Epilepsy is a condition defined by unprovoked seizures, sometimes with other neurodevelopmental consequences. Focal cortical dysplasia (FCD) is a common cause of focal epilepsy. FCDs are associated with genes in the mammalian target of rapamycin (mTOR) pathway, involved in cell growth and signaling. Genomic analysis studies from our group and others have demonstrated that germline and somatic mosaic variants in the DEPDC5 gene are associated with inherited and non-inherited focal epilepsy and contribute to Sudden Unexpected Death in Epilepsy. Germline, germline mosaic, or brain somatic variants in DEPDC5 are associated with FCD and other focal brain malformations associated with focal epilepsy. Disease-associated loss-of-function variants in DEPDC5 lead to mTORC1 activation in dysmorphic neurons. The DEPDC5 protein is part of the GATOR complex, an upstream repressor of the mTOR pathway. Since existing mouse models with Depdc5 germline variants demonstrate early death, we are generating mosaic models of Depdc5. Here, we demonstrate generating a Depdc5 mosaic loss-of-function zebrafish model to understand the role of mosaicism in this and related genes. Mature zebrafish were crossed, and the fertilized eggs were microinjected at 1-cell and 2-cell stages with Depdc5 gRNA, Depdc5 dTomato+UFlip construct, and CRISPR/Cas9mRNA (2nl/per embryo). The larvae were sorted under red fluorescence for a successful cut in Depdc5 and studied for survival up to 14dpf, demonstrating early death, recapitulating human DEPDC5-related epilepsy. In parallel, we quantified the morphological abnormalities in these larvae, including smaller head size and total body length when compared to controls. We demonstrate that the crispants display neuronal hyperexcitability using local field potential recordings, as well as neuronal cell death (early apoptosis). We additionally demonstrate abnormal levels of mTOR pathway downstream proteins.

Cohesin protein Smc3 contributes to kinociliary dynamics

Poster Number: 92

Theme: Disease Models

Presenting Author: **Mary Iovine, PhD** - Lehigh University

Co-Author(s): Fiona Mensching – Lehigh University; Niusha Banoukh – Lehigh University

Abstract: Cohesinopathies and ciliopathies are congenital disorders affecting overlapping body systems. The extent to which these syndromes may be linked remains largely untested. Recently, reduced expression of a cohesin core subunit, Smc3, was found to result in abnormal otolith development in zebrafish embryos. This finding suggests that Smc3 may contribute to kinociliary development and function, which would represent a novel role for Smc3. Here, we report that Smc3 indeed localizes to the kinocilium of hair cells found in neuromasts of the lateral line. Moreover, Smc3 knockdown resulted in reduced kinociliary length. To address the role of Smc3 in kinocilia function, we monitored neomycin resistance of neuromasts (associated with several cilia gene mutants) and FM1-43X uptake in hair cells (associated with mechanotransduction). We found that Smc3 knockdown indeed led to neomycin resistance of the posterior lateral line neuromasts, suggesting impaired kinocilium function. However, neuromast hair cells did not have defects in FM1-43X uptake. This study suggests a novel role of the cohesin subunit Smc3 beyond its nuclear functions and provides a preliminary link between cohesinopathy and ciliopathy etiologies.

Investigating the Effect of Diet-Induced Obesity on Cognitive Function in Zebrafish

Poster Number: 93

Theme: Disease Models

Presenting Author: **Manisha Gupte, PhD** - Austin Peay State University

Co-Author(s): Comfort Ogbu – Student, Biology, Austin Peay State university; Sai Nesanuru – Student, Biology, Austin Peay State university

Abstract: Dementia, particularly Alzheimer's disease, is a progressive neurodegenerative disorder with metabolic dysfunctions such as obesity and type 2 diabetes being significant risk factors. High-fat diets (HFD) contribute to cognitive decline through insulin resistance, neuroinflammation, and oxidative stress. This study investigates the impact of diet-induced obesity on cognitive function in adult zebrafish (Danio rerio), a model with conserved metabolic and neurological features. Following eight weeks of HFD feeding, zebrafish exhibited increased body weight and adiposity, mirroring mammalian obesity pathology. Cognitive function was assessed using the active avoidance test, revealing that male zebrafish on a control diet demonstrated significantly higher avoidance responses to negative stimuli and learned faster compared to their HFD-fed counterparts. To understand the mechanisms behind HFD-induced cognitive decline, we investigated the expression of genes associated with cognitive impairment. These findings indicate that HFD exacerbates cognitive decline, reinforcing zebrafish as a viable model for studying obesity-induced neurodegeneration and metabolic contributions to cognitive impairment.

Modeling MRKH syndrome in zebrafish through deleterious nr6a1a/b mutations

Poster Number: 94

Theme: Disease Models

Presenting Author: **Romane Vanhaeren, MS** - University of Liège

Co-Author(s): Manon Dohet – University of Liège; Lydie Flasse – University of Liège; Adeline Jacquinet – University of Liège; Frédéric Kerff – University of Liège; Bernard Peers – University of Liège

The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is a rare disorder, affecting approximately 1 in 4500 women, characterized by uterine and vaginal aplasia, often associated with kidney and skeletal defects. While TBX6 and GREB1L mutations cause similar anomalies, many MRKH patients lack mutations in these genes, suggesting the involvement of other genes. Exome sequencing identified two highly pathogenic variants in NR6A1, encoding an orphan nuclear receptor. AlphaFold predictions suggest these mutations impair DNA binding or destabilize the protein structure. Gel shift assays revealed that mutant NR6A1 proteins bind DNA less efficiently than the wild-type protein. To explore the link between these variants and the disease, a zebrafish model was generated by inactivating the orthologous genes nr6a1a and nr6a1b. Phenotypic analysis of the nr6a1a mutants revealed reduced body and trunk size, fewer thoracic vertebrae, and absence of the anal fin. In these mutants, the mesonephros displayed altered morphology and reduced size, while pronephric segments and cloacal

regions were disorganized. Double nr6a1a-/- , nr6a1b-/- mutants were more severely affected, showing unilateral or bilateral pectoral fin loss, posterior pharyngeal arch defects, and early lethality (12–15 dpf). Gene expression analysis through in situ hybridization revealed altered expression of posterior Hox genes, which are crucial to genital tract and kidney development. These findings suggest that NR6A1 mutations contribute to MRKH syndrome, partly through Hox dysregulation. To further investigate the disease mechanisms, RNA-seq was performed on WT and mutant zebrafish embryos at the 20-somite stage, confirming the upregulation of posterior Hox genes and the dysregulation of many other genes. In situ hybridization experiments are ongoing to validate the differential expression of these genes. Future studies will include ChIP-seq to identify genomic loci directly regulated by nr6a1a, shedding light on its transcriptional network.

Genetic diagnosis of inherited kidney diseases by modelization in zebrafish

Poster Number: 95

Theme: Disease Models

Presenting Author: **Manon Dohet** - University of Liège

Co-Author(s): Vincent Bours – University of Liège; Vinciane Dideberg – University of Liège; Lydie Flasse – University of Liège; Bernard Grisart – University of Liège; Tilman Jobst-Schwan – University of Erlangen; Bernard Peers – University of Liège

Abstract: Inherited kidney diseases affect approximately 70 per 100,000 individuals in Europe. Despite advances in exome sequencing, pathogenic variants can only be identified in about 30% of affected patients, indicating that numerous causative genes remain undiscovered. This challenge stems from various factors, including phenotypic and genotypic heterogeneity, often leading to multisystemic complications. In this context, zebrafish (Danio rerio) has emerged as a powerful model for validating human genetic variants, elucidating molecular mechanisms, investigating disease pathophysiology, and facilitating drug discovery. The primary objective of this study is to enhance the genetic diagnosis of congenital kidney diseases by identifying novel disease-associated genes. To achieve this, exome sequencing is performed on patients and their relatives to detect deleterious variants in genes not previously linked to these disorders. The pathogenicity of these candidate genes is evaluated through zebrafish models carrying patient-specific mutations, allowing functional analyses related to kidney development. Currently, our research focuses on two patients for whom exome sequencing has been performed. The first patient, from a consanguineous family, has kidney and ovarian cysts, microcephaly, and facial dysmorphism. Two homozygous variants predicted to be deleterious were identified in the WWC1 and PPIL2 genes. The second patient exhibits congenital anomalies of the kidney and urinary tract (CAKUT), with a frameshift mutation detected in the CHD1L gene, which has previously been suspected to be associated with CAKUT but without conclusive evidence. To investigate the potential roles of these three candidate genes, we have generated multiple zebrafish mutant lines using CRISPR/Cas9. Phenotypic analyses are currently underway, particularly focusing on the pronephros. These investigations will provide critical insights into whether these genes contribute to human kidney diseases.

Assessing differences in Vibrio cholerae biotype colonization timelines using a larval zebrafish model

Poster Number: 96

Theme: Disease Models

Presenting Author: **Bhavita Bhaya, MS** - Wayne State University

Co-Author(s): Isabella Cubillejo – Wayne State University; Jonathan Panzer – Wayne State University; Kevin Theis – Wayne State University; Jeffrey Withey – Wayne State University

Abstract: Cholera is an acute diarrheal disease that largely affects areas of the world that lack sanitation infrastructure. Vibrio cholerae is a gram-negative bacterium that emerges seasonally in endemic areas to cause cholera outbreaks. Classical and El Tor are two biotypes belonging to the V. cholerae O1 serogroup that can cause epidemic cholera. The El Tor biotype persists significantly longer in the host even after the diarrheal symptoms have cleared. Zebrafish are useful as a natural host model for V. cholerae infection as the entire disease cycle can be recapitulated in the presence of an intact intestinal microbiome and immune responses. Previous, unpublished studies from our lab indicate that El Tor can colonize adult zebrafish for up to two weeks, but classical gets cleared by 3 days post-infection (dpi). Although both caused perturbation of the intestinal microbiome during infection in adult zebrafish, the microbiome composition reverted by 3 dpi in fish infected with the classical biotype, whereas fish infected with the El Tor biotype had prolonged dysbiosis. We hypothesized that a combination of gut microbiome perturbation and differential immune responses to classical and El Tor biotypes contribute to the colonization duration differences observed in zebrafish. Since it takes about 5-6 weeks for development of the adaptive immune system after zebrafish hatch, using larvae helps us assess the effect of innate immune responses independently of the adaptive. It also provides some insight into how the larval microbiome develops simultaneously with infection. We observed that the larvae were able to clear the classical infection between 28-33 dpf/23-28 dpi whereas the El Tor infection cleared between 54-55 dpf/49-50 dpi. This timeline for clearance is consistent with classical being cleared by innate immune responses and/or microbiota competition, suggesting that El Tor evades innate immune responses and is cleared by adaptive immune responses.

Modelling Fetal Growth Restriction in Zebrafish: Pathway Dysregulation and Therapeutic Targets

Poster Number: 97

Theme: Disease Models

Presenting Author: **Giovanni Risato, PhD** - University of Padua

Co-Author(s): Silvia Visentin – University of Padua; Rudy Celeghin – University of Padua; Raquel Brañas Casas – University of Padua; Pierpaolo Zorzato – University of Padua; Manuela Simonato – University of Padua; Francesco Argenton – University of Padua; Erich Cosmi – University of Padua; Natascia Tiso – University of Padua

Abstract: Fetal growth restriction (FGR) is a pathological condition in which the fetus fails to reach its biological potential due to insufficient oxygen or nutrient supply. It can result from maternal, placental, or fetal factors. FGR is the second most common cause of perinatal mortality and is associated with long-term health complications, including neurological and cardiovascular disorders, diabetes, and

chronic inflammation. It affects 3-10% of fetuses in industrialized countries and 6-30% in developing ones. Despite limited intrauterine diagnostic techniques and therapeutic options, early detection of FGR is crucial for identifying its underlying causes and reducing perinatal mortality. This study utilizes transgenic zebrafish lines, specific to affected tissues and signaling pathways, to investigate developmental alterations in hypoxia-induced FGR larvae. These models exhibit traits similar to human FGR, including growth retardation, delayed nutrient uptake, and impaired movement. Additionally, a reduction in several brain cell types and global leukopenia are observed. Disorganized blood vessels (dilated with thickened endothelial walls) correlate with reduced blood flow and cardiac dysfunction, including lower ejection fractions and impaired contractility. After confirming that the FGR phenotype in zebrafish mirrors the human condition, we investigated possible changes in a set of signaling pathways associated with embryonic growth. Notably, Wnt/Beta-catenin and Jak/Stat3 pathways are consistently dysregulated in both human and zebrafish FGR samples. Pharmacological interventions reveal that modulating Jak/Stat3 has minimal impact on hypoxia-induced FGR phenotypes. In contrast, activating the Wnt/Beta-catenin pathway significantly rescues many FGR-associated abnormalities. Further investigations, using fluorescent transgenic zebrafish lines and in situ hybridization, aim to explore the involvement of the liver and pancreas. In conclusion, zebrafish emerges as a powerful model for elucidating FGR pathogenesis and conducting in vivo screenings of pathway-targeted drugs, offering insights into potential therapeutic strategies for rescuing specific pathological phenotypes.

Unveiling Molecular Mechanisms of Tricho-Hepato-Enteric Syndrome: Insights from a Novel ttc37 Knockout Zebrafish Model

Poster Number: 98

Theme: Disease Models

Presenting Author: **Angela Piersanti** - Department of Biology, University of Padua

Co-Author(s): Annachiara Tesoriere – Department of Biology – University of Padua; Franceso Sernesi – Department of Biology – University of Padua; Luisa Dalla Valle – Department of Biology – University of Padua; Luca Bosa – Department Women's and Children's Health – University Hospital of Padua; Mara Cananzi – Department Women's and Children's Health – University Hospital of Padua; Franceso Argenton – Department of Biology – University of Padua

Abstract: Tricho-hepato-enteric syndrome (THES) is an ultra-rare multisystem disorder resulting from biallelic mutations in TTC37 or SKIV2L genes, which encode components of the SKI complex, a cytosolic cofactor of the RNA exosome. THES is characterized by intractable diarrhea requiring parenteral nutrition for survival, with many patients developing a condition similar to inflammatory bowel disease (IBD) complicated by frequent strictures. The primary causes of mortality are intestinal failure and infections. Despite its severity, our understanding of THES remains limited. This is largely due to a lack of comprehensive data on the SKI complex's function in human physiology and an absence of established disease models. These limitations have impeded progress in unraveling the mechanisms underlying THES and developing effective treatments. To address this, we developed and characterized a ttc37 knock-out (ttc37-/-) zebrafish line to investigate THES pathogenesis and elucidate the mechanisms underlying its enteropathy. The model was created with a 4-nucleotide deletion in the coding sequence to disrupt gene function. Morphological, histological, and transcriptomic analyses were conducted on

this model. While able to reach adulthood and fertile, ttc37-/- zebrafish exhibited significant gut abnormalities. Transcriptomic analysis of 6-day-old larvae revealed altered expression of genes related to cell cycle, apoptosis and immunity, subsequently validated in adult mutant guts. Further investigation through histological analysis of adult zebrafish guts showed severe morphological changes, including absent or enlarged villus structures, goblet cell hyperplasia, epithelial cell stratification and mucosal layer breaches. This novel in vivo model closely mimics THES, providing a robust platform to investigate the molecular mechanisms underlying the condition. It serves as a valuable tool for identifying therapeutic targets, potentially leading to effective treatments. Further research into altered pathways could provide crucial insights for the development of targeted THES therapies, advancing both our understanding and treatment of this severe condition.

Disruption of corneal endothelium morphogenesis in a model of Axenfeld-Rieger Syndrome

Poster Number: 99

Theme: Disease Models

Presenting Author: **Emily Woodruff, PhD** - University of Utah

Co-Author(s): Ella Habbeshaw, BA – Undergraduate Research Assistant, Department of Human Genetics, University of Utah; Kristen Kwan, PhD – Associate Professor, Department of Human Genetics, University of Utah

Abstract: The cornea is the transparent outermost tissue of the eye, consisting of three layers: epithelium, stroma, and endothelium. While all layers are essential for vision, the neural crest-derived corneal endothelium is of particular interest for its function maintaining corneal transparency. Despite its importance, little is known about endothelium formation or its disruption in diseases such as Axenfeld-Rieger Syndrome (ARS). ARS is a congenital condition caused by genetic mutations in Pitx2 or Foxc1 and characterized by cornea and iris malformations. Our goal is to uncover the cellular mechanisms underlying corneal endothelium formation under normal conditions and in a model of ARS, the zebrafish pitx2 mutant. In the pitx2 mutant cornea at 4 dpf, the endothelium appeared indistinct or possibly absent, although the epithelium appeared normal. Quantification of prospective endothelial cells revealed fewer cells in the mutant than in wild type. Immunostaining for markers of proliferation (phospho-histone H3) and cell death (activated caspase-3) indicated that neither plays a substantial role in normal corneal development nor was significantly altered in pitx2 mutants. These results suggested that disrupted neural crest cell movement into the developing cornea might underlie the phenotype. To directly examine cell movement, we performed multidimensional timelapse imaging and 4D cell tracking using LongTracker software. Cell tracking revealed that in wild type, corneal endothelial cells move in front of the lens, while in pitx2 mutants, cells remain at the lens periphery, failing to reach the correct position. pitx2 mutant cells also appear to move faster but non-processively. These results suggest that failure of corneal endothelial cells to move in front of the lens may underlie the mutant corneal phenotype. Moving forward, we aim to uncover the molecular mechanism underlying the pitx2 mutant phenotype by examining potential adhesive interactions between the cornea and lens, and potential disruptions to Wnt signaling.

Protein Turnover Downstream of the Nipbl/CRL4 axis Contributes to Abnormal Development in zebrafish embryos

Poster Number: 100

Theme: Disease Models

Presenting Author: **Niusha Banoukh** - Lehigh University

Co-Author(s): Annie C. Sanchez – Lehigh University; Fiona Mensching – Lehigh University; Robert V. Skibbens – Lehigh University; M. Kathyrn Iovine – Lehigh University

Abstract: Cohesinopathies, including Cornelia de Lange Syndrome (CdLS) and Roberts Syndrome (RBS), are caused by mutations in cohesin complex components or cohesin regulators. While mutations in SMC3—a core cohesin subunit—are implicated in CdLS, they account for only a small fraction of cases. On the other hand, mutations in the cohesin regulator, Nipbl, accounts for 65% of the CdLS cases. Prior studies indicate that cohesin proteins, such as Esco2 and Smc3, regulate the CRL4 E3 ubiquitin ligase. This raises the possibility that other key cohesin regulators, such as Nipbl, also impact CRL4.To test this, we examined the effects of Nipbl knockdown in zebrafish embryos using morpholinos. Knockdown of Nipbl resulted in developmental abnormalities and a marked reduction in the transcription of CRL4 components such as ddb1, a critical component of the CRL4 ligase. The severity of these defects was partially rescued by exogenous ddb1 mRNA, suggesting that CRL4 is regulated downstream of Nipbl. Given that CRL4 mediates the degradation of various proteins during development, its disruption may lead to the accumulation of key substrates that drive disease phenotypes. To investigate this, we performed LC-MS analysis and identified various candidate substrates in cohesin and Ddb1 knockdowns. Moreover, we showed that overexpressing one of the candidate substrates exogenously, namely pparαa is sufficient to recapitulate some aspects of the developmental defects. These findings provide evidence that aberrant accumulation of CRL4 substrates contributes to developmental abnormalities, reinforcing a link between cohesin dysfunction, ubiquitin-mediated protein turnover, and the pathogenesis of CdLS and RBS.

Dpp9 regulates hematopoiesis and craniofacial development

Poster Number: 101

Theme: Disease Models

Presenting Author: **Sarah LaPotin** - University of Utah

Co-Author(s): Madeleine Servais – Graduate Student, University of Utah; Kristen Kwan – Professor, Human Genetics, University of Utah

Abstract: Mutations in Dipeptidyl Peptidase 9 (DPP9) cause DPP9 deficiency, an autoinflammatory disease characterized by persistent inflammasome activation. DPP9 deficient patients can present with a variety of phenotypes, including pancytopenia and skeletal abnormalities, which appear developmental in origin. It is not currently known how or why loss of DPP9 impacts embryonic development and whether these phenotypes are the result of the dysregulation and activation of the inflammatory response in these patients. To investigate the role of DPP9 during development, I generated stable dpp9

mutant zebrafish, which, similar to patients, display transcriptional signatures of inflammation and inflammasome activation. Dpp9 mutants are largely neutrophil deficient past 3 dpf despite possessing an outwardly normal population of hematopoietic stem cells. Interestingly, the dpp9 mutant neutrophil deficiency is not transient and does not appear to depend on canonical inflammasome formation. In addition to disruptions to hematopoiesis, dpp9 mutants display craniofacial defects, notably, an accelerated timeline of bone development in the lower jaw. Current efforts are focused on determining the mechanisms underlying these developmental disruptions and whether the roles dpp9 plays in regulating hematopoiesis and craniofacial development are dependent or independent of its function as a negative regulator of the inflammatory response.

AI-Guided Phenotypic Landscapes Reveal Optimal WE Medicine-Based Combinational Therapies in Zebrafish Models of Hepatocellular Carcinoma

Poster Number: 102

Theme: Disease Models

Presenting Author: **Chiou-Hwa Yuh** - Institute of Molecular and Genomic Medicine National Health Research Institutes (NHRI) Zhunan, Miaoli County, Taiwan

Co-Author(s):

Abstract: Hepatocellular carcinoma (HCC) is a complex and heterogeneous malignancy with limited therapeutic options. General HCC, often driven by TERT activation, remains the predominant form, but the rising prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) is shifting disease etiology. MASLD-associated HCC is increasing in parallel with global obesity, while HBV- and HCV-related HCC cases decline due to vaccination and antiviral therapy. Addressing this dual landscape requires models and tools capable of capturing disease subtype differences and optimizing combinational therapies. We developed an AI-powered phenotypic response surface (AI-PRS) platform that integrates high-throughput in vitro screening with in vivo validation in zebrafish. Using hepatoma (Huh7, PLC5, Hep3B) and normal hepatocyte (THLE-2) cell lines, we measured viability across drug-dose matrices to train neural network models that generate high-resolution 3D phenotypic response landscapes. Two transgenic zebrafish models were used: CD36*abcg1 knockout under high-fat diet to model MASLD-associated HCC, and tert*p53- mutants under normal diet to model general HCC. By 15 days post-fertilization, both models developed liver tumors. These were crossed with hepatocyte-specific fluorescent lines to quantify nuclear-to-cytoplasmic (N:C) ratio changes as a measure of tumor burden. Additionally, macrophage-red and neutrophil-green reporters enabled real-time visualization of immune cell infiltration following treatment. Critically, this study applies a WE Medicine strategy, combining the molecular specificity of Western therapeutics (lenvatinib, regorafenib, metformin) with the holistic, multi-targeted nature of Eastern medicine compounds (oligo-fucoidan, propolis). AI-PRS revealed optimal dosing combinations that significantly enhanced tumor suppression while minimizing toxicity, outperforming conventional strategies. This integrative approach demonstrates how zebrafish phenomics and AI analytics can accelerate personalized HCC therapy development. It offers the zebrafish research community a robust platform for evaluating complex, mechanism-informed, and culturally relevant treatment regimens.

Enhancing transcript representation and integration with other data in ZFIN

Poster Number: 103

Theme: Other

Presenting Author: **Sridhar Ramachandran** - Zebrafish Information Network, University of Oregon

Co-Author(s): Yvonne Bradford – Zebrafish Information Network; Christian Pich – Zebrafish Information Network; ZFIN Staff – Zebrafish Information Network; Ryan Taylor – Zebrafish Information Network

Abstract: Transcript data provides detailed insights into gene structure, alternative splicing and isoforms, and enriches gene annotations. The Zebrafish Information Network (ZFIN) is the central resource for genetic, genomic and phenotypic data from zebrafish research. Since transcripts link genomic sequences to functional outcomes, robust coverage of transcripts is essential for enabling cross-species comparisons and providing context for conserved or divergent gene functions and evolutionary relationships. Transcript data in ZFIN is made available through data exchanges with Ensembl. Historically, the transcript data made available from ZFIN were transcript records which were manually annotated by the Human and Vertebrate Analysis and Annotations team (HAVANA). In an effort to expand the ZFIN/Ensembl data exchange, as well as provide better gene models, ZFIN is now incorporating zebrafish transcript records which are solely generated by the Ensembl gene annotations pipeline (https://ensembl.org/Danio_rerio/Info/Index). The inclusion of transcripts from the Ensembl gene annotation pipeline has allowed for the resolution of ambiguous or incorrect Ensembl gene and transcript ID associations as well as greatly increased overlap of genes and transcripts between ZFIN and Ensembl. In addition, ZFIN exchanges transcript data with the Alliance of Genome Resources (http://alliancegenome.org) which facilitates the processing of allele and variant data associated with zebrafish genes via the Variant Effect Predictor (VEP) pipeline, generating molecular consequences as well as unique HGVS nomenclature for the variant. Here we will describe ZFIN transcript pages, the transcript data and metadata provided on those pages, how to access transcript records at ZFIN through regular searches, BLAST and the genome browser (JBrowse), as well as VEP allele/variant data at the Alliance. The increased integration of transcript data at ZFIN will help refine gene models, accurately map gene boundaries and will help maintain strong cross-references to data from Ensembl, NCBI and UniProt in the upcoming GRCZ12 Tu reference genome.

New at ZFIN: Expression, Phenotype, and Human Disease Model Annotations for Chemical Exposure Data

Poster Number: 104

Theme: Other

Presenting Author: **Yvonne Bradford, PhD** - ZFIN, University of Oregon

Co-Author(s): Ceri Van Slyke – ZFIN, University of Oregon; Jonathan Muyskens – ZFIN, University of Oregon; Wei-Chia Tseng – ZFIN, University of Oregon; Christian Pich – ZFIN, University of Oregon; Ryan Taylor – ZFIN, University of Oregon; Monte Westerfield – ZFIN, University of Oregon; ZFIN Staff – ZFIN, University of Oregon

Abstract: Over the past several decades zebrafish have increasingly been used to investigate the effects of chemical exposures, becoming an ideal model to study toxicity, phenotypic outcomes, and gene-

chemical interactions. Despite this increase in zebrafish-chemical exposure data, database resources supporting zebrafish toxicology and environmental exposure research are limited. To fill this gap, ZFIN is now providing annotation support for zebrafish gene expression, phenotype, and human disease model data that are the result of chemical exposure. To support the annotation of experimental conditions, ZFIN utilizes the Chemical Entities of Biological Interest Ontology (ChEBI) along with the Zebrafish Experimental Conditions Ontology (ZECO) to denote the chemical used and route of exposure, respectively. Chemical data are aggregated and viewed on newly created chemical term pages which list gene expression, phenotype and human disease model data for experiments which include chemicals. We will present information on how ZFIN annotates chemicals in experimental conditions, how to view and search for these data on ZFIN web pages and the download files available for bulk data use.

Notch Receptors Involved In The choice Between Secretory and Enterocyte Cells Within The Embryonic Intestinal Epithelium

Poster Number: 105

Theme: Other

Presenting Author: **Anisa Iftikhar** - Clarkson University

Co-Author(s): Kenneth Wallace – Professor, Biology, Clarkson University

Abstract: During the first half of embryogenesis, the intestinal epithelium is a simple layer ofcuboidal cells surrounded by lateral plate splanchnic mesoderm. As embryogenesisproceeds, the epithelium differentiates to include a variety of enterocytes andsecretory cells. Using a Notch driven Cre ERT2 combined with a nuclear mCherryreporter, we identify at least three different periods where Notch signaling is active.The first two periods involve the choice between secretory and enterocyte cells. Thefirst period occurs between 30 to 34 hpf but is unclear as to how this Notch signalingevent affects numbers of secretory cells as this occurs before the lateral inhibitionphase. The second period of Notch signaling occurs between 64 to 74 hpf whenthere is active choice between secretory and enterocytes. The third period of Notchsignaling does not appear to involve lateral inhibition but instead involves formationof a unique secretory cell subtype that begins differentiating after 74 hpf andcontinues throughout the post embryonic period. Although we have identified thetiming of Notch activation within the intestine, we do not know which of the receptorsparticipate in these events. Zebrafish have four receptors (Notch 1a/1b, Notch 2, andNotch 3) which are expressed homogeneously within the intestine. Here we use bothsingle and double mutant combinations of null Notch mutants to identify which ofthese receptors play a role in each of the intestinal epithelial signaling events. Wefind that the choice between secretory and enterocytes utilizes two receptors while asingle receptor is involved in formation of the unique secretory cell subtype. Thisproject was funded by EUNICE KENNEDY SHRIVER NATIONAL INSTITUTE OFCHILD HEALTH AND HUMAN DEVELOPMENT (Grant # 1R15HD108689-01).

Establishment of a Zebrafish Xenograft Model to Investigate Microbiome-Mediated Macrophage Polarization

Poster Number: 106

Theme: Disease Models

Presenting Author: **Huijung Han** - KRIBB

Co-Author(s): Jae-Geun Lee – KRIBB; Jeong-Soo Lee – KRIBB

Abstract: Macrophages are critical immune cells involved in inflammatory responses, including antigen presentation, phagocytosis, and immune regulation. They are polarized into two phenotypes:M1 and M2. M1 macrophages are pro-inflammatory playing a key role in pathogen elimination and initiating immune responses, while M2 macrophages exhibit anti-inflammatory functions, contributing to tissue repair and immune modulation. In the tumor microenvironment, M2 macrophages increase and contribute to immune-suppression and secrete growth factors that promote the proliferation and metastasis of tumor cells. In this study, we aim to elucidate the underlying mechanisms of interaction between human cancer cells and polarized macrophages using a zebrafish xenograft model. First, we compared tumor growth using zebrafish xenograft models by transplanting SW480 and SW620 colorectal cancer cell lines into Tg(mfap4:QF3, 13xQUAS:YFP-2A-NTR2.0)) zebrafish transgenic embryos at 2 days post-fertilization, this approach enabled us to observe the recruitment of macrophages to cancer cells in vivo. The different cancer cell lines exhibited distinct patterns of survival, proliferation, and dissemination. Additionally, to examine the influence of the microbiome on macrophage behavior, zebrafish were immersed in cultures of bacteria following fin amputation. To assess the impact of microbial exposure on macrophage polarization, we quantified the ratio of M1 to M2 macrophages in control versus bacteria-treated groups, revealing strain-specific shifts in macrophage phenotype. Currently, macrophage polarization in the tumor microenvironment is assessed using fluorescence in situ hybridization (FISH) and will be visualized in vivo through a 3D imaging system. This zebrafish xenograft model enhances our understanding of cancer progression and roles of macrophages. Furthermore, with growing interest in microbiome-immune interactions, this model also provides insights into how gut microbiota influence macrophage polarization and cancer progression. By integrating microbiome research with the zebrafish xenograft model, this study aims to establish a platform for microbiome-driven cancer immunotherapy.

Poster Session III

Themes: Cancer & Growth Control, Circuits & Behavior, Evolution & Comparative Biology, Lifespan & Aging, Neurobiology, and Regeneration

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Cadherin-16 promotes sensory gating through a Calcium-regulatory pathway and the endocrine corpuscles of Stannius

Poster Number: 1

Theme: Neurobiology

Presenting Author: **Jessica Nelson** - University of Colorado, Anschutz Medical Campus

Co-Author(s): Susannah Schloss – University of Colorado, Anschutz Medical Campus; Zackary Marshall – University of British Columbia; Nicholas Santistevan – University of Colorado, Anschutz Medical Campus;

Stefani Gjorcheska – Cincinnati Children's Hospital Medical Center; Amanda Stenzel – University of Colorado, Anschutz Medical Campus; Lindsey Barske – Cincinnati Children's Hospital Medical Center

Abstract: We are constantly flooded with sensory information. How do we decide which stimuli are critical and which can be ignored? While a handful of neural mechanisms regulating the process of sensory gating have been identified, brain-extrinsic regulators of internal state also play key roles. We identified cdh16, a gene encoding the cell-cell adhesion molecule Cadherin-16, as a regulator of sensory gating. cdh16 mutants are hypersensitive to acoustic stimuli, responding at high rates to stimuli their siblings are able to filter out and ignore. Although Cadherins often regulate neural development, we find that Cadherin-16 is primarily expressed in the corpuscles of Stannius (CS), endocrine organs that release the hormone Stanniocalcin 1l (encoded by stc1l). Using in situ hybridization, genetic epistasis, and quantitative assays of behavior, we find that when Cadherin-16 function is impaired, the CS is larger and more stc1l is produced. Excess Stanniocalcin 1l then suppresses the function of Pregnancy-Associated Plasma Protein-aa (Papp-aa). But how does this endocrine pathway regulate function within the brain and ultimately sensory gating? One key role for the pathway comprising Stanniocalcin 1l and Papp-aa is to regulate the uptake of environmental Ca2+. We find that in cdh16 mutants, whole-body Ca2+ is reduced, as are epithelial cells (called NaR ionocytes) specialized for Ca2+ uptake. We additionally find that reducing environmental Ca2+ behaviorally phenocopies the loss of Cadherin-16, consistent with a role for this endocrine pathway in regulating behavior through the regulation of cation homeostasis. Finally, underscoring the newly discovered role of the endocrine corpuscle of Stannius in the regulation of behavior, we find that ablation of these structures results in disrupted sensory gating. These data highlight the role that brain-non-autonomous regulators can play in sensory gating and the emerging importance of proper ion homeostasis for behavioral regulation.

Fishing for Neurocircuitry: Identifying and Characterizing Multisensory Integrating Neurons in the Optic Tectum of Zebrafish

Poster Number: 2

Theme: Neurobiology

Presenting Author: **Suehelen Garcia, BS** - BYU

Co-Author(s): Adeline Hamilton – BYU; Logan Carson – BYU; KariAnne Jex – BYU; Erika Marks – BYU; Jordan Yorgason – BYU; Tracianne Neilson – BYU; Arminda Suli – BYU

Abstract: The superior colliculus (SC) is a mammalian midbrain structure involved in multimodal sensory integration and is implicated to have a role in neurodevelopmental disorders. Although the presence of multisensory integrating neurons (MINs) in the SC has been well documented by electrophysiology techniques, little is known about their morphological or molecular characterization. To identify and study MINs, we utilized SC's non-mammalian homologous structure–the optic tectum (OT)–in the genetically tractable model organism zebrafish. In this process, we generated transgenic lines that allowed for fluorescent detection of neuronal activity by expressing the genetically engineered calcium indicators: H2B-jRGECO1a and cytoRGECO, respectively in the OT and mechanosensory hair cells of the ear. To activate the vestibular sensory pads in 7 days-post-fertilization (dpf) larvae, we used a piezoelectric actuator probe. We found that upon the application of this vibration stimulus to the skin and posterior cristae (PC), some OT neurons showed time-locked activation. This indicates that OT

circuitry in 7dpf larvae is mature enough to receive vestibular and somatosensory stimuli. Furthermore, when 7dpf larvae were sequentially and simultaneously exposed to vibration and light stimulations (1 second pulse of 488nm light), we found OT neurons that responded to concurrent stimuli and showed characteristics of multisensory integration. Identification of MINs in the zebrafish OT sets the stage for better morphological and molecular characterization and their role in neurodevelopmental disorders.

Induction of neuronal mitophagy at the synapse

Poster Number: 3

Theme: Neurobiology

Presenting Author: **Serena Wisner** - University of Wisconsin-Madison

Co-Author(s): Catherine Drerup – Department of Integrative Biology – University of Wisconsin-Madison

Abstract: Mitochondria at the synapse are critical for synaptic function. These organelles buffer calcium and produce ATP required for synaptic vesicle recruitment and recycling, among lesser known functions. It is no surprise then that defects in their maintenance are highly linked to neurodegenerative disease. As a key example, mutations in mitophagy related genes Pink1 and Parkin cause Parkinson's disease. Important work over the past few decades has made numerous discoveries delineating the mechanisms of mitochondria turnover and its dysfunction in disease. Yet despite what we have learned about mitophagy, we know strikingly little about mitophagy in neuron populations, especially at the synapse. We used a genetic indicator of mitophagy and serial block face scanning electron microscopy to look at mitophagy in the intact, defined circuit of sensory and motor neuron populations in zebrafish larvae. Through these methods, we found mitophagy occurs at the synapse of these neurons at similar rates. Additionally, we find that the mitophagic rate does not change with physiological levels of stimulation or genetically induced mitochondria damage. Only when mitochondria are genetically damaged and neurons are pushed towards higher activity does the rate and type of mitophagy change. Currently, we are investigating the role of Parkinson's risk genes PINK1 and PARKIN in this increased mitophagic demand. Together, our work contributes to our understanding of mitophagy in neurons, how it changes with mitochondria damage and neuronal activity, and how this relates to our understanding of the dynamics of this pathway in neurodegenerative disease.

Inhibition of Retrograde Transport Impairs Mitochondrial Biogenesis in Neurons

Poster Number: 4

Theme: Neurobiology

Presenting Author: **Angelica Lang** - University of Wisconsin-Madison

Co-Author(s): Catherine Drerup – University of Wisconsin-Madison; Chris Stein – University of Wisconsin-Madison

Abstract: Neurons are highly dependent on mitochondria to function and survive. Impaired mitochondrial health correlates with a range of neurodegenerative diseases, including Parkinson's and Alzheimer's. To maintain a healthy mitochondrial population in a long-lived cell like the neuron, mitochondria must be continuously replenished through the process of mitochondrial biogenesis. As the

majority of mitochondrial proteins are nuclear encoded, mitochondrial biogenesis requires communication between mitochondria and the nucleus. This can be a challenge in a large, compartmentalized cell like a neuron in which a large portion of the mitochondrial population is in the distal axon, far from the nucleus. Given the size and complexity of the neuron, it remains unclear how mitochondrial biogenesis is regulated to maintain the proper density of mitochondria throughout the entire cell. We hypothesized that mitochondrial transport between the cell body and the distal axonal compartments could serve as a signal to regulate mitochondrial biogenesis. To test this, we genetically manipulated zebrafish posterior lateral line neurons to disrupt the retrograde transport of mitochondria from axons back to the cell body and measured mitochondrial biogenesis markers in vivo. We found that the expression of transcriptional regulators of biogenesis, mtDNA replication, and cell body derived mitochondrial biomass were all significantly decreased when mitochondrial retrograde transport was disrupted. This resulted in impaired mitochondrial turnover in the axon terminal. Transcriptomics confirmed widespread loss of mitochondrial gene expression in neurons lacking mitochondrial retrograde transport. In silico transcription factor enrichment analyses and in vivo rescue analyses demonstrated that estrogen-related receptor activation links mitochondrial transport to the nuclear gene expression necessary for mitochondrial biogenesis. Together, our data support a role for retrograde feedback between axonal mitochondria and the nucleus through estrogen-related receptors for regulation of mitochondrial biogenesis in neurons.

Expansion of the zebrafish anterior lateral line depends on innervation

Poster Number: 5

Theme: Neurobiology

Presenting Author: **Theresa Christiansen** - University of Chicago

Co-Author(s): Vishruth Venkataraman – Graduate Student, Integrative Biology, University of Chicago; Victoria Prince, PhD – Professor, Organismal Biology & Anatomy, University of Chicago

Abstract: Lateral lines are a sensory system used by almost all aquatic vertebrates to sense hydrodynamic information in their environments. The system comprises distributed sense organs called neuromasts and their innervating ganglia. These are organized into anterior lateral lines (ALL) around the eye and jaw versus posterior lateral lines (PLL) on the trunk. At postembryonic stages, the early-forming neuromasts expand in size and become encased by bony canals, while late-forming superficial neuromasts are added to maintain sensory density with growth. In contrast to the well-studied PLL, there has been limited study of postembryonic development of the ALL to date. To fill this gap we have characterized the developmental mechanisms and innervation patterns driving the expansion of the zebrafish ALL. Using a tissue clearing protocol to observe transgenic and antibody markers of nerves and neuromasts through ontogeny, we find continuous neuromast addition in the ALL. Superficial neuromast lines form parallel to existing canal lines at late larval stages, with new neuromasts added through both budding and a novel nerve-driven mechanism. Despite most canal lines being innervated by the anterodorsal ganglion, we find that the ALL superficial lines are innervated by the anteroventral ganglion. Using unilateral ALL ganglion ablation we have demonstrated that innervation is necessary for both superficial neuromast formation and growth of the canal neuromasts. Our study establishes that innervation plays a critical role in the expansion of the ALL and highlights mechanistic differences

between the ALL and PLL systems. Moreover, our findings reveal a “developmental switch” for the ALL, in which innervation becomes necessary for a secondary phase of sense organ development.

Uncovering convergent retinal and pineal pathways for functional asymmetry in teleost fish

Poster Number: 6

Theme: Neurobiology

Presenting Author: **Jacob Starkey, PhD** - West Virginia University

Co-Author(s): John Hageter – West Virginia University; Rob Kozol – Florida Atlantic University; Kevin Emmerich – Johns Hopkins University; Jeff Mumm – Johns Hopkins University; Erik Duboué – Florida Atlantic University; Eric Horstick – West Virginia University

Abstract: Brain lateralization is a conserved characteristic of Bilateria, where neural functions are primarily processed in one hemisphere. These hemispheric specializations are believed to enhance behavioral efficiency and may underlie behavioral asymmetries, like handedness. Despite the widespread occurrence of behavioral lateralization, our understanding of the neural and molecular mechanisms underlying functional lateralization remains limited. Furthermore, the evolutionary selection for and modulation of lateralization is poorly understood. Comparative approaches provide a powerful means to address fundamental biological questions, yet a significant challenge in studying laterality has been the absence of a conserved lateralized behavior in laboratory-accessible species. We have previously identified a robust visuomotor asymmetry in larval zebrafish in which individuals will conduct a persistent left or rightward turn bias following the loss of illumination. Here, we demonstrate that this turn bias is conserved across a diversity of species in sighted larval teleost but is absent in naturally blind cavefish. Through our comparative approach, we reveal that sighted teleost exhibit distinct retina-dependent and -independent pathways supporting behavioral asymmetry. We find this retinal independent pathway is regulated by the pineal, and we further demonstrate that both pineal and retinal mechanisms converge on the same thalamic region. We show that the newly identified pineal-dependent pathway induces functional asymmetry in the thalamus through a distinct mechanism and its output is sufficient to dictate motor asymmetry direction. Last, comparison of the pineal between sighted and blind teleost suggests an evolutionary mechanism regulating asymmetry. Our findings uncover a previously unknown pathway that governs brain lateralization. More broadly, these results suggest that convergent neural circuits may serve as a mechanism for behavioral robustness and highlight the thalamus as a conserved neural substrate regulating brain functional asymmetry.

Single-cell analysis of mechanisms underlying the functional diversification of motor neurons that arise in the same place

Poster Number: 7

Theme: Neurobiology

Presenting Author: **Riku Yasutomi** - Department of Genome Sciences, University of Washington & Basic Sciences Division, Fred Hutchinson Cancer Center

Co-Author(s): Austin Seroka – Basic Sciences Division – Fred Hutchinson Cancer Center; Italia DiChristina – Basic Sciences Division – Fred Hutchinson Cancer Center; Cole Trapnell – Department of Genome Sciences – University of Washington; Cecilia Moens – Basic Sciences Division – Fred Hutchinson Cancer Center

Abstract: During development, neurons that share a common spatial origin go on to acquire unique properties and targets to carry out distinct functions. A fundamental goal of developmental neurobiology is to understand the mechanisms by which these functional differences arise. In zebrafish, neurons expressing LIM/homeobox gene, isl1, arise in the 4th and 5th rhombomeres, migrate caudally, and become either a facial branchiomotor neuron (FBMN) or octavolateral efferent neuron (OEN). In humans, the FBMNs innervate muscles of facial movement and expression, while OENs innervate sensory hair cells in the cochlea to suppress damage triggered by excessive auditory stimuli. In fish, FBMNs innervate muscles involved in feeding and ventilation, while OENs directly innervate neuromast hair cells to suppress sensory stimulation triggered by self-movement. While the shared developmental programs between FBMNs and OENs are well established, the mechanisms that determine their unique targets and functions remain poorly understood. Furthermore, the development of functional differences among FBMNs has not been studied. To assess heterogeneity between these developmentally similar neurons, we used single-cell RNA-sequencing and identified a unique trajectory corresponding to the FBMN/OEN lineage. Hybridization chain reactions revealed a changing gene expression program as neurons migrate to their final positions in the brainstem, with earlier-born FBMN/OENs arriving first, taking up ventral positions and expressing different genes than later-born FBMN/OENs that take up more dorsal positions. This sequence of events is not triggered by changes in FBMN position over time, since they occur in PCP mutants in which FBMNs fail to migrate. We are currently using retrograde labeling to determine whether these gene expression domains correspond with neurons that innervate distinct targets. Our results suggest the possibility of targeting differences based on gene expression differences that may themselves be specified by birth order.

Two Lef1 isoforms with distinct functions have unique required roles in hypothalamic gene expression and larval behavior

Poster Number: 8

Theme: Neurobiology

Presenting Author: **Guangning Wang** - University of Utah

Co-Author(s): Richard Dorsky – University of Utah; Priscilla Figueroa – University of Utah

Abstract: In previous work we discovered that Lymphoid enhancer factor 1 (Lef1), a transcriptional mediator of Wnt signaling, regulates hypothalamic neurogenesis and stress-related behaviors in zebrafish and mice, and may function similarly in other species including humans. We now want to understand whether the hypothalamic expression of different Lef1 protein isoforms could provide a mechanism for specifically modulating behavior through neurogenesis without affecting its many other critical roles. Alternative splicing creates Lef1 isoforms with two highly conserved C-terminal N-tail or B-tail polypeptides, which have unknown functions and differential expression. We found that overexpression of the human Lef1 B-tail and N-tail isoforms in zebrafish embryos produces phenotypes consistent with Wnt pathway activation at distinct developmental timepoints. Furthermore, a consensus

kinase recognition sequence in the B-tail suggests that this isoform may be uniquely regulated through phosphorylation that is altered by a human SNP-encoded LEF1 variant allele. We are now using zebrafish overexpression assays to determine how the C-termini and B-tail phosphorylation can directly affect Lef1 function. The highest relative level of B-tail-encoding Lef1 mRNA is expressed in the developing brains of mice and zebrafish, consistent with its conserved roles in regulating neurogenesis. To determine the required functions of individual Lef1 isoforms. we used CRISPR/Cas9 mutagenesis to generate isoform-specific zebrafish mutant lines. Our preliminary analysis reveals B-tail isoform-dependent gene expression in the developing hypothalamus, and we are currently pursuing several candidates with links to human brain disorders. We have also observed allele-specific behavioral phenotypes in mutant larvae, and will now test their relationship to hypothalamic stress response circuitry using physiological and additional behavioral assays. Our ultimate goal is to create allele-specific zebrafish models that can provide genetic links between human behavioral disorders and Wnt pathway dysfunction.

The effect of housing conditions on glial bridging and anxiety in larval zebrafish after spinal cord injury (SCI)

Poster Number: 9

Theme: Neurobiology

Presenting Author: **Lawson Cross** - East Carolina University

Co-Author(s): Karen Mruk, PhD – Assistant Professor, Pharmacology and Toxicology, East Carolina University

Abstract: Spinal cord injury (SCI) is a major public health burden. There is currently no cure for SCI and up to 30% of people with SCI will develop anxiety. Regenerative models, like the zebrafish, may be our best resource for finding new treatments. Our lab previously demonstrated that when given a food source that is alive and moves (rotifers), a larger percentage of larval zebrafish form a glial bridge and recover motor skills after SCI than larvae given a pellet diet. However, it was unclear whether increased motor recovery was from a difference in nutrition or the visual stimulation of moving prey. In addition, whether the zebrafish exhibited anxiety-like behavior after SCI and during recovery, particularly in the presence of live food, was never determined. In this study, we test whether larvae individually housed or housed together affect motor recovery post SCI. Measuring both total swim and thigmotaxis, we observed no difference between housing conditions for uninjured animals. However, large swimming increased with time in different rearing conditions. We next used manual transection to induce SCI in larval zebrafish and are currently analyzing total swim and thigmotaxis in the above conditions. We anticipate these studies will provide additional information regarding how environmental cues affect the recovery process after SCI.

Loss of m6A mRNA readers in zebrafish model impacts head size and brain size phenotypes implicated in autism

Poster Number: 10

Theme: Neurobiology

Presenting Author: **Gabriana La** - University of California, Davis

Co-Author(s): Megan Dennis – Associate Professor, PI, Biochemistry & Molecular Medicine, University of California, Davis

Abstract: Autism (ASD) is a complex neurodevelopmental condition encompassing a diverse range of phenotypes. ASD is highly heterogeneous in both phenotypic presentation and genetic predisposition, leaving much to be uncovered regarding its genetic etiology. Focusing on disproportionate megalencephaly (DM), a subphenotype of ASD where individuals have an enlarged brain disproportionate to their height, we identified two probands harboring cases of de novo mutations in two m6A-mRNA reader genes with no prior association with ASD — a duplication of YTHDF2 and a loss of function mutation in YTHDC1, respectively. Our recently published data using zebrafish show that knocking out ythdf2 yielded smaller brain area and overexpression via injected transcripts resulted in larger brain area, recapitulating patient phenotypes. Following these results, we aimed to functionally assay other m6A mRNA reader genes including ythdc1, ythdc2, ythdf1, and ythdf3, to see how loss of function may also impact head and brain size phenotypes in our zebrafish model. Upon knocking out these genes using CRISPR mutagenesis, we observe significantly smaller head sizes in ythdc1 knockouts and significantly larger head sizes in ythdf3 knockouts at 3 days post fertilization. Additionally, ythdc1, ythdf1, and ythdf3 knockout mutants all exhibit shorter body lengths. Measuring insignificant changes in head trunk angle for all mutants confirmed these differences were not due to developmental delay. Within the m6A mRNA pathway, readers interact with m6A-modified transcripts in the nucleus and cytoplasm, dictating the balance and timely export, translation, stabilization, and degradation of their target transcripts. To test the hypothesis that m6A-modified transcripts relevant to neurogenesis may be impacted by the loss of function of any of these m6A mRNA readers, we are actively characterizing mutant model transcriptomes. Our work will expand our mechanistic understanding of ASD etiology, potentially connecting m6A mRNA modifications as a contributor to this complex condition.

Vagus nerve development: a transcriptional code for viscerotopy?

Poster Number: 11

Theme: Neurobiology

Presenting Author: **Austin Seroka, PhD.** - Fred Hutchinson Cancer Center

Co-Author(s): Adam Isabella – University of Minnesota; Cecilia Moens – Fred Hutchinson Cancer Center

Abstract: During development, the central nervous system establishes precise connections with the body to coordinate organ function. A crucial component of communication between the brain and body is the vagus nerve (cranial nerve X), which innervates multiple organ systems including the heart, lungs and digestive tract to regulate blood pressure, heart rate, respiration and digestion. Despite this important role, the molecular mechanisms guiding the vagus nerve to these organ targets during development remain unknown. We have developed the zebrafish embryo as a powerful model for interrogating vagus nerve development, taking advantage of its optical clarity and genetic accessibility. Using a novel photoconversion-based retrograde axon tracing approach we show that vagal motor neurons (mXns) that project to different organs (e.g. gallbladder, stomach, intestines) are spatially segregated within the hindbrain vagus nucleus. We hypothesize that these distinct mXn "target groups" have distinct molecular identities that guide axon targeting. To test this hypothesis we have generated a

developmental scRNAseq atlas focused on cranial motor neurons and have validated the spatially restricted expression of transcription factors and cell-surface molecules within the vagus motor nucleus. We are generating genetic tools to correlate gene expression with target groups, and performing a reverse mutagenesis screen to test the role of these candidates in topographic map formation, revealing preliminary mXn identity phenotypes. We have identified a role for the tbox transcripton factor, tbx5b, in guiding visceral vagus motor neuron identity. We have also observed that mXn axons contact specific subsets of enteric neurons (ENS) during motor axon pathfinding and have begun testing the role of these contacts in guiding topographic motor targeting.

Axon targeting of transcriptionally distinct pioneer neurons is regulated by retinoic acid signaling

Poster Number: 12

Theme: Neurobiology

Presenting Author: **Alex Nechiporuk** - Oregon Health & Science University

Co-Author(s): Benjamin Woodruff – Oregon Health and Science Univ; Lauren Miller – Oregon Health and Science Univ; Nicholas Calistri – Oregon Health and Science Univ; Jacqueline Mcvay – Oregon Health and Science Univ; Laura Heiser – Oregon Health and Science Univ

Abstract: During nervous system development, pioneer neurons (pioneers) extend their axons towards distant targets, creating a scaffold for follower neurons and defining the initial structure of the nervous system. Despite years of study, whether pioneer neurons are molecularly distinct from followers is unknown. To address this question, we performed single-cell RNA sequencing (scRNA-seq) of zebrafish posterior lateral line (pLL) sensory neurons and found that pioneers and followers are transcriptionally distinct populations. Interestingly, expression profiling of differentiating pLL progenitors defines “follower” as the ground state and “pioneer” as a later developmental state, with retinoic acid (RA) signaling active in all pLL progenitors. Modulation of RA signaling within single pLL neurons showed that its downregulation is necessary for expression of a neurotrophic factor receptor ret, which is required for correct targeting of pioneer axons. Our study reveals molecular heterogeneity between pioneer and follower neurons and implicates RA signaling in correct pioneer axonal targeting.

Profiling Chromatin Accessibility and Regulatory Regions in Zebrafish Neurogenesis

Poster Number: 13

Theme: Neurobiology

Presenting Author: **Jessie Greenslade** - University of Pennsylvania

Co-Author(s): Bushra Raj – University of Pennsylvania; Marisa Reed – University of Pennsylvania

Abstract: During development, regulation of gene expression underlies proper establishment of cell identity and function across diverse cell types. Chromatin accessibility is a key component of this gene regulation. Accessible regions often comprise active cis-regulatory elements (CREs) along the DNA, which modulate transcriptional activity. However, the nature of chromatin landscapes during brain

development is poorly understood. I have profiled chromatin accessibility of over 50,000 cells in the zebrafish brain and retina to identify active CREs during neurogenesis. Using singe cell Assay for Transposase Accessible Chromatin (scATAC), I collected data at 3 days post fertilization (dpf) and 21 dpf, representing early and later neurogenesis. In total, this data reveals 378,950 consensus peaks along the genome. Using integration of scATAC-seq data with previously collected scRNA-seq data, I annotated up to 54 unique cell clusters at 3 dpf and 60 unique clusters at 21 dpf. The annotated cell types include several classes of brain progenitors and neurons, retinal cells, and non-neuronal cell types. I compared changes in chromatin accessibility between the two timepoints and identified timepoint-specific putative CREs. For example, a comparison of retinal cone cells at 3 dpf versus 21 dpf revealed 22,662 differentially accessible regions. Regions of accessibility in the cone cells were proximal to genes such as syne2a, known to function in nuclear migration and photoreceptor maintenance, and pde6ha, a cone photoreceptor marker shown to regulate photopic sensitivity. The former showed increased accessibility at 3 dpf but diminished accessibility by 21 dpf. In contrast, regions around pde6ha gained accessibility at 21dpf. Importantly, I am using a fluorescent reporter assay to functionally assess which putative CREs drive timepoint specific activity in vivo. These chromatin accessibility datasets provide a rich resource for the discovery of functional CREs and elucidating their roles in the developing zebrafish brain.

CYFIP2 MEDIATES SENSORIMOTOR INTEGRATION OF VISUAL INPUT THROUGH RAC1-DEPENDENT ACTIN REMODELING

Poster Number: 14

Theme: Neurobiology

Presenting Author: **Kimberly Charron** - North Carolina State University

Co-Author(s): D. Christopher Cole – North Carolina State University; Katya Frazier – California State University San Marcos; Audrey Johnson – North Carolina State University; Kurt Marsden – North Carolina State University

Abstract: The development of neural circuits that enable visual stimulus detection, processing, and behavioral responses requires a complex genetic program that orchestrates neuronal migration, axonal growth and guidance, and synapse formation and function. cytoplasmic FMRP-interacting protein 2 (cyfip2) has well-established roles in all of these developmental processes, and cyfip2 mutants have defects in patterning of retinotectal axonal projections. However, it is not known whether cyfip2 is required for visually-driven behavior. Here we show that prey capture behavior and responses to sudden changes in illumination are almost completely absent in cyfip2 mutant. However, the optokinetic response in cyfip2 mutants remains intact, indicating that retinal phototransduction is unaffected. Thus, cyfip2 mutants' severe defects in prey capture and dark- and light-flash responses indicate that cyfip2 has important roles in downstream sensorimotor integration rather than stimulus detection. To identify the precise location(s) where cyfip2 controls visual system function, we are currently measuring brain-wide activity mapping with phosphorylated ERK immunostaining and pan-neuronal calcium imaging . To define the critical window when cyfip2 acts in visual system development we established a heatshock-inducible transgenic line and found that transient expression of Cyfip2 from 30 to 50 hours post-fertilization restores visually-mediated responses in cyfip2 mutants. To determine if Cyfip2 regulates visual behavior circuitry through its role in modulating actin dynamics as part of the WAVE regulatory

complex and/or through its role in translation regulation through interactions with FMRP and eIF4E, we used heatshock-inducible transgenic lines with point mutations in Cyfip2's protein binding domains that disrupt actin remodeling (ΔRac1) or translational repression (ΔFMRP) and found that Cyfip2 regulates the development of visual behavior circuitry through actin remodeling pathways but not through translation regulation. Together, these experiments will both spatially and temporally define Cyfip2's roles in the neural circuits required for sensorimotor integration of visual stimuli.

Esrr localization regulates mitochondrial biogenesis in neurons

Poster Number: 15

Theme: Neurobiology

Presenting Author: **Roger Schultz** - University of Wisconsin-Madison

Co-Author(s): Angelica Lang – University of Wisconsin-Madison; Catherine Drerup – Associate Professor, Integrative Biology, University of Wisconsin-Madison

Abstract: Mitochondria are important contributors to the generation of a cell's energy, regulation of signaling molecules like calcium, and the mediation of cell growth and death. Therefore, it is imperative for cells to be able to maintain their mitochondrial pool. This is done through mitochondrial biogenesis, which requires tightly regulated nuclear gene transcription to produce essential mitochondrial proteins. A multitude of key transcriptional regulators within the biogenesis pathway have been identified and investigated thoroughly in cells like myocytes or adipocytes. These pathways rely on measures of local mitochondrial health and function to operate effectively. How a neuron, with mitochondrial populations stretched up to a meter from the nucleus, measures and responds to biogenic demands is, however, less clear. Our work has identified the localization of two key transcription factors, the Estrogen-related receptor (Esrr) and Proliferator-activated receptor gamma coactivator 1-alpha (Pgc1a), as critical for regulation of mitochondrial biogenesis in neurons. Using live imaging and transcriptomic approaches, we discovered a relationship between Esrr activation and localization, mitochondrial transport, and mitochondrial biogenesis in neurons. Specifically, disruption of mitochondrial transport from the axon terminal back to the cell body (retrograde) disrupts Esrr-mediated mitochondrial biogenesis. Live imaging of Esrr and its transcriptional coactivator Pgc1a demonstrated Esrr localization in the axon terminal, far removed from the cell body. Somewhat surprisingly, the disruption of retrograde mitochondrial transport also alters Esrr localization, leading to loss of nuclear localization and accumulation of this transcription factor in the axon terminal. Our future work will address activation, localization, and transcriptional regulation of Esrr by mitochondrial transport and function. Overall, these findings challenge the current view of Pgc1a and Esrr being strictly nuclear actors and highlight the potential importance of their extranuclear signaling and retrograde dynamics.

Disc1-Dependent Mitochondrial Transport and Morphology in Neurons

Poster Number: 16

Theme: Neurobiology

Presenting Author: **Brooke Weiler** - University of Wisconsin-Madison

Co-Author(s): Katie Drerup, Assistant Professor – Department of Integrative Biology – University of Wisconsin-Madison

Mitochondria are essential to neuronal function and survival. They are actively transported toward the synapse (anterograde) and toward the cell body (retrograde) via microtubule-based transport. Mitochondria are linked to motors by scaffold proteins to accomplish this movement; one emerging scaffold protein of interest is Disrupted in Schizophrenia 1 (Disc1). Though the cellular function of Disc1 remains unclear, studies in mammalian cell lines have demonstrated that Disc1 localizes to mitochondria and proteomics data link Disc1 to molecular motors. Therefore, we hypothesized that Disc1 mediates mitochondrial transport in axons. To test this, we used the zebrafish posterior lateral line (pLL) sensory system to analyze Disc1-mediated mitochondrial transport. Utilizing transient transgenesis, I overexpressed eGFP-tagged disc1 (Disc1 OE) and eGFP-tagged disc1 with a premature stop codon after exon 8 (truncDisc1). This truncation models a highly conserved human mutation linked to schizophrenia in a Scottish family. Live confocal imaging at 4 days post-fertilization demonstrated that Disc1 preferentially colocalized with stationary mitochondria in the pLL axon of Disc1 OE and truncDisc1 larvae. Additional assessment of mitochondrial motility in Disc1 OE and truncDisc1 larvae revealed no alterations to transport rates or the number of mitochondria moving in either direction. Because colocalization of Disc1 with stationary mitochondria suggests a role in mitochondrial function or maintenance, I analyzed mitochondrial length in the pLL axon. Stationary and anterograde mitochondria were decreased in length in truncDisc1 larvae compared to wild-type controls. As mitochondrial length is directly altered by fission and fusion dynamics, these data support a possible role of Disc1 in mitochondrial dynamics. Future experiments in novel knockout lines will determine if Disc1 is necessary for mitochondrial size, localization, and function. Furthermore, we will also determine if there are downstream effects on synaptic transmission. Together, this will elucidate the role of the Disc1 protein in mitochondrial and neuronal structure and function.

Investigating the role of Cadherin-16 in brain non-autonomous regulation of sensory gating

Poster Number: 17

Theme: Neurobiology

Presenting Author: **Susannah Schloss** - University of Colorado Anschutz Medical Campus School of Medicine

Co-Author(s): Zackary Marshall – Graduate Student, Zoology, University of British Columbia; Nicholas Santistevan – Cell and Developmental Biology – University of Colorado Anschutz Medical Campus School of Medicine; Stefani Gjorcheska – Department of Pediatrics – University of Cincinnati College of Medicine; Amanda Stenzel – Graduate Student, Cell and Developmental Biology, University of Colorado Anschutz Medical Campus School of Medicine; Lindsey Barske – Department of Pediatrics – University of Cincinnati College of Medicine; Jessica Nelson – Cell and Developmental Biology – University of Colorado Anschutz Medical Campus School of Medicine

Abstract: Appropriately filtering environmental stimuli is critical for organismal survival. Studies examining sensory filtering have largely focused on molecular mechanisms that function in neurons, while brain-extrinsic homeostatic regulators of internal state remain understudied. For example,

disrupted serum ion homeostasis has been linked to psychotic symptoms in humans, but its role in the process of sensory filtering has not been thoroughly investigated. We identified a cadherin gene, encoding Cadherin-16 (Cdh16), as a critical non-brain-autonomous regulator of sensory filtering in larval zebrafish. Interestingly, Cdh16 is expressed in the mammalian kidney and had not been studied for its role in sensory filtering. We found that wild type larval zebrafish express Cdh16 throughout their development in a teleost-specific set of endocrine organs called the corpuscles of Stannius (CS), which release a calcium-regulatory hormone called Stanniocalcin 1, like (Stc1l). Next, we determined via genetic epistasis that Cdh16 functions within the CS to exert its effect on sensory filtering, through suppression of Stc1l, which in turn inhibits another sensory filtering regulator Pregnancy-associated plasma protein-a (Papp-aa). As Stc1l is critical for calcium regulation, we hypothesized that the Cdh16-Stc1l-Papp-aa pathway functions in sensory gating by regulating calcium homeostasis and used a colorimetric calcium assay to show cdh16 mutant larvae have significantly decreased whole-body calcium levels. Stc1l and Papp-aa have been shown to regulate Calcium ion uptake by tuning the proliferation of epithelial ionocytes specialized for calcium transport. We hypothesized that Cdh16 loss-of-function and overexpression of Stc1l would cause increased suppression of Papp-aa and decreased proliferation of these ionocytes. Through in situ hybridization chain reaction experiments, we indeed found that cdh16 mutant larvae have fewer ionocytes. Altogether, these data support a novel role for Cdh16 in regulating sensory filtering via control of Calcium ion homeostasis.

Compensatory Regenerative Axon Growth in a Nerve Ablation Model

Poster Number: 18

Theme: Neurobiology

Presenting Author: **Olivia Carraher** - University of Minnesota

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

Abstract: The human nervous system is susceptible to damage from clinical conditions or traumatic injuries, and the outcomes range in symptoms and severity. The peripheral nervous system has some intrinsic regenerative mechanisms, but regeneration is often incomplete and non-functional. In contrast, zebrafish have highly regenerative nervous systems, making them an ideal model organism to study neural repair. Our work focuses on the regeneration of the vagus motor nerve, which has five major branches projecting to the pharyngeal arches (PAs) and visceral organs. Previous work has shown vagus motor axons can reinnervate appropriate tissue targets and restore function following transient axonal injury. However, this model does not fully reflect permanent neuron loss that is characteristic of many human conditions. We aim to investigate the regenerative response to the permanent loss of vagus motor axon branches in a neuronal ablation injury model. Left and right vagus motor axons normally innervate ipsilateral ventral pharyngeal muscles. We have observed that unilateral removal of axon branches causes axons on the opposite side to reinitiate growth and extend across the ventral midline towards contralateral denervated muscles. We hypothesize that this represents a compensatory process to restore bilateral pharyngeal muscle function. We aim to use molecular, functional, and behavioral assays to assess whether these axons can synapse with, and restore function to, denervated contralateral muscles. Finally, we will perform gene expression analyses to identify candidate genes that promote or inhibit this compensatory growth. This study will provide insight into the mechanisms of

vagus nerve regeneration and may highlight therapeutic strategies for enhancing functional recovery in humans.

Identifying Key Molecules Involved in the Biogenesis, Transport, and Recycling of Synaptic Vesicles at Ribbon Synapses

Poster Number: 19

Theme: Neurobiology

Presenting Author: **Sandeep David** - NIDCD/NIH

Co-Author(s): Katie Kindt – NIDCD/NIH; Katherine Pinter – NIDCD/NIH

Abstract: Introduction Sensory hair cells utilize specialized ribbon synapses to reliably transmit sensory information to the brain. Ribbon synapses have high rates of spontaneous vesicle release and function without fatigue. To sustain this level of release, a continuous supply of synaptic vesicles (SVs) must be trafficked to the presynapse. Recently we showed that Kif1a-mediated transport delivers new SVs along microtubules to the hair cell presynapse. Loss of Kif1a resulted in a depletion of SVs at the synapse, along with impaired neurotransmission. However, many other factors are needed to ensure that SVs are made, transported and recycled appropriately. Methods To study SV distributions in fixed hair cells in the lateral line, we use the immunohistochemistry against SV markers such as Vglut3, Rab3a, and CSP. To visualize SV populations in vivo, we use the vital dye Lysotracker. We are using RNA-FISH to investigate expression of candidate genes. Then, to assess gene function we are using CRISPR-Cas9 to create mutations in candidate genes involved in SV packaging, transport, and recycling in zebrafish. Further, we will assess ribbon synapse function in our mutants using in vivo calcium imaging and electrophysiology. Results Currently we are using CRISPR-Cas9 to test the following candidate genes: Ap3, Rab3a/Madd, and Dynamin2, that we hypothesize are critical for SV biogenesis, transport, and endocytosis, respectively. Preliminarily, we have found that first-generation ap3m2 CRISPR mutants show less Lysotracker label throughout the cell when compared to wild-type zebrafish hair cells, indicating Ap3m2 may be important to produce new vesicles. We are currently testing our other candidate genes using a similar approach. Conclusion/Future Work Characterizing these mutants will allow us to shed light on the process of vesicle packaging, movement, and recycling in hair cells. This work will deepen our understanding of the multiple pathways that converge to supply SVs at ribbon synapses.

Establishing novel CRISPR-generated htr2b mutant zebrafish lines to investigate how the 5-HT2B receptor regulates acoustic behavior selection

Poster Number: 20

Theme: Neurobiology

Presenting Author: **Rebecca Voss** - Haverford College

Co-Author(s): Rebecca Osbaldeston – Department of Biology – Haverford College; Kevin Villafañe – Department of Biology – Haverford College; Matt Curran – Department of Biology – Haverford College; Roshan Jain – Department of Biology – Haverford College

Abstract: Serotonin (5-HT) regulates many aspects of behavior including mood, sleep, appetite, social interactions, and decision-making. A major challenge in understanding serotonin's function is determining which of its 15 receptors are responsible for these diverse behaviors. In zebrafish, serotonin helps larvae select between two different acoustically evoked escape behaviors: an explosive short-latency response (SLC) and a kinematically distinct and weaker long-latency response (LLC). However, the specific receptors involved in regulating this decision-making are not yet known. Through a pharmacological screen, we found that 5-HT2B/2C receptor agonists bias fish towards SLC responses, while 5-HT2B/2C receptor antagonists bias fish towards LLC responses. Interestingly, CRISPR-directed mosaic mutagenesis of 5-HT2B receptors produced the opposite shift in escape behavior selection bias phenotype. Together these results suggest that 5-HT2B and 5-HT2C receptors bi-directionally modulate vertebrate decision-making following acoustic threat. Since our mosaic G0 individuals may have inconsistent disruptions, we generated F1 and F2 individuals carrying novel germline-transmitted htr2b mutations. We will present the isolation and our initial characterization of several of these htr2b mutations. This set of mutants will clarify the specific roles of htr2b in simple acoustic decision-making, and provide tools for future dissection of serotonin-regulated behavior.

Investigating the Effect of Nitric Oxide Mimetics on a Multiple Sclerosis Zebrafish Model

Poster Number: 21

Theme: Neurobiology

Presenting Author: **Briana Maktabi, PhD** - Center for Drug Design and Development

Co-Author(s): Jose Moreno-Lopez – Department of Medicinal and Biological Chemistry – University of Toledo; Alexander Wisner, Ph.D. – Research Assistant Professor, University of Toledo; Frederick Williams, Ph.D. – Professor, University of Toledo; Isaac Schiefer, Ph.D. – Professor, University of Toledo

Abstract: Multiple sclerosis (MS) is the most prevalent demyelinating and neurodegenerative disease of the central nervous system in young adults. Inflammatory MS lesions were found to have higher-than-normal concentrations of the free radical nitric oxide (NO). In patients with MS, the concentrations of markers of NO production are elevated in the CSF, blood, and urine. The net effect of NO production in MS is not necessarily deleterious as it has several beneficial and immunomodulatory effects. To that, the full idea of how NO affects MS pathogenesis is not completely understood. Evidence supporting or refuting a link between the two is lacking. No currently available therapy for MS involves NO targeting. Recent findings about Furoxan(1,2,5-oxidiazole-N-oxides), an NO mimetic, showed neuroprotective effects in vitro. This data influenced the hypothesis that furoxans may have such effects in an MS zebrafish model. The goal of this project is to broaden our understanding of the relationship between NO and MS. A copper chelator toxin, cuprizone (CPZ), was used to induce inflammation and demyelination in zebrafish. Then, furoxans, IS-1-41 and AH-2-36, were tested and compared to other agents on the market (isosorbide dinitrate, teriflunomide). Locomotor assay was performed in zebrafish following treatment with CPZ, IS-1-41, AH-2-36, isosorbide dinitrate, and teriflunomide. This assay assesses locomotor activity and anxiety related behaviors. Parameters of interest included total distance moved, turn angle, time spent in the outer zone vs inner zone, etc. Results showed that CPZ decreased locomotor activity, IS-1-41 significantly increased locomotor activity, overcoming cuprizone effects, but

caused hyperactivity at higher doses. AH-2-36 and teriflunomide increased locomotor activity. Results illustrated by the furoxans herein advocate for the prospective role of targeting an NO pathway in the development of new therapies for MS. Funded by the Center for Drug Design and Development and National Institutes of Health (R01AG057598).

Visualizing how Activity Shapes Presynapse Formation in Sensory Hair cells

Poster Number: 22

Theme: Neurobiology

Presenting Author: **Olivia Molano** - NIH/Brown

Co-Author(s): Katie Kindt – NIH

Abstract: Hair cells are the sensory receptors in auditory, vestibular and lateral-line sensory systems. Hair cells rely on specialized ribbon synapses that are essential for the rapid and precise transmission of sensory stimuli. At mature ribbon synapses, the voltage-gated calcium channel Cav1.3 plays a critical role in mediating this transmission. While the role of Cav1.3 at mature ribbon synapses is relatively well understood, the role these channels play during synapse formation is not well understood. Previous research has demonstrated that Cav1.3 channels are required for spontaneous calcium responses in developing hair cells. Further, loss of this activity influences presynapse size during development, and may play a role in synapse maintenance (Wong et. al., 2019, Sheets et al., 2012). How activity impacts synapse development in hair cells remains unclear. Recent work using live imaging has demonstrated that two processes, transport along microtubules, and presynapse fusion are key events that define the dynamics of presynapse formation in hair cells (Hussain et. al., 2024). But whether Cav1.3-mediated activity impacts either of these processes is not known. To study these processes and presynapse formation in vivo, we are using Zeiss Airyscan confocal imaging using Tg[myo6b:Ribeye-tagRFP] and Tg[myo6b:YFP-Tubulin] transgenic lines, to mark presynapses and microtubules in developing hair cells in the lateral-line system. After image capture, we are quantifying presynapse fusion events and presynapse transport in Imaris. We found that Cav1.3-mediated activity does not disrupt presynaptic transport on microtubules. Therefore, we hypothesize that Cav1.3-mediated activity may be important to regulate the fusion of presynaptic precursors during development. Our work will highlight how activity shapes the formation of presynapses in hair cells. Understanding how presynapses are established at the active zone can help us to develop strategies to repair synapses after damage and ultimately restore sensory system function.

Optimization of a Photomotor Response Assay in Larval Zebrafish for High-Throughput Screening of Compounds Counteracting Fentanyl's Effects

Poster Number: 23

Theme: Neurobiology

Presenting Author: **Jovana Duric, MS** - University of Toledo

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Therapeutics, University of Toledo; Isaac Schiefer – Professor and Vice Chair, Medicinal and Biological Chemistry, University of Toledo

Abstract: Fentanyl is one of the most potent synthetic opioids, requiring only a small dose to elicit significant pharmacological effects. While it is widely used for pain management and anesthesia, fentanyl poses a high risk for addiction. Its illicit use—often in combination with heroin, methamphetamine, or cocaine—has contributed to a surge in fatal overdoses, making it a prominent member of the new psychoactive substances. Naloxone, an FDA-approved opioid antagonist, is currently used to reverse the effects of fentanyl overdose; however, fatality rates continue to rise despite its availability. The aim of this study is to optimize a photomotor response (PMR) assay in 7-days-post-fertilization (dpf) larval zebrafish to facilitate high-throughput screening of novel compounds that can counteract the effects of fentanyl. Precision analysis of larval zebrafish locomotion provides an ideal platform for neuroactive drug discovery. Among established behavioral assays, the light-dark locomotion test has proven to be particularly effective. This assay utilizes a multi-well plate placed in a closed chamber, where individual larvae are exposed to alternating light and dark conditions. Such set up enables automated, high-resolution tracking of locomotor activity while also supporting scalable behavioral phenotyping for efficient screening across large sample sizes. Additionally, adult zebrafish were exposed to varying concentrations of fentanyl over five consecutive days to determine a safe and tolerable dose range. This preliminary toxicity and tolerance assessment is essential for informing the development of a future zebrafish self-administration model aimed at studying opioid-seeking behavior and evaluating the efficacy of candidate compounds in reducing fentanyl intake.

Cranial Motor Neuron Diversity in Zebrafish Development

Poster Number: 24

Theme: Neurobiology

Presenting Author: **Italia DiChristina** - Fred Hutchinson Cancer Center

Co-Author(s): Austin Seroka – Fred Hutchinson Cancer Center; Riku Yasutomi – Fred Hutchinson Cancer Center and University of Washington; Adam Isabella – University of Minnesota; Cecilia Moens – Fred Hutchinson Cancer Center

Abstract: In the vertebrate nervous system, most neurons are classified into three distinct cell populations: motor neurons (mns), sensory neurons, and interneurons, each of which can be further divided into numerous subpopulations that carry out specialized functions based on a combination of morphological and molecular identities. Mns are cholinergic neurons that express the transcription factor isl1 and innervate muscles and visceral organs throughout the body in order to control their voluntary and involuntary movements. Spinal mns in the trunk express mnx1 and innervate body wall and limb muscles, while cranial mns in the brainstem express tbx20 and innervate muscles in the head and neck as well as glands, sensory organs and parasympathetic ganglia in the visceral organs. While the molecular determinants that shape the identities of spinal motor neurons have been well characterized, less is known about how the distinct identities of the cranial motor neurons are specified during vertebrate development. Using the zebrafish embryo, in which cranial nerve organization is evolutionarily conserved, we have begun to define the molecular identities of cranial mns that contribute to different cranial nerves. We have generated a developmental scRNAseq atlas focused on

mapping distinct populations of isl1+, tbx20+ cranial mns and have identified candidate marker genes for each population. We performed a series of hybridization chain reactions (HCRs) for candidate genes across three developmental stages (1, 3 and 6 days post fertilization) in Tg(isl1GFP-CAAX) zebrafish embryos to visualize the spatiotemporal expression patterns. We have identified unique molecular markers for oculomotor (cranial nerve III), trigeminal (V), facial (VII) and vagal (X) neurons and have begun to assemble a gene expression atlas to provide a valuable resource for further understanding cmn functional diversity.

Innate metabolic profiles are not correlated with variation in the exploratory behavior of zebrafish (Danio rerio)

Poster Number: 26

Theme: Circuits and Behavior

Presenting Author: **Aliyah Goldson** - Wayne State University

Co-Author(s): Jacob Hudock – Wayne State University; Justin Kenney – Wayne State University

Abstract: Examining how physiological factors impact individual differences in behavior can shed light on the mechanisms driving behavioral variability. A potential contributor to behavioral variation is individual differences in metabolic rate. The pace of life syndrome (POLS) hypothesis proposes that physiological traits of an individual, such as metabolic rate, influence their behavioral traits (i.e., risk-taking, aggressiveness, or exploration). This study aimed to explore if baseline metabolism affects individual differences in the exploratory behavior of Danio rerio (zebrafish). We hypothesized that fish with greater metabolic rates would exhibit more bold and exploratory behaviors in a novel environment due to their need to acquire more resources. To test this, we employed the novel tank test (NTT), an assay in which fish were placed in a novel environment and their exploratory behavior was recorded. Two days later, metabolic activity was captured by measuring oxygen consumption of individual fish over 30 minutes. We found a positive correlation between oxygen consumption and body weight, with female fish having higher metabolic profiles than males. However, there were no significant correlations between the metabolic profiles of fish and their behavior in the NTT. Finally, to directly manipulate metabolism, we fasted fish for 16 hours prior to the NTT. We found that 16-hour starvation significantly reduced metabolism in fish but had no effect on their behavior. This challenges the POLS hypothesis' proposed link between metabolic rate and behavior. The lack of significant correlation suggests metabolism may have less of an influence on behavior than initially thought.

Individual differences in fear memory expression engages distinct functional networks

Poster Number: 27

Theme: Circuits and Behavior

Presenting Author: **Justin Kenney, PhD** - Wayne State University

Co-Author(s): Barbara Fontana – Wayne State University; Jacob Hudock – Wayne State University; Neha Rajput – Wayne State University; Dea Kanani – Wayne State University

Abstract: Fear is a fundamental emotional state that is highly conserved across the animal kingdom. Despite the intrinsic importance of fear for survival, its behavioral manifestation varies between individuals where the choice of response is key to survival. However, we know little about the biological basis for these individual differences in fear behavior. Adult zebrafish were trained to associate a new environment with fear by exposing them to conspecific alarm substance (CAS), an ethologically relevant chemical stimulus released from the epithelial cells of injured fish as a danger signal. After tracking with DeepLabCut, we trained a random forest machine learning model to identify different behaviors (e.g., freezing, bursting, and erratic movements) to greater than 94% accuracy. We collected data from over 400 animals from four different inbred strains (AB, TU, TL, and WIK) and both sexes. An unsupervised machine learning approach identified four distinct behavioral clusters: (1) low fear responsivity, (2) erratic behavior, (3) high freezing interspersed with erratic behavior, and (4) high freezing interspersed with normal swimming. We found that both background strain and sex had an influence on the type of fear behavior exhibited. Finally, we performed whole-brain activity mapping to identify the neural basis individual differences in fear behavior. To do this, we used a combination of in situ hybridization chain reaction for c-fos, tissue clearing, light-sheet microscopy, and image registration to the adult zebrafish brain atlas (azba.wayne.edu). We found that freezing covaries strongly with the cerebellum and parts of the dorsal and ventral telencephalon whereas evasive behavior is characterized by elevated activity in the nucleus isthmi. At the functional network level, freezers engaged the cerebellum, preglomerular nuclei, and pretectal nuclei whereas evading freezers had more activation in the preoptic and hypothalamic areas. Both freezers and evading freezers had strong engagement of the telencephalon.

Investigating Test-Retest Reliability of Automated Video-Tracking-Based and Manual Observation-Based Behavioural Quantification Methods

Poster Number: 28

Theme: Circuits and Behavior

Presenting Author: **Kelly Cheung** - University of Toronto

Co-Author(s): Steven Li – University of Toronto; Ryan Ugovsek – University of Toronto; Benjamin Tsang – University of Toronto; Robert Gerlai – University of Toronto

Abstract: The replicability crisis is an ongoing issue in the sciences, including in behavioural neuroscience. In the latter, behavioural assays are used to quantify altered brain function in response to various experimental conditions. However, methods to quantify animal behaviour are highly diverse. Some employ an automated video-tracking approach while others utilize the expertise of human observers. These methods, combined with complex and nuanced animal behaviour, present challenges in quantifying behaviour not only across laboratories but also within the same researcher. Interestingly, there is an absence of literature assessing the test-retest reliability of these two behavioural quantification methods. Here, we address this gap by examining the test-retest reliability of behavioural testing of zebrafish (Danio rerio) using two software packages: EthoVision XT 17.5, which relies on automated video-tracking to quantify movement path parameters, and Observer XT, which leverages human observation to quantify complex motor and posture patterns. We extracted behavioural measures from the same 18 adult wild-type zebrafish placed in a novel tank. Using EthoVision, one group of raters analyzed each fish at two frame rates (5Hz and 25Hz), i.e., twice, and extracted several

swim-path parameters from each recording. Independently, a second group of raters used Observer to measure the frequency and duration of multiple behavioural parameters in the same recorded fish, performing two passes. We found that analyses at different temporal resolutions in EthoVision had pronounced effects on behavioural results, due to specific temporal characteristics of path parameters. Unlike intuitively assumed, higher frame rate was not found more suitable for all behaviours. For Observer, no significant differences were seen in behavioural scoring between the first and second pass, suggesting that raters scored behaviours consistently between their first and their second session. Ultimately, these results will help elucidate the reliability of the automated and manual approaches to behavioural analysis.

Anxiolytic Drug Screening - Tools and Strategies for Reliable Predictions

Poster Number: 29

Theme: Circuits and Behavior

Presenting Author: **Małgorzata Potoczna** - 1. Transpharmation Poland Ltd., ul. Michała Oczapowskiego 13/105D, 10-719 Olsztyn, Poland 2. Faculty of Veterinary Medicine, Department of Pathophysiology, Forensic Veterinary Medicine and Administration, University of Warmia and Mazury in Olsztyn, ul. Oc

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Abstract: Anxiety disorders are the world's most common mental disorders. Despite the availability of various clinically approved anxiolytics, there remains an urgent need to identify novel compounds with improved efficacy and minimal side effects. Zebrafish (Danio rerio) larvae have emerged as a powerful in vivo platform for high-throughput behavioural and molecular screening of anxiolytic substances. In this research, we used zebrafish larvae to evaluate well-characterized anxiolytics: diazepam, amitriptyline, fluoxetine, a new compound TP003 and novel psychedelic drugs: DOI, 5-Me-DMT, and psilocybin. Initially, the Fish Embryo Toxicity (FET) test was conducted to determine all drugs tolerability. Behavioural studies comprised two complementary assays: the Light–Dark Challenge (L-DC) and the Light–Dark Preference (L-DP) tests. Substances that showed clear behavioural effects were then analysed at the molecular level. The whole-body cortisol levels were measured by ELISA. Expression of key hypothalamic–pituitary–adrenal (HPA) axis genes (crha, crhbp, hsd11b2, ucn3l, ppp3r1a) was quantified using RT-qPCR method. Although the FET test reliably determined developmental toxicity thresholds, we suggest this may not necessarily reflect toxicity in matured organisms. In behavioural assays, both the L-DC and L-DP tests detected anxiolytic properties of drugs; however, the L-DC assay exhibited greater sensitivity and reproducibility. Molecular endpoints only partially aligned with behavioural outcomes: while cortisol reductions mirrored the most robust behavioural responses. The HPA axis gene-expression changes were variable and, in some cases, did not reach statistical significance. These findings suggest that gene-expression profiling alone may be insufficient to fully characterize anxiolytic potential. Zebrafish larvae screening offers a versatile, cost-effective approach for early-stage evaluation of anxiolytic drug candidates, combining toxicity, behavioural, and molecular endpoints. Our study underscores the importance of integrating multiple assays to capture a compound's pharmacological profile comprehensively. Careful experimental design and detailed

interpretation of results are essential to maximize the potential of this model, while recognizing its translational limitations.

The stability control of cdkn2 mRNA in muscle determines systemic aging speed

Poster Number: 31

Theme: Lifespan and Aging

Presenting Author: **Shohei Ogamino** - Department of Homeostatic Regulation, Research Institute for Microbial Diseases (RIMD), The University of Osaka

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Abstract: The accumulation of senescent cells in vivo accelerates to physiological aging and age-related pathology, as demonstrated by studies in which senescent cells were removed from the whole body of mice. However, it remains unclear how senescent cells control systemic aging and lifespan. To investigate them, we use the ultra-short-lived small fish Nothobranchius furzeri (N. furzeri). By performing the reporter and genomic analyses in the shorter-lived and longer-lived N. furzeri, which have lifespans of 3-4 and 6-8 months, respectively, we reveal that, only in skeletal muscle, the senescent cells accumulate faster in the shorter-lived strain, and that these differences are caused by three base SNPs in the untranslated region (UTR) of cdkn2, a positive regulator of cellular senescence, between the shorter-lived strain and the longer-lived strain. CRISPR/Cas9-mediated base-editing of these SNPs from the shorter-lived-type to longer-lived-type reduced cdkn2 mRNA expression and suppressed senescence in muscle tissue, extending lifespan by 128 % and improving aging phenotypes in the shorter-lived N. furzeri strain, indicating that three base SNPs have a significant impact on lifespan regulation. Furthermore, we found that an RNA-binding protein binds and degrades the longer-lived-type cdkn2 mRNA but not shorter-lived-type one, and that the number of this RNA-binding protein binding sequences in the cdkn2 UTR is positively correlated with lifespan of various mammals. Collectively, our study suggests that the stability control of cdkn2 mRNA in muscle determines vertebrate systemic aging speed and lifespan.

Accessing Zebrafish and Comparative Genome Data at ZFIN and the Alliance of Genome Resources

Poster Number: 32

Theme: Other

Presenting Author: **Ceri Van Slyke** - ZFIN

Co-Author(s): Yvonne Bradford – ZFIN – University of Oregon; Holly Paddock – ZFIN – University of Oregon; Christian Pich – ZFIN – University of Oregon; Sridhar Ramachandran – ZFIN – University of Oregon; Leyla Ruzicka – ZFIN – University of Oregon; Alliance Staff – Alliance of Genome Resources; ZFIN Staff – ZFIN – University of Oregon; Monte Westerfield – Institute of Neuroscience – University of Oregon

Cross-species comparisons are invaluable in understanding conserved gene function, analyzing mutations, modeling human disease and discovering treatments for disease. The Zebrafish Information Network (ZFIN, zfin.org) contributes expertly curated zebrafish data to the Alliance of Genome Resources (the Alliance, www.alliancegenome.org) where it is integrated with data from six other Model Organism Databases (MODs) and the Gene Ontology (GO) Consortium. The Alliance provides a platform where users can access harmonized data enabling researchers to perform robust comparative analysis on various data types including genes, gene expression, human disease models, variants, orthology, paralogy, and gene function. We will present how to navigate between ZFIN and Alliance pages, where to find zebrafish data at the Alliance website, differences in data availability, and how to download data directly and through APIs from the Alliance. The goal of ZFIN and the Alliance is to streamline cross-species analysis and enhance the research done by our communities.

Defining the role of SOX4 as a transcriptional regulator of melanoma onset & progression

Poster Number: 33

Theme: Cancer & Growth Control

Presenting Author: **Megan Glaeser** - Washington University in St. Louis School of Medicine

Co-Author(s): Charles Kaufman – Washington University in St. Louis School of Medicine

Abstract: Melanoma is a deadly skin cancer that arises from the malignant transformation of pigmented, neural crest-derived skin cells called melanocytes. Melanoma incidence is rising and is especially dangerous due to its high metastatic potential. Considering the difficulty of treating late-stage melanoma, critical goals for melanoma treatment include targeting regulators of melanoma initiation to prevent or shrink early tumors, as well as identifying additional diagnostic or prognostic biomarkers for characterizing melanomas. Zebrafish models of melanoma have been crucial in understanding biological processes and transcriptional programs that influence melanoma initiation, including reactivation of neural crest gene expression. The SRY-related HMG-box transcription factor SOX4 is one neural crest-associated gene that is significantly upregulated in both zebrafish and human melanoma. However, the effects of SOX4 upregulation in melanoma are not fully understood; specifically, the effect of increased SOX4 expression on melanoma initiation and the transcriptomic consequences of SOX4 upregulation are unknown. Additionally, the specific enhancer elements and upstream regulatory inputs that cause SOX4 upregulation in melanoma are unknown. We aim to answer these questions by combining studies in a zebrafish model of melanoma and in cultured human melanoma cells. Preliminary studies revealed that overexpression of a zebrafish SOX4 ortholog specifically in melanocytes of zebrafish accelerates melanoma initiation in vivo. We also identified a putative enhancer upstream of zebrafish SOX4 drives reporter activity in neural crest cells and melanoma tumors in our zebrafish model. Ongoing work aims to further define 1) the effect of human SOX4 upregulation on melanoma onset, 2) the gene regulatory networks that act downstream of SOX4 in pre-malignant melanocytes or melanoma, and 3) the mechanism resulting in human SOX4 upregulation in melanoma.

Investigating the effect of thyroid hormone on melanoma initiation and behavior.

Poster Number: 34

Theme: Cancer & Growth Control

Presenting Author: **Michael Hilzendeger** - Washington University in St. Louis School of Medicine

Co-Author(s): Charles Kaufman, MD, PhD – Associate Professor, Medicine, Washington University in St. Louis School of Medicine

Abstract: Hypothyroidism is up to 50% more prevalent in melanoma patients than in the general population. Interestingly, zebrafish models of thyroid hormone deficiency or excess exhibit aberrant numbers and patterns of pigment-producing cells in the developing skin, suggesting a link between thyroid hormone signaling and melanocyte development. Although clinical observations and epidemiologic data suggest a relationship between hypothyroidism and melanoma, research thus far has focused on the coincidence hypothyroidism and melanoma rather than a functional link between the two. Previous research has uncovered a role for thyroid hormone in other malignancies, including colorectal carcinoma and squamous cell carcinoma, but the role of thyroid hormone in melanoma remains to be studied. Thus, the cellular mechanisms by which hypothyroidism could promote melanoma initiation are currently unknown. As melanoma incidence increases and hypothyroidism secondary to immunotherapy-induced hypophysitis becomes more common, it is critical to develop a mechanistic, organism-level understanding of how thyroid hormone impacts melanoma tumorigenesis. Herein, the proposed studies aim to dissect the link between thyroid hormone and cutaneous melanoma using a combination of in vivo vertebrate modeling using zebrafish and in vitro cell culture to investigate the effect of thyroid hormone on melanoma initiation and melanoma proliferation and invasiveness. Our preliminary findings affirm a role for thyroid hormone in modulating melanocyte proliferation and cell identity. Understanding how thyroid hormone impacts melanoma onset and behavior will help provide a mechanistic understanding of the link between thyroid hormone and melanoma and potentially provide avenues for new therapeutic strategies for prevention and treatment of melanoma in the clinic. Altogether, the proposed studies offer an innovative approach to further uncover metabolic modulators of melanoma onset.

Functional Characterization of a CML Progression Gene in the Zebrafish Hematopoietic System

Poster Number: 35

Theme: Cancer & Growth Control

Presenting Author: **Uijeong Nam** - Department of Biomedical Science and Technology, Kyung Hee University

Co-Author(s): Chang-Kyu Oh – Department of Biochemistry, School of Medicine, Pusan National University; Semin Lee – Department of Biomedical Engineering, UNIST; Dong-Wook Kim – Hematology Center, Uijeongbu Eulji Medical Center, Eulji University; Yoonsung Lee – Clinical Research Institute, Kyung Hee University Hospital at Gangdong, College of Medicine, Kyung Hee University

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the excessive proliferation of myeloblasts in the bone marrow. Although tyrosine kinase inhibitors such as imatinib

have significantly improved clinical outcomes, drug resistance and limited understanding of disease progression remain major challenges. To uncover genes and mechanisms associated with CML progression, we performed RNA-seq analysis on patient samples from the chronic phase (CP) and blast crisis (BC), leading to the identification of a novel candidate, Gene A. To functionally characterize this gene in vivo, we utilized zebrafish (Danio rerio), a genetically tractable model with conserved hematopoietic pathways. Knock-down (KD) and CRISPR-mediated knock-out (KO) of Gene A revealed its essential role in embryonic myelopoiesis and in maintaining hematopoietic stem and progenitor cell (HSPC) proliferation. Conversely, overexpression of Gene A in zebrafish induced hematopoietic expansion phenotype resembling features of CML progression. Further analysis indicated that Gene A modulates HSPC development through EGFR signaling. These findings establish Gene A as a novel regulator of primitive and definitive hematopoiesis and a potential therapeutic target in CML progression.

Using Transgenic Zebrafish to Profile Metabolic Changes in Hepatocellular Carcinoma

Poster Number: 36

Theme: Cancer & Growth Control

Presenting Author: **Jessye Castro, MS** - University of Utah

Co-Author(s): Greg Ducker – University of Utah; Kimberley Evason – University of Utah; Ryan O'Connell – University of Utah; Chad Van Sant-Webb – University of Utah

Abstract: Hepatocellular carcinoma (HCC) is a leading cause of cancer death worldwide. A major cause of HCC is metabolic dysfunction-associated steatotic liver disease (MASLD), characterized by inflammation and altered hepatocyte metabolism. About 30% of HCCs regardless of etiology show activating mutations in CTNNB1 which encodes beta-catenin. Transgenic zebrafish expressing hepatocyte-specific activated β-catenin (Tg-ABC) have liver overgrowth as larvae and HCC as adults. MicroRNAs negatively regulate expression of target mRNAs through direct binding in the cytoplasm and influence various aspects of cellular function such as mitochondrial function, metabolism, and growth signaling. We performed micro-RNA profiling using NanoString and RNAseq and found that microRNA-21 (miR-21) was upregulated in human HCC and Tg-ABC zebrafish. To delineate the role of miR-21 in lipid metabolism and hepatocarcinogenesis, we made transgenic zebrafish that overexpress (miR-21OE) or sponge (miR-21SP) miR-21. We found that miR-21OE enhanced the larval liver overgrowth of Tg-ABC, while miR-21SP suppressed beta-catenin-driven larval liver overgrowth. To understand the role of miR-21 in progression from MASLD to HCC, we examined Tg-ABC and miR-21OE zebrafish larvae and adults fed a high fat diet (HFD, 10% cholesterol). Tg-ABC and miR-21OE larvae and adults showed significantly less hepatic steatosis than non-transgenic control siblings in response to HFD. Lipidomics analysis of Tg-ABC and miR-21OE adult livers on HFD and normal control diet (NCD) revealed overlap in changes to key lipid species including triglycerides and acyl carnitines. RNAseq of miR-21OE larvae revealed decreased expression of lipid metabolic genes such as ldlr and fads2. The finding that miR-21 and ABC cause similar aberrations to the hepatocyte lipidome suggests that miR-21 may mediate some of the metabolic dysregulation and tumor-promoting effects of ABC and support tumor initiation and progression.

Tetracycline-On Twist: A Game-Changer in Melanoma Treatment Discovery

Poster Number: 37

Theme: Cancer & Growth Control

Presenting Author: **Melissa Spigelman, BS/MS in Molecular Biology** - Montclair State University

Co-Author(s): Carlos Molina – Montclair State University

Abstract: Melanoma is associated with poor outcomes and the rapid acquisition of drug resistance. Existing zebrafish models, while valuable, suffer from a crucial limitation: uncontrolled gene expression of candidate genes against melanoma. To address this, we propose a novel inducible plasmid-vector system leveraging the Tet-on system to precisely regulate gene expression in melanocytes only with the addition of doxycycline (DOX). This advancement aims to align with the treatment resistant nature of human melanoma, enabling initiation of gene expression only upon melanoma confirmation. By overcoming the limitations of uncontrolled gene expression, this model offers enhanced translational relevance and facilitates investigation into candidate tumor suppressor proteins across distinct stages of tumorigenesis.

Lipid dysregulation in B-catenin-driven Hepatocellular Carcinoma

Poster Number: 38

Theme: Cancer & Growth Control

Presenting Author: **Aavrati Saxena, MS** - UNIVERSITY OF UTAH

Co-Author(s): Greg Ducker – University of Utah; Kim Evason – University of Utah; Alexis Ross – N/A; Richard Smith – University of Utah; Chad Van Sant Webb – University of Utah; Junko Kuramoto – Keio University School of Medicine

Abstract: Introduction/Background: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer and affects ~1 million people per year worldwide. Metabolic reprogramming is fundamental in cancer, including HCC, as it enables a healthy normal cell to transform into a malignant one. Essential lipids such as phosphatidylcholine (PC) are commonly dysregulated in human HCC patient samples. PC is a significant constituent of biological membranes and is synthesized via the cytidine diphosphate (CDP)-choline and phosphatidylethanolamine N-methyltransferase (PEMT) pathways in the liver. The goal of this study is to elucidate the role of PC lipid metabolism in HCC. Methods/Results: Our lab developed transgenic zebrafish expressing hepatocyte-specific activated beta-catenin (ABC), which show liver overgrowth as early as 6 days post fertilization (dpf) and as adults develop HCCs that are histologically and transcriptomically similar to human HCC. We performed lipidomics analysis of male and female transgenic ABC zebrafish with HCC and non-transgenic sibling control zebrafish livers using LC-MS and found significant differences in numerous lipid species, including acyl carnitines, ceramides, and PCs. We also performed isotope tracing in ABC-HCC and non-transgenic control zebrafish liver tissues to quantify the metabolic pathways contributing to these changes. We discovered that PC flux was downregulated in zebrafish HCC via sex-specific mechanisms. Ongoing studies are focused on defining the effects of manipulating PC metabolism on zebrafish hepatocarcinogenesis using genetic tools. Using CRISPR-Cas9 we generated chpt-1 deletion mutants, knocking out a gene necessary for PC synthesis. Our preliminary data show that loss of chpt-1 decreases larval liver size and decreases HCC

incidence in ABC zebrafish. We are currently characterizing transgenic zebrafish lines with overexpression of chpt-1 to further evaluate the role of PC in HCC tumorigenesis. Conclusions: Our results suggest that PC lipid metabolism contributes to ABC-driven liver tumor formation.

PDZRN3-RAF1, a plausible oncogenic fusion protein, drives embryonic malformation by potentiating MAPK signaling.

Poster Number: 39

Theme: Cancer & Growth Control

Presenting Author: **Hyunju Ro** - Department of Biological Sciences, College of Biological Sciences and Biotechnology, Chungnam National University

Co-Author(s): Hyunyoung Kim – graduate student, Department of Biological Sciences, College of Biological Sciences and Biotechnology, Chungnam National University

Abstract: The PDZRN3-RAF1 fusion gene, identified in pancreatic adenocarcinoma, results from the fusion of the N-terminal region of PDZRN3 with the C-terminal kinase domain of RAF1. Although the detailed oncogenic mechanisms of this fusion have not yet been fully elucidated, it may aberrantly activate MAPK signaling by bypassing normal regulatory inputs, given that the N-terminal portion of RAF1—typically acting as an autoinhibitory domain—is absent in the fusion. In this study, we demonstrated that overexpression of PDZRN3-RAF1 significantly potentiates MAPK signaling during zebrafish embryogenesis and in human cell culture, an effect that could be suppressed by treatment with either a pan-RAF inhibitor or a RAF1-specific inhibitor. More importantly, PDZRN3-RAF1 is capable of activating MAPK signaling independently of endogenous RAF proteins. In addition, a monomeric mutant version of PDZRN3-RAF1 remained as active in stimulating MAPK signaling as the wild-type protein. Collectively, our data provide a novel research platform for studying cancers driven by gene fusions involving the C-terminus of RAF1.

Characterizing the Role of Unc-45a in Zebrafish Gut Development

Poster Number: 40

Theme: Cell Biology

Presenting Author: **Madyson Syvenky** - University of Alberta

Co-Author(s): Dave Pilgrim – Faculty of Science – University of Alberta

Myosin proteins are vital for the development and maintenance of the GI tract. Myosin motor heads must be chaperoned to reach a functional state as they are unable to fold independently. One such chaperone is UNC45A, homologs of which have been identified in all metazoans, demonstrating its essential nature in acting as a molecular chaperone. Recently, rare human diseases, O2HE Syndrome and Aagenaes Syndrome, have been linked to UNC45A mutations. While O2HE patients present similar symptoms, like cholestasis and congenital diarrhea, there are no overlapping mutations across all studies. In contrast, all Aagenaes patients carry a 5'UTR causative mutation, with some also carrying unique coding mutations. Despite the shared cholestasis symptom between these two rare diseases, there is a notable absence of other symptoms in Aagenaes patients. Altogether this suggests a

complicated pathogenicity underlying UNC45A mutations. The unc45a mutant zebrafish line is a good model of this disease as the smooth GI phenotype is apparent. However, a thorough analysis of gut morphology has not been conducted. To address this limited understanding, I am generating novel mutations using CRISPR/Cas9 followed by in-depth histological characterization of the GI tract. Bioinformatic analysis indicates that mutations in human UNC45A are not limited to the myosin head binding site, co-chaperone binding sites and other conserved motifs, suggesting that domain disruption is not necessary for disease phenotype. Moreover, in-silico modeling of the amino acid changes in patients demonstrates a range of effects on protein stability, suggesting that the pathogenicity of these variants is complex. Together, my work can provide an animal model and information to aid in researching the pathogenicity of UNC45A mutations and effect on protein function. This, in turn, can provide insight into the mechanism of pathogenesis of UNC45A mutations in O2HE syndrome, thereby allowing for effective diagnosis, counselling, and treatment of O2HE patients.

Loss of nudt7 regulate lipid metabolism through suborganelles altered by H4 deacetylation in zebrafish

Poster Number: 42

Theme: Cell Biology

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Abstract: Coenzyme A (CoA) must be maintained at an appropriate concentration in cells. NUDT7 encodes an enzyme required to maintain intracellular CoA level by degrading CoA and CoA species present in peroxisomes. Peroxisomes play an important role in lipid metabolism , such as fatty acid oxidation, scavenging reactive oxygen species, ether phospholipid synthesis and cholesterol synthesis. We confirmed that Nudt7 is localized in peroxisome in zebrafish. To investigate the role of Nudt7 in regulating metabolism in zebrafish, we generated a zebrafish nudt7 mutant (nudt7 KO) line harboring a premature stop codon. Interestingly, the nudt7 KO liver contained more peroxisomes and showed decreased level of mitochondrial respiratory chain complex IV under fasting conditions at 8 dpf. We performed a diet experiment and observed morphological changes in mitochondria and peroxisomes in adults. More liver lipids were evident in the nudt7 KO group with higher BMI compared to the WT control group. In addition, nudt7 KO liver contained abnormally swollen mitochondria with peroxisomes being located nearby mitochondria. Transcriptome and proteome analyses of adult zebrafish liver confirmed the significant changes in both lipid and carbohydrate metabolism. Notably, we found that the histone H4 acetylation level was significantly decreased in nudt7 KO based on the omics analysis as well as immunostaining assay. Taken together, Nudt7 plays an important role in the control of cellular metabolism by regulating histone acetylation levels in zebrafish.

Wound-induced endogenous electric fields guide neutrophils to sites of injury in zebrafish epidermis

Poster Number: 43

Theme: Cell Biology

Presenting Author: **Christopher Prinz** - University of Washington/HHMI

Co-Author(s): Adithan Kandasamy, PhD – Post doctoral canididate, Stanford Cardiovascular Institute and Department of Medicine, Standford; Mugdha Sathe, PhD – Post doctoral canididate, Biology, University of Washington/HHMI; Julie Theriot, PhD – Professor, Biology, University of Washington/HHMI

Abstract: Skin wound-healing requires precise spatio-temporal coordination among multiple cell types, including epidermal epithelial cells and immune cells such as neutrophils. Intact skin maintains a trans-epithelial potential (TEP) due to asymmetric ion transport. Wounding the epithelium disrupts the TEP and induces a lateral electric field (EF) with the cathodal pole at the site of injury. Isolated neutrophils and isolated basal epidermal cells have been shown to migrate toward the cathode of an applied electric field in tissue culture, and we hypothesize that the wound-induced EF may contribute to directional migration of both cell types in wounded skin. To distinguish between directional migration guided by the wound-induced EF versus other wound-induced signals, including osmotic shock, we compared the speed and directionality of migration for these two cell types in the first 30 minutes following epidermal laceration in larvae at 11-14 days post-fertilization under varying media conditions. In comparison to the rapid, dramatic, wound-directed migration of epidermal cells in normal hypotonic media (E3), we find that wounding in isotonic media containing NaCl (eliminating both the osmotic shock and the EF) fails to induce any wound-directed epidermal cell migration at all, while wounding in isotonic media containing sorbitol (eliminating osmotic shock while preserving the EF) induces migration with normal directionality but reduced cell speed. In contrast, neutrophils migrate with increased speed (as compared to E3) after wounding in both isotonic NaCl and isotonic sorbitol. Most strikingly, rapidly migrating neutrophils in animals wounded in isotonic NaCl move in completely random directions, with no preference for migration toward the wound, while neutrophils in animals wounded in isotonic sorbitol have normal wound-directed movement. These results strongly suggest that both basal epidermal cells and neutrophils use the endogenous wound-induced EF as a directional signal, dominating their migration direction in the critical minutes immediately following epidermal injury.

A mutation in the Ceramide Transfer Protein (CERT) disrupts ciliated tissues

Poster Number: 44

Theme: Cell Biology

Presenting Author: **Sarah Christian, MS** - University of Missouri Kansas City

Co-Author(s): Olivia Fritz – University of Missouri Kansas City; Roe Hendricks – University of Missouri Kansas City; Hillary McGraw – Assistant Professor, Division of Biology and Biomedical Systems, University of Missouri Kansas City; XiaoLan Yao – Associate Professor, Division of Biology and Biomedical Systems, University of Missouri Kansas City

Abstract: The ceramide transfer protein (CERT) regulates sphingolipid homeostasis in cells by extracting ceramide from the endoplasmic reticulum and moving it to the trans-Golgi network. This sphingolipid balance is important for maintaining appropriate sphingomyelin concentration for development and cellular signaling. Previous studies in animal models show that loss of CERT function leads to developmental defects and shortened lifespan. Several specific alleles have been identified in human

patients that link CERT function to neural deficits, though the precise nature of the mutations is still under investigation. To better understand the developmental role of CERT protein, we have established a zebrafish model using CRISPR-Cas9 genome editing to target exons 1 and 2 of the zebrafish gene cert1a and have generated a null mutant allele. Analysis of the F2 generation shows recessive inheritance of a cert1a mutant phenotype including a small body, eyes, and brain. Embryos homozygous for the cert1a mutation rarely survive past 5dpf. Early analysis shows disruption of cilia formation, leading to developmental defects in ciliated tissues, including severe dorsal body curvature, swelling in brain ventricles, small eyes, and defects in pronephros formation, all of which are characteristic of ciliopathies. These results suggest a previously undescribed link between CERT function and ciliogenesis. Using our cert1a mutant line we will begin to address how CERT functions during embryonic development and disease, with an emphasis on sphingolipid function and cilia formation.

Mechanisms underlying adaptation to protein malnutrition

Poster Number: 45

Theme: Cell Biology

Presenting Author: **Siyao Wang** - Duke University

Co-Author(s): Michel Bagnat – Duke University; Jieun Park – University of North Carolina at Chapel Hill; Laura Childers – Duke University; Fernando Martinez – Duke University

Abstract: Lysosome-rich enterocytes (LREs) are a specialized population of intestinal cells that mediate the uptake and absorption of dietary proteins in zebrafish and neonatal mammals. Loss of LRE function causes severe stunting and poor survival due to protein malnutrition. Previously, we reported that loss of Plasmolipin (pllp), an endosomal membrane protein highly expressed in LREs, impairs LRE differentiation and dietary protein absorption, resulting in marked reduction in survival rates. Raising pllp homozygous mutants surviving to adulthood and in-crossing them for multiple generations resulted in their adaptation to malnutrition, with pllp mutants showing robust suppression of survival deficits. To uncover mechanisms underlying this adaptive response, we conducted transcriptome profiling and protein absorption assays to compare the older adapted pllp allele with genetically related wild type (WT) fish and a newly generated pllp mutant allele. We found that adapted pllp mutants exhibit upregulation of LRE endocytic components and have a capacity for protein absorption that exceeds that of WT. This hyperactivation of LRE endocytic and absorptive activity, appears also to be aided by the suppression of anti-microbial responses that may contribute to the enhanced survival of pllp mutants in the face of increased exposure to environmental antigens. Overall, our study illustrates a genetic adaptation mechanism for enhancing organ function and organismal survival in response to severe protein malnutrition.

Reactive Oxygen Species (ROS) Relax Wounds to Allow Healing & Regrowth

Poster Number: 47

Theme: Cell Biology

Presenting Author: **Chang Ding** - Purdue University

Co-Author(s): Yueyang Wang – Harvard Medical School and Massachusetts General Hospital; Qing Deng – Purdue University

Abstract: Moderate level of reactive oxygen species (ROS) plays pivotal roles in facilitating wound closure by mediating various cellular processes, including kinases activation, protein tyrosine phosphatases inhibition, and cytoskeleton reorganization. However, the detailed underlying mechanism by which ROS regulate wound dynamics to drive wound closure largely remain unknown. Here, we report that ROS relax wounds to promote wound closure utilizing zebrafish tailfin amputation model. ROS inhibition with pharmacological and genetic perturbations lead to wound over-contraction, delayed wound closure, and impaired wound regrowth following amputation. ROS supplementation with H2O2 or optogenetic stimulation with mini-SOG results in enhanced wound relaxation without impairing wound regrowth, highlighting the essential role of ROS in mediating wound closure through the regulation of wound dynamics. Excessive contraction observed in DPI-treated wound results from significantly elevated phosphorylated myosin regulatory light chain (p-MRLC) levels at the wound margin, while myosin light chain kinase (MLCK) or Rho-associated protein kinase (ROCK) inhibitor-treated wounds display relaxed tailfin dynamics and unimpaired wound closure and regrowth, resembling ROS supplemented wounds' phenotype. These findings indicate that contraction does not facilitate efficient wound closure post amputation, and over-contraction can, in fact, impede wound closure. Moreover, long-term ROS depletion during embryonic development alters actin abundance and tissue stiffness, which ultimately disrupts wound dynamics and closure due to reduced tissue elasticity and rigidity. Together, ROS is critical for preventing tissue from over-contraction and promote wound healing, which uncovers a new role of ROS in ensuring efficient wound closure.

Phosphorylation regulates NudC function in post-mitotic neurons

Poster Number: 48

Theme: Cell Biology

Presenting Author: **Brittany Salazar, PhD** - University of Wisconsin - Madison

Co-Author(s): Catherine Drerup – Integrative Biology – University of Wisconsin - Madison

Abstract: Maintenance of healthy neural circuits is crucial for sustained neurological health, including cognition and motor control. To connect to targets, neurons extend axons, which can reach up to a meter in length in humans. Formation and maintenance of these axons relies on active transport of organelles, proteins and mRNAs between the cell body and axon terminal. Retrograde transport of a variety of cargoes is driven by the molecular motor cytoplasmic dynein. To initiate movement, the dynein motor complex must first be "activated" by the protein Lis1. Lis1 requires an accessory protein, NudC, for local stability to facilitate active dynein complex formation. It is not understood how the NudC-Lis1 cascade is regulated to promote dynein activation to initiate cargo transport with spatio-temporal specificity. In the Drerup lab, we previously identified a NudC mutation in zebrafish which disrupts dynein activation through impaired Lis1 protein stability. This mutation results in the loss of a C-terminal phosphorylation site which is linked to regulation of NudC localization during mitosis but is unexplored in post-mitotic neurons. Interestingly, this NudC phosphorylation mutation (nudc:phos) does not fully phenocopy dynein, Lis1, or NudC null (nudc:null) mutations, indicating a unique role for NudC phosphorylation in its function. In the long axons of the posterior lateral line (pLL), nudc:phos larvae

demonstrate axon terminal swellings containing autophagosomes and nets of acetylated microtubules, indicating altered dynein activation in this neuronal compartment. Transient expression of phosphomimetic NudC in nudc:phos mutant larvae increases Lis1 and NudC colocalization in pLL axon terminals, indicating phosphorylation of NudC may regulate Lis1 and NudC interaction. These data suggest that local phosphorylation of NudC in axon terminals may promote Lis1 protein stabilization and facilitate active dynein complex formation. Ultimately, our work will delineate the compartment-specific regulation of dynein activation necessary for dynein-driven transport initiation in neurons.

Distinct dermal cell populations contribute to burn wound healing in larval zebrafish

Poster Number: 49

Theme: Cell Biology

Presenting Author: **Adam Horn** - University of Wisconsin - Madison

Co-Author(s): Alexandra Fister – University of Wisconsin - Madison; Yiran Hou – University of Wisconsin - Madison; Anna Huttenlocher – University of Wisconsin - Madison

While epithelial tissue is well-adapted to heal from mechanical injury, thermal burn injuries heal poorly. Defects in extracellular matrix remodeling can result in scarring and permanent loss of tissue function in patients, suggesting the function of dermal fibroblasts is perturbed in burn wounded tissue. In larval zebrafish, little is known about the cellular composition of the developing dermis. Previous reports by our lab and others have identified mesenchymal cell populations in the dermis that are associated with collagen fibrils during development and tissue repair. Here, we use novel collagen reporter fish lines, combined with existing mesenchymal cell reporter lines, to investigate the dynamics of dermal cell populations in larvae during development and burn wound healing. Live imaging of early larval development revealed that the dermis contains multiple distinct cell populations, labeled by the expression of both collagen and the fibroblast marker vimentin, suggesting a complex cellular environment is required for normal fin growth. Following burn injury, dynamic morphological changes in collagen-expressing mesenchymal cells was associated with co-expression of the intermediate filament protein vimentin. Successful expression of vimentin was required to restore homeostatic organization of mesenchymal cells following injury. Treatment of burn injuries with isotonic medium, known to improve burn wound healing, was associated with faster expression of vimentin by collagen-positive cells. We speculated that reduced inflammation of burn wounded tissue caused by isotonic medium may result in improved mesenchymal cell function during healing. Indeed, we found that preventing neutrophil migration to burned tissue phenocopied the vimentin expression and morphological changes among collagen-positive mesenchymal cells that we observed with isotonic medium treatment. Our results highlight the complexity of dermal cell populations in the developing zebrafish larvae. Further, our findings suggest that neutrophil interactions with developing dermal cells may inhibit wound healing by perturbing mesenchymal cell dynamics and subsequent extracellular matrix remodeling.

Regulation of endoplasmic reticulum stress induced apoptosis in caudal fin epidermal cells

Poster Number: 50

Theme: Cell Biology

Presenting Author: **Douglas Weiser** - University of the Pacific

Co-Author(s): Danielle Hicks – Modesto Junior College; Krithika Giresh – University of the Pacific; Lauren Kim – University of the Pacific; Ayano Ohata – University of the Pacific; Lisa Wrischnik – University of the Pacific

Abstract: The Unfolded Protein Response (UPR) is a complex transcriptional and translational pathway that responds to the accumulation of unfolded proteins in the endoplasmic reticulum (ER-stress). One branch of the UPR involves phosphorylation of eukaryotic translation initiation factor 2 (eIF2α) by to attenuate global protein translation and reduce the accumulation of proteins in the secretory pathway. This pathway is multifunctional and can be both protective, allowing for recovery from ER stress, or promote ER-stress induced apoptosis. Two scaffolding proteins, GADD34 and CReP, bind to Protein Phosphatase 1 and promote the dephosphorylation of eIF2α. Pharmacological inhibitors of GADD34 and CReP delay onset and severity of neurodegenerative diseases. In vitro, GADD34 and CReP behave similarly, and the degree to which they overlap genetically is unclear. We use zebrafish as a model for GADD34 and CReP function. Epidermal cells in the caudal fin of 24 hpf zebrafish embryos are highly sensitive to ER-stress induced apoptosis. Inducing ER-stress induces two distinct genetic programs. The acute phase induces apoptosis within 4 hours of stress induction and activates apoptosis through p63 and Puma. Prolonged stress induces CHOP-dependent apoptosis after 24 hours of stress induction. We use a combination of loss-of-function alleles in GADD34 and CReP, morpholino-knockdown and pharmacological inhibition and observe that knockdown of GADD34 protects the epidermal cells from the long-term stress-induced apoptosis but the acute phase was unchanged. Knockdown of CReP had no effect on apoptosis. Double knockdown of GADD34 and CReP resulted in protection against the acute and chronic stress-induced apoptotic phases. We also observed that both GADD34 and CReP were transcriptionally upregulated by stress-induction. We believe this system will provide an ideal model elucidate the mechanism of action of GADD34 and CReP and lead to a better understanding of ER-stress induced apoptosis.

Uncovering the Role of α-Neurexin3 in Sensory Synapse Formation

Poster Number: 51

Theme: Cell Biology

Presenting Author: **Yommi Tadesse** - National Institute on Deafness and Other Communication Disorders, National Institutes of Health

Co-Author(s): Kate Pinter – National Institute on Deafness and Other Communication Disorders, National Institutes of Health; Lucia Salatino – National Institute on Deafness and Other Communication Disorders, National Institutes of Health; Katie Kindt – National Institute on Deafness and Other Communication Disorders, National Institutes of Health

Abstract: Hair cells (HC) are the primary sensory receptors of auditory, vestibular and lateral line systems. Specialized ribbon synapses between HCs and afferent neurons transmit these stimuli the central nervous system. The molecular mechanisms behind ribbon synapse formation remain largely unknown. Previous research has demonstrated that the longer isoform of the presynaptic adhesion

molecule Neurexin 3 (α-Nrxn3) is required for HC synapse assembly in both mice and zebrafish; however, its precise role in this process remains unclear. We investigated the dynamics underlying ribbon synapse formation in wild-type and α-Nrxn3 knockout (KO) larvae at different stages of HC development. We first validated the use of transgenic lines that label pre- and postsynaptic components. We found that live imaging of our transgenic lines revealed no impact on synapse counts in wild-type larvae. Further, we observed a similar synapse loss in α-Nrxn3 KOs larvae in our live samples compared to published immunostaining studies. After this validation we acquired in vivo time lapses using an Airyscan confocal microscope to visualize ribbon synapse formation over two different time intervals (4 min for 1-2 hrs and 30 min for 6 hrs). From our longer timelapses (6 hrs), we found significantly reduced paired synapses and a higher number of unpaired presynapses throughout HC development. Moreover, we identified an accumulation of postsynaptic components in the afferent dendrites that failed to reach the synaptic terminals. Additionally, our faster timelapses (1-2 hrs) revealed a delayed and unstable binding between pre- and postsynaptic components in α-Nrxn3 KO larvae. Together our timelapses indicate that α-Nrxn3 plays an early role in synapse stabilization, rather than a later role in synapse maintenance. Overall, understanding the molecular foundation of ribbon synapse formation is important for the development of novel therapeutics to treat noise-induced and age-related hearing loss caused by loss of HC synapses.

Bcl2l1 backfires as a retinal ganglion cell pro-survival factor

Poster Number: 52

Theme: Cell Biology

Presenting Author: **Antonia Amidon** - Medical College of Wisconsin

Co-Author(s): Joel Miesfeld – Ophthalmology and Visual Sciences – Medical College of Wisconsin; Matthew Veldman – Cell Biology, Neurobiology and Anatomy – Medical College of Wisconsin

Abstract: Retinal ganglion cell (RGC) genesis begins as multipotent retinal progenitor cells (RPCs) transition to a neurogenic state due to the expression of the RGC genesis and survival transcription factor, Atoh7. In mice and zebrafish, Atoh7 loss of function leads to a >95% reduction in mature RGCs leading to blindness. Atoh7 mediated RGC death in mice can be rescued by inhibition of apoptosis through the loss of the pro-apoptotic Bax gene, however it is unknown if this mechanism is shared by zebrafish. To determine if Bax mediated apoptosis is responsible for the loss of RGCs in atoh7 mutant zebrafish, we utilize cell type specific transgenes to overexpress the Bax inhibitor Bcl2l1 at three critical timepoints - prior to atoh7 expression in RPCs (vsx2), in atoh7+ neurogenic RPCs (atoh7), and in postmitotic RGCs (isl2b). Bcl2 mediated RGC survival will be assessed using the pan-RGC marker Rbpms2 at the conclusion of the initial wave of RGC genesis, 48hpf, and 5dpf once all retinal layers are formed and visual processing begins. Our initial experiments to assess the pro-survival effects of Bcl2l1 take advantage of the gal4;UAS system, utilizing the transgenic line vsx2:gal4;dsRed:UAS:Bcl2l1 in wild type and atoh7 mutants, where the dsRed reporter enables visualization of cells ovexpressing Bcl2l1. Assessment of embryos positive for Bcl2l1 expression in RPCs revealed surprising results. Instead of a rescue of RGCs in atoh7 mutants, we observed increased apoptosis in the wild type and mutant background, including a decrease in wild type RGCs, and no detectable Rbpms2+ RGCs in atoh7 mutant retinae. This paradoxical outcome highlights a context-dependent function of Bcl2l1 in the retina and future studies refining its overexpression to the neurogenic RPC and RGC population will provide further

mechanistic insight into Bcl2l1 function. Together, our data underscores the importance of tuning survival signals precisely during development.

Classifying the zebrafish atoh7 retinal lineage in wild-type and atoh7 mutants

Poster Number: 53

Theme: Cell Biology

Presenting Author: **Darby Bennett** - Medical College of Wisconsin

Co-Author(s): Joel Miesfeld – Medical College of Wisconsin; Robert Newland – Medical College of Wisconsin; Matthew Veldman – Medical College of Wisconsin

The Atoh7 transcription factor is expressed during retinal neurogenesis in transitional retinal progenitor cells, which give rise to all 7 major retinal cell types, but is interestingly only required for retinal ganglion cell (RGC) genesis. Atoh7 mutant zebrafish and mice lack optic nerves and lose >95% of RGCs. However, only ~55% of total RGCs come from the Atoh7 lineage in mice, suggesting the presence of signals released from atoh7+ populations that influence atoh7- RGC survival. It remains unknown if zebrafish have a similar RGC survival phenomenon and proportion of atoh7+ RGCs. To determine the zebrafish atoh7+ retinal lineage, we developed atoh7:iCre transgenic zebrafish and bred them to ubiquitously expressing ubi:loxP-eGFP-loxP-mCherry (ubi:Switch) transgenics, which upon Cre recombination permanently change the expression of eGFP to mCherry. Three atoh7:iCre founders were identified and their F2 offspring were confirmed to share the same expression pattern. Further transgene analysis in both wild-type and atoh7 mutants was performed at multiple timepoints consistent with endogenous atoh7 expression, including the onset of expression between 28-30 hours post fertilization, at 5 days post fertilization, when all cell types of the retina are present and functional, and in adult retinas. Timepoint analysis revealed that the atoh7 lineage gives rise to all retinal cell types and the proportion of atoh7+ cells remains consistent in the adult retina but shift in atoh7 mutants. Like mice, there are a subset of RGCs which are atoh7-, although at different proportions. We identified previously unknown populations of atoh7+ neurons within the forebrain and hindbrain. Together, our data confirm the presence of atoh7+ and atoh7- RGCs, allowing for further characterization of RGC survival mechanisms in atoh7 mutants, and provides a new and important tool for genetic fate mapping in the CNS.

Investigating mia2/mia3 function in zebrafish photoreceptors

Poster Number: 54

Theme: Cell Biology

Presenting Author: **Michael Donohue** - Medical College of Wisconsin

Co-Author(s): Steven Farber – John Hopkins University; Mckenna Feltes – John Hopkins University; Brian Link – Medical College of Wisconsin

Abstract: From a mutational screen in zebrafish, we identified two related genes that are essential for maintaining age-related photoreceptor health, mia2 and mia3. Of significance, mutations in human and dog MIA3 have recently been shown to cause retinopathies. How the splice isoforms of mia2 (Ctage5 and Tali) and mia3 (Tango1L and Tango1S) affect photoreceptor health is uncharacterized.

Mechanistically, mia2/mia3 protein isoforms act at ER exit sites to expand the budding COPII vesicle and facilitate large cargo secretion to the Golgi. Photoreceptor health is dependent upon intrinsic and extrinsic large proteins. Disrupted secretion of several of these large proteins has been shown to cause photoreceptor degeneration. Usherin (Ush2a), which bridges the gap between the inner segment and connecting cilium, is a candidate for large protein secretion. We hypothesize mia2/mia3 function within photoreceptors to accommodate secretion of large proteins, but also plays a role in the unconventional secretion pathway. To test this hypothesis, we assessed photoreceptors in mia2/mia3 mutants created using Crispr Cas9 deletions. Preliminary results indicate Ush2a is mislocalized in both Ctage5 and Tali mutants. Both mia2 (Ctage5/Tali double knockout) and mia3 (Tango1S/Tango1L double knockout) mutants resulted in aberrant photoreceptor morphology and degeneration. Interestingly, Tango1L mutants did not result in disruptions to photoreceptors or Ush2a localization. Our results validated previous research that shows altered protein secretion in photoreceptors leads to degeneration and underlies the importance of mia2/mia3 in photoreceptor health. Future experiments will evaluate all isoforms of mia2/mia3 by immuno-based markers and track fluorescently tagged candidate proteins. In addition, we will apply an in vivo protein proximity labeling assay (TurboID) to identify Mia2/Mia3 interactants within the photoreceptors to identify proteins involved in unconventional or large protein secretion. Finally, planned experiments include conditional gene deletions of mia2/mia3 to determine cell autonomous effects.

Surviving Isn't Thriving: Impacts of Low-Dose Cisplatin on Lateral-Line Hair Cells

Poster Number: 55

Theme: Cell Biology

Presenting Author: **Neva Bergemann** - Washington University School of Medicine in St. Louis

Co-Author(s): Lavinia Sheets – Department of Otolaryngology – Head and Neck Surgery – Washington University School of Medicine in St. Louis; Josef Trapani – Department of Biology – Amherst College; Elayna Malak – Department of Biology – Amherst College

Abstract: Cisplatin is an effective chemotherapy agent against solid tumors, but it also causes progressive and permanent hearing loss by damaging mechanosensory hair cells responsible for hearing. There is a limited understanding of cellular mechanisms behind cisplatin-induced hearing loss and a need for further research to develop effective therapies. Previous zebrafish studies used cisplatin dosages that result in maximal hair cell death. Using lower doses of cisplatin allows for hair cell survival and creates a more reflective model of progressive hearing loss seen in patients. Our work aims to characterize the effects of lower doses of cisplatin on zebrafish lateral line hair cells to illuminate mechanisms of cisplatin-induced hair cell damage and causes of dysfunction at a subcellular level. Zebrafish at 6 days post-fertilization (dpf) were treated with low and mid dose levels of cisplatin for two hours followed by a 24-hour recovery period, the time required for maximal hair cell loss. Confocal live imaging was then performed to assess mitochondrial morphology, mitochondrial membrane potential, and hair cell mechanotransduction function. Mitochondrial live imaging results revealed altered mitochondrial morphology in hair cells that survive cisplatin exposure. Low dose cisplatin was shown to induce mitochondrial donuts within hair cells, a formation that is associated with cellular and oxidative stress. Live imaging results of mechanotransduction revealed that mid dose cisplatin had significantly reduced mechanotransduction, whereas the low dose cisplatin groups had no significant effect

compared to controls. Correspondingly, we also observed depolarized mitochondrial membrane potential. Cumulatively, these results suggest that hair cells which survive cisplatin display significant mitochondrial damage which impacts function. Identifying mechanisms of hair cell mitochondrial damage and dysregulation of mitochondrial turnover will provide avenues for future research on ways to promote mitochondrial biogenesis and repair damaged hair cells, mitigating cisplatin-induced hearing loss.

Investigating the Roles of Jam2a and Mymk in Muscle Regeneration in Zebrafish

Poster Number: 56

Theme: Cell Biology

Presenting Author: **Yingying Hu, Ph.D** - University of Texas Southwestern Medical Center

Co-Author(s): Zhou Luo – University of Texas Southwestern Medical Center; Elizabeth Chen – University of Texas Southwestern Medical Center

Abstract: Myoblast fusion is an indispensable process in skeletal muscle development and regeneration. The cell adhesion molecules (Jam2a and Jam3b) and bi-partite vertebrate fusogens (Myomaker and Myomixer) are known to play essential roles in myoblast fusion during zebrafish embryonic muscle development. However, their involvement in adult muscle regeneration remains unclear. To address this, we utilized the col1a2:Gal4; UAS:mCherry transgenic line to visualize muscle progenitor cells following injury. A localized needle-injury model targeting 1–2 somite areas at 3 days post-fertilization (dpf) was employed, and regeneration was monitored every 24 hours over a 3-day period. By comparing the cellular dynamics in jam2a and mymk mutant fish, we aim to determine the roles of Jam2a and Mymk in muscle regeneration. These findings may shed light on the cellular mechanisms underlying regenerative muscle repair and inform future studies on fusion-related processes in vertebrate systems.

ATP contribution to wound edge calcium signaling in embryonic zebrafish

Poster Number: 57

Theme: Cell Biology

Presenting Author: **Shelly Tan** -

Co-Author(s): Bin Dong – Chemistry – Purdue University; Jesse Zhang – Chemistry – Purdue University; Qing Deng – Biological Sciences

Abstract: Rapid wound response is critical for maintaining multicellular life, allowing organisms to survive damage from the surrounding environment; thus, its pathways and molecular players are highly conserved. A transient elevation in intracellular calcium levels around the wound site, seen across many models at various scales, is one of the most immediate signals released in response to injury. This elevation begins the moment the wound occurs and spreads outwards from damaged tissue, dissipating several minutes later. However, the critical molecular mechanism that encodes and decodes this rapid calcium transient in vivo is unknown. A recently developed real-time precision opto-control (RPOC) technology enables laser activation during scanning to control chemical processes with high spatiotemporal precision. As a result, simultaneous stimulation and acquisition in this system allows for

the immediate cessation of ATP uncaging upon calcium wave initiation, effectively mimicking ATP release under wounding conditions. Using this platform, we demonstrate that localized ATP release triggers calcium waves via P2Y receptor activation, with wave initiation dependent on ATP release in a dose-dependent manner. In contrast, IP3 photorelease induces localized calcium release without wave propagation. Disruption of P2Y signaling prevents immune cell trafficking to the injury site in the first hour after the wound occurs, suggesting a mechanism by which damaged tissue can coordinate multiple complex factors to initiate regeneration and repair.

Role of mechanics in shaping epithelial cell migration during zebrafish embryogenesis

Poster Number: 58

Theme: Cell Biology

Presenting Author: **Sukriti Kapoor** - UCLA

Co-Author(s): Alvaro Sagasti – Group Leader, UCLA

Abstract: The mechanisms by which environmentally imposed mechanical constraints influence cell migration have been characterized primarily in cells migrating through the extracellular matrix (ECM). I am investigating the migration of two developing cell types in the zebrafish epidermis. At early embryonic stages, the epidermis consists of two epithelial layers, the inner basal and outer periderm layers. These cells originate from the basal layer, migrate in the ECM-poor intraepithelial region between basal and periderm cells, before intercalating into the periderm and differentiating into mucus cells and ionocytes. These cells are found in many mucosal tissues in humans, and their appropriate dispersal is required for tissue health. Our observations indicate that cells prefer to migrate along cell borders and avoid the nuclei of underlying basal cells. However, in the epidermis covering the yolk, migratory cells sometimes cross-over and deform the nuclei of basal cells, indicating a sensitivity to the mechanical nature of the tissue. To determine if migratory cell avoidance or crossing of epithelial nuclei correlates with cell density or nuclear roundness, a measure of compaction and stiffness, I am quantifying migratory behavior in different areas of the body at different developmental stages. Stiffness can be influenced by cell adhesiveness and nuclear deformability. To test these possibilities, I will knockdown and over-express cadherin and laminA and analyze how these manipulations impact migration and distribution of mucus cells and ionocytes. Finally, to determine if these factors are regulated by the cell cycle, I am using FUCCI to test if cell-cycle stage correlates with migration behavior. These studies will describe the role of cell junctions, nuclear stiffness and cell-cycle stage in the migration of mucus cells and ionocytes in the zebrafish epidermis.

Merkel cell development and regeneration involve an epithelial-to-mesenchymal transition

Poster Number: 59

Theme: Cell Biology

Presenting Author: **Ahlan Ferdous** - University of Washington

Co-Author(s): Eric Peterman – University of Washington; Elgene Quitevis – University of Washington; Jeffrey Rasmussen – University of Washington

Abstract: Merkel cells (MCs) are innervated mechanosensory cells in vertebrate skin that derive from epithelial progenitors. MCs detect light touch and texture, making them vital for fine motor control and social interactions. A reduction in MCs following injury or in age-related diseases can result in irregular sensation. Abnormalities in MCs, or their progenitors, may lead to Merkel cell carcinoma, an aggressive form of skin cancer. Thus, understanding how MCs develop from progenitors could inform biomedical approaches to treat skin conditions. However, the exact mechanisms of MC development remain unclear, partly due to the challenges of studying in utero development and opaque skin in mammals. By contrast, the zebrafish epidermis allows for direct observation of cell behaviors via non-invasive confocal imaging. We previously identified MCs in the zebrafish epidermis and further leveraged this system to identify the direct progenitors of MCs termed "dendritic MCs (dMCs)". Using live imaging, we discovered that dMCs display mesenchymal-like behaviors before maturing into neuroepithelial-like MCs. To dissect the molecular mechanisms associated with MC lineage progression, we generated a single-cell RNA sequencing dataset from homeostatic and regenerating adult zebrafish skin. Pseudotemporal analysis combined with hybridization chain reaction revealed an MC developmental trajectory from keratinocyte precursors during regeneration. Consistent with our live imaging data, distinct states within this trajectory show an enrichment of epithelial-to-mesenchymal transition (EMT) associated factors. Notably, we observe a progressive switch of adhesion molecules within the MC lineage from E-Cadherin to N-cadherin to adherence junction associated protein (ajap-1) as cells progress through the developmental trajectory. Together, our findings implicate a natural EMT process in MC development and regeneration and highlight the value of zebrafish as a novel model for studies of the MC lineage.

Defining Serpine3-Associated Protein Interactions in the Zebrafish Eye Using TurboID-Based Proximity Labeling

Poster Number: 60

Theme: Cell Biology

Presenting Author: **Asher Boucher** - Medical College of Wisconsin

Co-Author(s): Ross Collery – Medical College of Wisconsin

Abstract: Defining Serpine3-Associated Protein Interactions in the Zebrafish Eye Using TurboID-Based Proximity Labeling Authors: Asher Boucher1, Ross F Collery2, 1 1Department of Cell Biology, Neurobiology, and Anatomy, Medical College of Wisconsin, Milwaukee, WI, 53226, 2Department of Ophthalmology and Visual Sciences, Medical College of Wisconsin, Milwaukee, WI, 53226 Dysregulation of eye growth and size control is central to refractive errors such as myopia and hyperopia. RNA sequencing data from zebrafish models of these conditions revealed that the gene serpine3 is upregulated in myopic eyes and downregulated in hyperopic eyes, suggesting a potential role in eye size regulation. To investigate the molecular interactions of Serpine3 in the developing and adult eye, we have developed a collection of TurboID-based proximity labeling constructs for transgenic expression in zebrafish. Using the RPE-specific rpe65a promoter, we generated plasmids expressing TurboID-eGFP fused to: (1) full-length zebrafish Serpine3 to identify proximal and potentially interacting proteins; (2) a modified Serpine3 lacking the reactive center loop (RCL), the known inhibitory domain, to distinguish

direct targets from co-factors or scaffolding proteins; (3) eGFP alone to serve as a negative control for non-specific labeling; (4) full-length human SERPINE3 to assess conservation of interactors across species. These transgenic lines will be used to perform TurboID-mediated biotinylation of proteins that are found within 10 nm of Serpine3 in vivo, followed by streptavidin pulldown and mass spectrometry to map Serpine3-associated proteomic landscapes. This toolkit provides a powerful, RPE-specific method for investigating protein networks such as those implicated in eye size regulation and demonstrates the utility of TurboID in resolving complex protein networks and signaling environments in zebrafish.

The Role of Periplakin in Microridge Morphogenesis

Poster Number: 61

Theme: Cell Biology

Presenting Author: **Evan Takayoshi, MS, PhC** - UCLA

Co-Author(s): Alvaro Sagasti – UCLA

Abstract: Cellular protrusions are fundamental features of cells whose formation and morphologies are determined by the organization of cytoskeletal elements. Plakin family cytolinker proteins directly interact with all three cytoskeletal networks and various regulatory proteins to coordinate cytoskeletal organization. We previously discovered that periplakin, a plakin protein, is required for the formation of microridges, elongated cellular protrusions arranged in maze-like patterns on the apical surfaces of mucosal epithelial cells, and that periplakin's N-terminal head region is necessary for the initiation of microridge morphogenesis. Microridges are found in a variety of tissues, including the outer epithelial layer of larval zebrafish skin which forms early in embryogenesis, allowing them to be imaged in live animals throughout development. Periplakin's head region contains many protein-protein interaction domains (PPIs) that are potential binding sites for cytoskeletal regulatory proteins, suggesting that periplakin may directly bind and coordinate cytoskeletal regulatory proteins to initiate microridge morphogenesis. The specific roles and binding partners of each PPI in microridge formation are not known. I am investigating the relationship between periplakin's structure and function, using a combination of mutagenesis, imaging, and proteomics. By expressing GFP-tagged periplakin domain deletion transgenes in periplakin knockout zebrafish embryos, I have found that the SH3 domain and a set of spectrin repeats (SPEC 1/2) are both necessary for microridge morphogenesis. Overexpression of either transgene dominantly induces unique neomorphic structures that disrupt microridge patterning. To identify proteins that may interact with periplakin in microridges, I am using proximity-dependent biotin labelling (BioID) and mass spectrometry using the BLITZ system (Xiong et al. 2021, PMID: 34722825). To determine the role of candidate periplakin-interacting proteins in microridge morphogenesis, I am using a crispant screen. My findings suggest that periplakin coordinates microridge morphogenesis and organization through interactions with other proteins, in part mediated by its SH3 and SPEC 1/2 domains.

Mechanisms of neutrophil interstitial migration within the larval zebrafish skin

Poster Number: 62

Theme: Cell Biology

Presenting Author: **Jonathan Schrope** - University of Wisconsin - Madison

Co-Author(s): Adam Horn – University of Wisconsin - Madison; Tanner Robertson – University of Wisconsin - Madison; Jack Stevens – University of Wisconsin - Madison; Emilie Rochon – University of Wisconsin - Madison; Clyde Tinnen – University of Wisconsin - Madison; Dave Beebe – University of Wisconsin - Madison; Anna Huttenlocher – University of Wisconsin - Madison

Abstract: Neutrophils are the most abundant leukocyte and play an integral role in coordinating the immune response in the context of tissue injury and infection. After exiting the vasculature, neutrophils forge paths through dense epithelial tissues to reach sites of focal inflammation. The mechanisms that regulate this interstitial motility in vivo are poorly understood, limiting our ability to develop therapeutic strategies to modulate the innate immune response. Here we employ a larval zebrafish model to capture neutrophil interstitial motility within distinct mechanical environments within the skin. Neutrophils migrate faster within the matrix-rich dermis and slower in the cell-packed epidermis where they must exert forces to deform surrounding keratinocytes to forge a path for motility. We find that neutrophils utilize a centralized actin burst mediated by the Rho-GTPase cdc42 and the actin nucleation factor Wiskott-Aldrich Syndrome protein (WASp) to deform surrounding cells and thus enable interstitial motility. Altogether, this works advances our understanding of how neutrophils forge paths through dense cellular tissues to reach sites of focal inflammation.

Phenotypic study of mitochondrial dysregulation on opa1 KO zebrafish

Poster Number: 63

Theme: Physiology & Metabolism

Presenting Author: **Seongjin Kim** - Wonkwang University

Co-Author(s): Seong-Kyu Choe – Professor, Department of Microbiology, Wonkwang University School of Medicine

Abstract: The OPA1 gene plays a critical role in regulating mitochondrial morphology, facilitating transitions between fusion and fission states. Additionally, it is involved in cristae remodeling and the release of cytochrome C during apoptosis. In this study, we employed the CRISPR/Cas9 system to establish a zebrafish model with opa1 homozygous knockout (KO) and investigated its role in zebrafish development. Consistent with its known role, opa1 gene knockout resulted in fragmented mitochondria within the cell. Notably, we found that opa1 KO zebrafish gradually experience starvation from 10 days post-fertilization (dpf), exhibiting fatigue and malnutrition due to anorexia and, consequently, show mortality at 13 dpf. To determine the cause of the lethal phenotype in opa1 KO larvae, we monitored food consumption under experimental feeding conditions. We found that opa1 KO larval food consumption declines from 12 dpf to 17 dpf, with eventual mortality at 20 dpf. Additionally, opa1 KO larvae exhibit systemic mitochondrial dysfunction in various metabolic organs due to the global loss of opa1. This includes reduced ATP production, mitochondrial DNA level and expression of metabolic genes including glucose, ketone, and lipid metabolism. Interestingly, we found a significant increase in the expression of fibroblast growth factor 21 (FGF21) mRNA in opa1 KO larvae. This induction of fgf21 coincides with alterations in the expression of genes associated with metabolic regulation. These findings strongly suggest that opa1 loss leads to mitochondrial dysfunction, which has systemic effects

on the metabolism at the organismal level. Consequently, mitochondrial dysfunction may contribute to larval anorexia in opa1 KO zebrafish.

Toward an understanding of vertebrate iris muscle development: developmental timecourse and comparative analysis in mouse and zebrafish

Poster Number: 65

Theme: Evolution and comparative biology

Presenting Author: **Jonathan Setzke, B.S.** - University of Utah

Co-Author(s): Emily Woodruff, Ph.D. – University of Utah; Paresh Hejmadi, B.S. – University of Utah; Kristen Kwan, Ph.D. – University of Utah

Abstract: The iris, the thin, circular tissue that regulates entry of light into the eye, is essential for proper vision. This regulation of light entry, known as the pupillary light reflex, is governed by the iris muscles, the sphincter and dilator pupillae. Disruption of these muscles in diseases such as aniridia and congenital mydriasis can lead to blurred vision and is associated with glaucoma. Despite the importance of the iris muscles, their development and morphogenesis are poorly understood both cellularly and molecularly. Here we are characterizing iris muscle development both cellularly and molecularly by performing a comparative analysis between mouse and zebrafish, species which possess or lack a pupillary light reflex. We first needed to establish the timecourse of iris muscle development. We assessed iris muscle formation using immunofluorescence staining for smooth muscle actin, Acta2, at crucial timepoints in mouse and zebrafish: initiation (E17.5 and 6 dpf, respectively), expansion (P4 and 17 dpf), and maturation (P21 and 28 dpf). Contrary to the prevailing model, our results indicate that despite lacking a pupillary light reflex, zebrafish appear to initiate iris musculature development. However, this process appears to stall: the iris of the adult fish lacks fully functional iris muscles but still contains some Acta2 staining. These results suggest that zebrafish may provide a model for furthering our understanding of iris muscle morphogenesis and how iris muscle development becomes disrupted in disease. Moving forward, we seek to complete the timeline of iris muscle development in both mouse and zebrafish, explore the functionality of the remaining tissue in the adult fish, and define cell populations and transcriptomic profiles at crucial timepoints in iris muscle development in each species to understand where iris muscle development may diverge.

Latitudinal variation in vertebral numbers of Oryzias latipes species complex is determined by genetic factors

Poster Number: 66

Theme: Evolution and comparative biology

Presenting Author: **Rie HARA** - Graduate School of Agriculture, Kyoto University

Co-Author(s): Satoshi Ansai – Professor, Ushimado Marine Institute, Okayama University; Masaru Matsuda – Professor, Center for Bioscience Research and Education, Utsunomiya University; Yasuhiro Kamei – Professor, Optics and Bioimaging Facility, Trans-Scale Biology Center, National Institute for Basic Biology; Masato Kinoshita – Associate Professor, Graduate School of Agriculture, Kyoto University

Abstract: Fish populations from higher latitude tend to have more vertebrae than those from lower latitude, which tendency known as “Jordan’s rule”. Vertebral number is based on the number of somites, yet the genetic mechanisms underlying latitudinal variation remain poorly understood. The medaka (Oryzias latipes) species comlex comprising O. latipes, O. sakaizumii, O. sinensis and an undescribed species from eastern Korea, is a freshwater teleost species complex that spans latitudes from 25° to 40°N. This geographical range makes medaka a valuable model for studying latitudinal variation. The National BioResource Project Medaka has maintained wild-derived medaka stocks as closed colonies in outdoor fields, which minimize environmental variability while retaining genetic diversity. Also, SNP data of these stocks are now available, which enable genomic analysis. We revealed the positive correlation between abdominal vertebral number and latitude of the original sampling sites of 90 wild-derived stocks, which follows the previous study using O. sakaizumii wild populations. However, wild-derived stocks were raised in different developmental temperatures, which influence somite and vertebral numbers. To assess the robustness of the variation of vertebral numbers, we examined vertebral traits in 10 stocks from various latitudes, reared at different water temperatures (22, 24, 26, 28 °C) until somite stage. As a result, stocks from higher latitude consistently exhibited more abdominal vertebrae across all temperatures within each phylogenetic subgroup. ART-ANOVA revealed that phylogeny significantly affected abdominal, caudal, and total vertebral numbers, while water temperature only influenced caudal vertebrae. However, we could not reproduce the known negative correlation between water temperature and vertebral numbers, likely due to the short period of temperature control until somite stage. These findings indicated that wild-derived stocks of O. latipes species complex kept the genetic basis of latitudinal variation in abdominal vertebral number.

Characterization of zebrafish RW strain

Poster Number: 67

Theme: Evolution and comparative biology

Presenting Author: **Hiromi Hirata, Ph.D** - Aoyama Gakuin University

Co-Author(s): Kenichiro Sadamitsu – Aoyama Gakuin University; Makoto Kashima – Toho University; Seiji Wada – Aoyama Gakuin University; Akiko Ishioka – RIKEN Center for Brain Science; Satomi Nakayama – RIKEN Center for Brain Science; Ryoko Nakayama – RIKEN Center for Brain Science; Hitoshi Okamoto – RIKEN Center for Brain Science

Abstract: Zebrafish have become an indispensable vertebrate model in both basic and applied biological research. Among the commonly used laboratory strains, AB and TU have been most extensively employed. However, accumulating evidence highlights that zebrafish strains exhibit notable differences in complex behaviors and susceptibility to adult phenotypes, indicating that genetic background plays a critical role in experimental outcomes. In response to increasing demands for genetically diverse models, we established a wild-type strain, termed the RIKEN Wild-type (RW), aimed at minimizing the impact of deleterious genetic variants and offering distinct phylogenetic characteristics. In this study, we performed comparative genomic analyses of the RW strain alongside ten other commonly used wild-type strains including AB, TU, TL, WIK, SAT, NHGRI-1, PET, IND, IM, and M-AB, with a focus on protein-coding regions. Our results identified numerous coding-region variants across all strains, many of which were strain-specific and likely contribute to phenotypic diversity. The RW strain was found to harbor unique variants in 13 protein-coding genes. Importantly, phylogenetic analysis based on whole-genome

SNP profiles revealed that RW forms a distinct cluster, genetically separated from other strains. The RW strain also demonstrates strong breeding performance and robust health, further supporting its utility in a wide range of experimental applications. Its distinct genetic profile and favorable husbandry traits make RW a valuable addition to the zebrafish research community, particularly for studies requiring strain-specific resolution or broader assessments of genetic diversity.

Characterizing brain functions of human duplicated NOMO paralogs using zebrafish

Poster Number: 68

Theme: Evolution and comparative biology

Presenting Author: **Aidan Baraban** - UC Davis Genome Center

Co-Author(s): Megan Dennis, Dr. – Principal Investigator, Department of Biochemistry and Molecular Medicine, UC Davis Genome Center; Nicholas Haghani – Graduate Student, Integrative Genetics and Genomics Graduate Group, UC Davis Genome Center; Zoeb Jamal – Graduate Student, Integrative Genetics and Genomics Graduate Group, UC Davis Genome Center

Abstract: Genetic drivers of human-specific neurological traits–including neocortical expansion and altered synaptic connections–remain largely undiscovered. Duplicated genes are a source of evolutionary innovation across the tree of life, including in humans. Several published examples have expressed human-specific genes in animal models and shown effects on brain development. Recently, we identified hundreds of human duplicate paralogs exhibiting brain expression, leaving us tasked with sorting functional genes from many likely non-functional pseudogenes. Hypothesizing that human genes implicated in neurocognitive conditions play a role in brain development, we focused on the NOMO gene family, encoding Nodal Modulator proteins, comprising three paralogs in the human T2T-CHM13 genome at chromosome 16p13.11. This locus is prone to large-scale deletions and duplications enriched in individuals with neuropsychiatric conditions and also associated with altered brain sizes. Previous work revealed reduced brain mass in homozygous nomo knockout adult mutant zebrafish (3 months), without reduced brain size. Here, we aimed to understand impacts of nomo knockout on neurodevelopment by generating CRISPR mosaic F0 knockouts and assessing morphological, behavioral, and brain phenotypes in 3 and 5 days post-fertilization larvae. These mosaic F0 knockouts exhibited smaller head size relative to body length and reduced motor behavior, suggestive of neurodevelopmental alterations. The behavioral results match those of adult nomo mutants' behavior, but expands our knowledge of an early development brain size phenotype. Additionally, we have in vitro transcribed mRNA encoding human-specific NOMO1 and NOMO2 paralogs, with ongoing work characterizing "humanized" larvae via transient, ectopic expression of these genes in early development. This will help us clarify NOMO's role in early brain development, and also establish a framework in zebrafish for studying novel duplicated genes relevant to human evolution and disease.

THAP7 is a transposase-derived transcription factor implicated in zebrafish development and human intellectual disability

Poster Number: 69

Theme: Evolution and comparative biology

Presenting Author: **Rachel Cosby, PhD** - National Institutes of Health/NICHD

Co-Author(s): Jennifer Sinclair – National Institutes of Health/NICHD; Zobia Umair – KAUST; Catrina Rateb – National Institutes of Health/NICHD; Steven Gay – National Institutes of Health/NICHD; Lisa Kratz – Kennedy Krieger Institute; Wolfgang Fischle – KAUST; Fowzan Alkurya – KFSHRC; Harold Burgess – National Institutes of Health/NICHD; Todd Macfarlan – National Institutes of Health/NICHD

Transcription factors (TF) and their networks can evolve via fusion between host- and DNA transposase-derived protein domains. Several fusions are implicated in vertebrate development and developmental disorders, but their functions remain largely unknown. We identified variants in human THAP7, a TF derived from a P-element-like transposase, that segregate with intellectual disability in five families. We tested whether THAP7 regulates development by modeling THAP7 loss in mice and zebrafish (CRISPR). The behavior and brain morphology of thap7-/- fish are normal, and Thap7-/- male mice are grossly normal, suggesting THAP7-associated intellectual disability is human specific. While Thap7-/- mice are viable, thap7-/- fish grow slowly and die as juveniles. These deficits appear due downregulation of the zebrafish homologs of GCDH (gcdha/b), the causative gene for human glutaric aciduria type I (GA1), and elevated GA1-associated metabolites. Neither human patients nor Thap7-/- mice show GA1 symptoms or GCDH downregulation. This suggests that THAP7's regulatory network, and resulting biological role, has diverged across vertebrates. To identify THAP7 target genes across species, we assessed THAP7 binding (ChIPseq/exo) in various contexts (juvenile zebrafish, juvenile mouse brains, and mouse, human, and zebrafish cell lines). While most (~50%) THAP7 targets are conserved across species, some are only bound in one species, including zebrafish specific gcdha. We determined this is due to gain of the THAP7 motif in the GCDH promoter prior to the teleost whole genome duplication, consistent with a lack of THAP7-dependent GA1 in mammals. Thus, while THAP7's molecular function as a TF is preserved, its biological function has diverged between fish and mammals. We are adapting our approach to identify human specific THAP7 targets in iPSC-derived THAP7-/- neurons to investigate the underlying molecular cause of THAP7-associated human intellectual disability. Our research underscores THAP7's role in vertebrate development and elaborates on the origin and function of host-transposase fusion genes.

Separation of church and state: dynamic developmental changes of the cloaca and the formation of an anus

Poster Number: 70

Theme: Evolution and comparative biology

Presenting Author: **M. Brent Hawkins** - Boston Children's Hospital

Co-Author(s): Matthew Harris – Orthopedic Research – Boston Children's Hospital

Abstract: For most vertebrates the urogenital and digestive tracts terminate in a shared exit opening in the body wall called the cloaca. However, placental mammals and some ray-finned fishes have evolved a derived configuration wherein the digestive tract ends in a distinct anus separated from the urogenital system. Despite the importance of these exits and their malformation in human conditions such as persistent cloaca, the developmental genetic controls that underlie cloaca formation and the changes in these controls that lead to an independent anus are poorly understood. We are investigating these

mechanisms using zebrafish, which exhibit a pseudo-cloacal arrangement where the hindgut (HG) and pronephric duct (PND) exit the body through adjacent but distinct openings of the vent. Using knock-in endogenous reporter lines, we show that Hox13 genes are expressed in the terminal regions, with different Hox clusters exhibiting different tissue specificity. We find that hoxa13b is expressed in more superficial gut layers and the hindgut sphincter, while hoxd13a is present in the intestinal lining and cloacal membrane. Combinatorial loss of Hox13 paralogs result in the fusion of the HG and PND prior to leaving the body through a single hole, recapitulating the cloaca phenotype also observed in mouse Hox13 mutants. Next, we assessed the effect of activating mutations in the Vav2/Wasl pathway, which modulates Hox activity in the fins, on cloacal development. Shockingly, we discovered that waslb gain-of-function resulted in the formation of a diastema separating the HG and PND exits. This morphology effectively phenocopies the independent anus of placental mammals. Altogether our findings reveal a conserved role of Hox13 genes in regulating terminal tube configurations across vertebrates and highlight the Vav2/Wasl pathway as a candidate mechanism in the evolution of independent urogenital opening and anus in mammals.

Fish phylomapping screens for identification of genetic regulators of trait development and evolution

Poster Number: 71

Theme: Evolution and comparative biology

Presenting Author: **Jacob Daane** - University of Houston

Co-Author(s):

Abstract: Despite advances in recent computational and experimental genetic tools, identifying the genetic basis of phenotypic traits remains a fundamental challenge in biology. Traditional approaches of gene discovery require random generation of variation in laboratory model systems (e.g., forward genetics) or sampling of population-level variation (e.g., QTL, GWAS). However, these approaches are limited by phenotypes that are viable in model systems or to cases where there is phenotypic variation within a species. Recently, phylomapping, or “forward genomic”, approaches have been developed that leverage the wide spectrum of natural variation that exists between species to identify novel gene function through comparative genomics. Here, I present our recent efforts to apply phylomapping approaches across the teleost radiation to identify the genetic basis of evolved traits. I will focus on a case study of coordinated evolution of low skeletal density and elevated corporeal lipids in perciform fishes, which has evolved multiple times across Antarctic notothenioids, snailfishes, and sculpins. As part of this work, we have assembled several new genomes, including two species of Baikal oilfish (genus Comephorus), one of which has enough corporeal lipids in adults that it makes up an astounding 40% of their total weight. We have integrated these genomes into a broad analysis of across a multiple alignment of over 35 whole perciform genome assemblies and a dataset that includes protein coding exons and conserved non-coding elements from targeted sequencing of 54 additional perciforms. Through analysis of both monomorphic and convergent evolution, we have identified potential roles for several genes and putative enhancer elements, which we are testing in the zebrafish using genome editing tools. These studies will shine light both on genetic and developmental mechanisms of adaptation and identify conserved modifiers of human disease.

Investigating Polymorphisms Associated with Skin Hypopigmentation in a Native American Caribbean Population

Poster Number: 72

Theme: Evolution and comparative biology

Presenting Author: **Khai Ang, PhD** - Penn State College of Meicine

Co-Author(s): Victor Canfield – Penn State College of Medicine; Keith Cheng – Penn State College of Medicine; Kathryn Early – Penn State College of Medicine; Thaddeus Harbaugh – Penn State College of Medicine

Abstract: Skin pigmentation is a polygenic, quantitative trait with high heritability and varies widely between populations of different genetic ancestries. It is hypothesized that the prevalence of fair complexion in environments with limited sun exposure is due to positive selection to increase UV-dependent Vitamin D synthesis. To investigate the genetic determinants of skin hypopigmentation variation between East Asians/Native Americans and Africans, we sought a population of primarily Native American and African ancestry with minimal European contribution that exhibited a wide range of skin color. The Kalinago, a population from the Commonwealth of Dominica, fulfill these criteria. Admixture analysis of 458 individuals revealed a genetic contribution of approximately 55% Native American, 32% African, and 12% European ancestry, reflecting the highest reported Native American genetic ancestry among Caribbean populations. Skin pigmentation measurements ranged from 20 to 80 melanin units and averaged 46. The most pervasive European hypopigmentation alleles, SLC24A5A111T and SLC45A2L374F had allele frequencies of 0.14 and 0.06, with calculated effect sizes of –6 and –4 melanin units, respectively. Three Kalinago with albinism were determined to be homozygous for a causative multi-nucleotide polymorphism, OCA2NW273KV; its allele frequency was 0.03 and the effect size was –8 melanin units. Native American genetic ancestry alone was associated with a pigmentation reduction of 24 to 29 melanin units. However, the genetic variants responsible for hypopigmentation remain to be identified, as the previously reported polymorphisms associated with skin color in the Native Americans did not cause a measurable reduction in pigmentation in the Kalinago. In particular, the variants proposed to contribute towards skin pigmentation differences between Native Americans and Africans in the genes EFR3B, CNKSR3, IPCEF1, and EFGR did not reach genome-wide significance. However, it is important to study the potential contributions of these genes towards human skin pigmentation differences and elucidate their role in melanogenesis.

Regional intestinal wounding during early life leads to imperfect regeneration and deployment of a cryptic regional program

Poster Number: 75

Theme: Regeneration

Presenting Author: **Peyton Moore** - Department of Molecular Genetics and Microbiology, Duke Microbiome Center, Duke University School of Medicine, Durham, NC, USA

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Abstract: The intestinal epithelium performs unique digestive physiologies facilitated by distinct regional developmental programs. Following substantial regional wounding, the intestine undergoes multiphase repair to restore homeostasis. During the acute repair phase, inflammatory cells are recruited to clear debris and infiltrating microbes while unwounded intestinal epithelial cells (IECs) from neighboring regions migrate to restore the barrier (reparative epithelia). This precedes a longer adaptive phase, where the reparative epithelium adapts to its new regional location along the anterior-posterior axis. However, it is unclear if the reparative epithelium adopts the program of its new regional location, retains its original regional program, or adopts a new cryptic program not found in unwounded animals. These knowledge gaps are applicable to all animals but are particularly relevant in humans experiencing intestinal resection, short bowel syndrome, necrotizing enterocolitis, or ulceration. We and others have previously demonstrated conservation of regional transcriptional, developmental, and physiological programs between zebrafish and mammalian small intestines. Here we report a new intestinal wounding model in zebrafish that selectively and conditionally ablates jejunal IECs using the nitroreductase system (NTR 2.0). This transient ablation during larval stages triggers an acute healing phase including barrier dysfunction, neutrophil and macrophage recruitment, and migration of neighboring ileal IECs into the jejunum resulting in a proximal relocation of the ileal boundary. Surprisingly, wounded larvae that survive into adulthood retain abnormal proximal expression of ileal markers while also co-expressing jejunal markers. This suggests that the reparative epithelium adopts and durably maintains a new cryptic regional program not observed in unwounded animals. Ongoing research aims to characterize the cryptic program of the reparative epithelium, and assess the role of microbiota and inflammation on acute and adaptive repair. Together this work indicates that regional intestinal wounding during early life can lead to imperfect regeneration and life-long alteration of intestinal regional anatomy.

Modulation of Small Conductance Calcium-activated Potassium (SK) Channel Activity Affects Regeneration after Spinal Cord Injury (SCI) in Larval Zebrafish

Poster Number: 76

Theme: Regeneration

Presenting Author: **Patrick Garrett** - East Carolina University

Co-Author(s): Karen Mruk – East Carolina University

Abstract: Small conductance calcium-activated potassium channels (SK) are activated by an increase in cytosolic Ca2+ and contribute to after-hyperpolarization following an action potential and regulate synaptic transmission and plasticity. Given their role in nervous system function, we sought to determine whether SK channels are involved in cellular regeneration and/or functional recovery after spinal cord injury using larval zebrafish. Using qRT-PCR and in situ hybridization, we found that SK1

channels are upregulated after SCI in larval zebrafish beginning at 3 days post injury (dpi) and remained upregulated.. Using hybridization chain reaction (HCR) we identified the cell types that express SK1 after injury.We next used pharmacological manipulation to measure functional recovery of different swim speeds after SCI Given the pharmacological effects on swim behavior, we next generated the transgenic line Tg(UAS:kcnn1a) to determine whether genetic overexpression of SK1 is the main driver of functional recovery, and the effect overexpression of SK1 has on the cellular response to SCI. This data is the first to elucidate the role SK channels have in spinal cord regeneration and functional recovery and provides a new therapeutic target to improve recovery from SCI.

Dysregulated immune and fibroblast signaling in spontaneous failure of spinal cord regeneration in adult zebrafish

Poster Number: 77

Theme: Regeneration

Presenting Author: **Pierre Gillotay, PhD** - Morgridge Institute for Research and Department of Cell and Regenerative Biology, University of Wisconsin-Madison, Madison, WI USA.

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Abstract: Primary and secondary tissue damage from spinal cord injury permanently impairs sensory and motor functions, causing irreversible paralysis. Following spinal cord injury, nerve cell death and scar formation inhibit regeneration. By contrast with most mammals, teleost zebrafish can form new neurons, regrow axons, and recover the ability to swim just 6 to 8 weeks after a paralyzing injury that completely severs the spinal cord. Importantly, these regenerative events proceed without massive scarring. Instead, following injury, specialized non-neural glia and other cells build a tissue bridge to connect the two severed ends, allowing axons to grow across the wound and reestablish crucial connections. In preliminary studies, we found that a reproducible percentage of zebrafish remain permanently paralyzed following spinal cord transection. Initial histological characterization indicated grossly normal bridge formation in these cases of failure, whereas axon growth from rostral to caudal stumps was disrupted. Using single nuclei RNA sequencing, we interrogated the cellular and molecular differences associated with either successful or failing regeneration across several timepoints. Our analysis of the failure-associated cellular landscape revealed an over-representation of fibroblasts concomitant with a depletion of T-cells, among other changes. Failure-associated fibroblasts display a transcriptomic signature enriched in expression of collagen genes, and signaling pathways associated with failure included dysregulation of the TIGIT/CD226 pathway, which controls T-cell survival and activity. Genetic perturbation of TIGIT/CD226 membered inhibited the capacity of injured zebrafish to recover from paralyzing injuries. Taken together, our findings highlight zebrafish as a model for both successful and failed spinal cord regeneration, and they indicate that each outcome is associated with a specific cellular and molecular and signaling landscape.

Sniffing Out Neuroregeneration: Olfactory Neurogenesis in the Context of Alzheimer's Disease

Poster Number: 78

Theme: Regeneration

Presenting Author: **Lynne Nacke** - UAB Heersink School of Medicine

Co-Author(s): Debangana Chakravorty, PhD – Postdoctoral researcher, UAB Heersink School of Medicine; Sriivatsan Govidna Rajan, PhD – Postdoctoral researcher, Memorial Sloan Kettering Cancer Center; Ankur Saxena, PhD – Associate Professor, UAB Heersink School of Medicine

Abstract: Anosmia, the loss of smell, is an early potential biomarker for Alzheimer's disease (AD), with many patients experiencing symptoms months to years before evidence of cognitive decline. This correlation is perplexing given that the olfactory system is highly regenerative, with a population of basal stem cells responsible for the continuous renewal of olfactory sensory neurons (OSNs). Building on findings of a Notch signaling-Insm1a feedback loop that drives developmental olfactory neurogenesis in vivo and the ability of AD-associated Aβ42 peptide to shift Notch signaling-Insm1a expression patterns in vitro, we hypothesized that Aβ42 would disrupt olfactory neurodifferentiation. To test this idea, we treated zebrafish embryos with exogenous Aβ42 peptide and found temporally-dynamic changes in the numbers of basal stem cells and OSNs. Next, we mosaically overexpressed Aβ42 in vivo during and post-olfactory development and compared individual cells with differing levels of Aβ42. We uncovered transcriptional changes indicating that Aβ42 cell autonomously shifts the stem cell-neuron balance towards neuronal differentiation during development. Moreover, after development is complete, Aβ42 also promotes reprogramming of neurogenesis in response to damage. Now, we are assaying the effects of Aβ42 overexpression in adults, including determining which signaling pathways drive self-renewal versus differentiation. In sum, we hope to discover neurogenic mechanisms that can be harnessed to improve outcomes for neurodegenerative diseases. This work was supported by the National Institute on Aging, National Institute of Child Health and Human Development, and the Alzheimer's Association.

Huntingtin is selectively required for Spinal Cord Regeneration in Zebrafish

Poster Number: 79

Theme: Regeneration

Presenting Author: **Jaffna Mathiaparanam** - University of Pennsylvania

Co-Author(s): Michael Granato – Professor, Cell and Developmental Biology, University of Pennsylvania; Jessica Nelson – Asisstant Professor, Cell Biology, University of Colorado

Abstract: Following injury, axons in the Central Nervous System (CNS) initiate molecular pathways to respond and regrow. Although blunted by inhibition, vertebrates display evidence that intrinsic mechanisms exist to promote spontaneous regeneration, however identifying the relevant functional pathways contributing to this regeneration remain largely unknown. Unlike most mammals, zebrafish exhibit robust and functional CNS regrowth, providing a powerful system to decipher cellular and

molecular mechanisms of spontaneous CNS regeneration. Using a candidate gene approach, we observed a loss-of-function mutation in huntingtin (htt) resulted in a severe deficit in regeneration of a spinal cord axon after laser-mediated transection. While wildtype siblings displayed robust regrowth of the Mauthner Cell (M-Cell) axon, crossing the injury site in 24h and regrowing to pre-injury length within 96 hours, 60% of mutant M-cell axons fail to cross the injury site, suggesting htt may promote initiation of the regenerative response. Initiation of the response to injury involves transcriptional upregulation of several identified regeneration associated genes. We observed delayed upregulation of several genes in this network in htt mutants compared to siblings, supporting that htt works upstream of the transcriptional injury response. Interestingly, regeneration of the M cell axon is significantly improved if injury occurs more proximally to the M-Cell body, suggesting that htt may be a selective factor of long-distance regeneration and involved in axonal trafficking. Supporting this assertion, I identified two other huntingtin associated proteins, HAP40 and HAP1, also involved in trafficking, as necessary for M-Cell regeneration. HTT has been recently identified to play a role in spinal cord regeneration in the mouse, however the endogenous role of htt in vertebrates remains undefined. Here, we directly show that htt is involved in the timely regenerative response to injury and begun to identify a novel network of associated genes with previously uncharacterized roles in axon regeneration

Identifying the cellular-molecular mechanisms of neuronal regeneration in the enteric nervous system

Poster Number: 80

Theme: Regeneration

Presenting Author: **Julia Ganz** - Michigan State University

Co-Author(s): Mujahid Shah – Michigan State University; Katherine Moran – Michigan State University; Helen Rueckert – Michigan State University

Abstract: The enteric nervous system (ENS), the largest subdivision of the peripheral nervous system, comprises an intricate network of neurons and glial cells. It controls all important gut functions including motility, digestion, and inflammatory responses. ENS dysfunction or loss is linked to various gastrointestinal disorders, including Hirschsprung disease (HSCR), which is characterized by a lack of ENS neurons in the distal gut. So far, the only treatment for HSCR is surgery, which commonly has detrimental post-surgical complications. Hence, restoring missing ENS neurons using regenerative approaches would represent a promising treatment option for ENS diseases. Previous studies on ENS regeneration revealed slow and incomplete neuronal regeneration in mammalian models. Work in zebrafish using laser ablation showed some neuronal recovery but this approach targeted only a few cells and caused unintended surrounding tissue damage. To overcome these challenges, we developed a chemical-genetic ablation model in zebrafish using the Gal4/UAS-nitroreductase (NTR) system for targeted, robust ENS neuron ablation. Metronidazole is converted into a cytotoxic compound by NTR, inducing spatially and temporally controlled cell death in NTR-expressing neurons, as confirmed by morphological alterations, significant neuronal loss, and TUNEL assays. Post-ablation, we quantified the regeneration of ENS neurons and found complete recovery by day 9 post ablation. Among the regenerated neurons, the proportion of nitrergic and cholinergic neurons fully recovered, whereas serotonergic and VIPergic neurons only displayed partial recovery, indicating subtype-specific

differences in the regenerative capacity and/or timing. To determine the cellular-molecular mechanisms underlying successful ENS regeneration we are currently performing single-cell RNA-sequencing in regenerating guts compared to controls to identify the cellular processes and genes important for neuronal regeneration. This study establishes a robust model for ENS regeneration, offering a valuable platform for investigating the molecular-cellular mechanisms of ENS regeneration as a first step to develop potential therapeutic strategies for ENS diseases in the future.

The Rhomboid Protease Rhbdl2 Modulates in vivo Wound Healing and Neutrophil Behavior

Poster Number: 81

Theme: Regeneration

Presenting Author: **Saroj Gourkanti** - University of California, San Diego

Co-Author(s): Yazmin Muñoz – National Institute of Environmental Health Sciences; Rosa Chavez – University of California, San Diego; Jacqueline Cheung – University of California, San Diego; Taylor Schoen – University of California, San Diego; Gayathri Ramakrishnan – University of California, San Diego; Thomas Whisenant – University of California, San Diego; Sonya Neal – University of California, San Diego

Abstract: Every year, millions of people suffer from irreparable wounds, with most treatment focusing on pain prevention and staving off infection. However, while characterization of the machineries involved in repair and regeneration is ongoing, the regulators associated with wound healing signaling events remain elusive. The highly conserved intramembranous protease RHBDL2 has emerged as a candidate for mediating wound healing based on in vitro tissue culture studies. Its ability to cleave plasma membrane-tethered ligands and their associated signaling pathways during wound healing make it an appealing therapeutic target for wound treatments. Currently, the physiological function of RHBDL2 is unknown due to the lack of a vertebrate knockout model. The objective of my project is to address this by generating a rhbdl2-deficient model in zebrafish via CRISPR/Cas9 genome editing. Zebrafish have the remarkable ability to regenerate various tissues, with both larvae and adults being ideal models to study wound repair mechanisms. Along with the benefits of established imaging methods, wounding assays and transgenic lines, zebrafish are advantageous in their ability to monitor developmental changes easily. To that end, we have successfully generated a stable rhbdl2-/- mutant line. Contrary to in vitro wound healing studies, we have found that loss of rhbdl2 leads to aberrant wound healing characterized by excessive regrowth in the developing larvae. Surprisingly, proteomic analysis revealed significant changes in proteins associated with neutrophil motility. Based on these observations, we hypothesize that rhbdl2 regulates wound healing through modulation of neutrophil behavior in the wound site. Currently, by utilizing confocal imaging and motility analyses, I am investigating how the wounding healing and neutrophil motility phenotypes are related using both morpholinos and neutrophil-deficient mutants. Together, these approaches will shed light on RHBDL2's role in innate immunology, regeneration and act as a foundation for novel studies on rhomboid proteases in zebrafish knockout models.

Revisiting Polycomb Repressive Complex 2 contributions to cell state transitions using zebrafish fin regeneration

Poster Number: 82

Theme: Regeneration

Presenting Author: **Rachael Giersch, B.S.** - University of Oregon

Co-Author(s): Gabriel Yette – University of Oregon; Scott Stewart – Research Associate Professor, University of Oregon; Bryson Ricamona – University of Oregon; Kryn Stankunas – Professor of Biology, University of Oregon

Abstract: Zebrafish rapidly and robustly regenerate amputated fins to their original shape and size. Tissue growth is balanced between proliferation of injury-activated, de-differentiated progenitors and their subsequent re-differentiation. Gene expression program changes facilitated by dynamic chromatin landscapes drive such bi-directional cell-state transitions. Histone modifications stabilize chromatin landscapes to support cell type- and state-distinguishing programs. For example, tri-methylation of Histone H3 lysine-27 (H3K27me3) by Polycomb Repressive Complex 2 (PRC2) promotes “stemness” by repressing terminal differentiation genes. Additionally, PRC2/H3K27me3-mediated gene silencing stabilizes differentiated cell identities. We investigated PRC2/H3K27me3 roles during adult zebrafish fin regeneration by genetic disruption of the PRC2 catalytic subunits ezh1 and ezh2. Near global loss of H3K27me3 was accompanied by greatly elevated, gene activation-associated H3K27 acetylation. Surprisingly, PRC2 mutant fins regenerated largely normally, including after re-amputation, despite an expectation for widespread, spurious gene activation. Less than 2% of gene transcripts analyzed by RNA-Seq were differentially expressed upon loss of H3K27me3. We then performed CUT&Tag to detail residual H3K27me3 across the epigenome that could accommodate regeneration in PRC2-deficient fish. H3K27me3 was variably retained at low levels at transcription start sites but otherwise largely depleted. Therefore, minimal local H3K27me3 marks may be sufficient to maintain essential gene repression. Alternatively, fin regeneration may be robust to the functional loss of H3K27me3. Regardless, PRC2/H3K27me3 seems unlikely to have a widespread role maintaining cell type- and state-distinguishing chromatin landscapes during fin regeneration. T32HD007348 and R21HD109670 provided support.

Live Imaging of Macrophage Response to Liver Injury and Regeneration

Poster Number: 83

Theme: Regeneration

Presenting Author: **Manjari Trivedi, PhD** - Harvard Medical School

Co-Author(s): Wolfram Goessling – Harvard medical school

Abstract:

The liver possesses a remarkable regenerative capacity as it can completely restore its structure and function after an injury. Nevertheless, liver diseases remain a major global health challenge. Both chronic and acute liver disease patients commonly exhibit hepatic inflammation, significantly

contributing to liver disease progression. Particularly, macrophages exacerbate liver damage but are also necessary for repair. Their activation can be detrimental to liver function as they release pro-inflammatory cytokines and chemokines but are also required to clear debris from the damaged liver. Hence, modulating macrophage-mediated inflammation can be an effective therapeutic strategy to enhance liver regeneration.

To study the role of macrophages, we established a robust hepatic inflammation model by inducing acute hepatotoxicity using the metronidazole/nitroreductase system in zebrafish larvae. We then used high-resolution, intravital imaging to investigate the interactions between fluorescently-tagged macrophages and hepatocytes during injury and regeneration.

A significant reduction in liver volume was observed after Mtz-induced liver toxicity, confirming hepatic damage. Macrophages swiftly responded to this damage and were recruited throughout the injury. Notably, they persisted around the regenerating livers until the liver volume was restored, suggesting their importance in liver regeneration. Interestingly, depleting macrophages delayed liver recovery, caused significant structural defects, and accumulation of debris and excessive collagen deposits. Additionally, comparing liver regeneration post-Mtz injury, with or without inhibiting phagocytosis, revealed no difference in liver volume recovery. This suggests that macrophage functions, such as cytokine production and growth factor secretion may play a more important role in liver repair.

These results establish a sequential and coordinated response of macrophages during the critical phases of liver injury and subsequent regeneration. We also highlight how crucial macrophage interactions with the damaged liver is in facilitating effective liver regeneration. Further research is needed to elucidate the molecular mechanism underlying the critical role of macrophages in liver regeneration.

Wnt-signaling drives prrx1a/b expression in meningeal fibroblasts to promote zebrafish spinal cord regeneration

Poster Number: 84

Theme: Regeneration

Presenting Author: **Sam Alper** - University of Utah

Co-Author(s): Heather Hamilos – University of Utah; Deeptha Vasudevan – University of Utah; Maya Wheeler – University of Utah; Richard Dorsky – University of Utah

Abstract: In mammals, spinal cord injury (SCI) is poorly repaired and leads to permanent sensorimotor dysfunction. However, many fish, including zebrafish, display spontaneous spinal cord regeneration. This difference in regenerative capacity may be underpinned by distinct fibrotic responses between mammals and fish. In both organisms, SCI leads to neuronal cell death, severed axons, and a resulting loss in sensorimotor function. In mammals, a diverse set of non-neural factors, including meningeal fibroblasts, inhibit axon regrowth in part through the creation of a chronic fibrotic scar. In zebrafish, however, the fibrotic response appears to be transient and facilitates robust axon regrowth and sensorimotor recovery. While prior research in zebrafish shows that Wnt/β-Catenin signaling drives pro-regenerative functions in fibroblast-like cells, the precise identity of these cells and the Wnt-dependent genes that mediate their pro-regenerative functions remain unknown. My research shows meningeal fibroblasts respond to both SCI and Wnt-signaling. After SCI, fibroblasts (col1a1a+/fmoda+)

proliferate and accumulate at the injury site. In addition, SCI and wnt-signaling drive meningeal fibroblast expression of the homeodomain transcription factors prrx1a/b. Larval zebrafish lacking the prrx1a/b transcripts show deficits in axon regrowth and glial bridge formation. While the gene products of PRRX1 are known to promote regeneration across several tissues, this research is the first to associate PRRX1 with nervous system repair. Together, these data reveal key differences between the meningeal fibroblast response to mammalian vs fish SCI. Future work on this project will seek to further characterize the SCI- and Wnt-induced behavior of meningeal fibroblasts and determine the transcriptional targets of prrx1a/b. Results from these studies will provide valuable guiding information for the development of treatments for SCI including gene, stem cell, and matrisome-based therapies.

Enhancing Functional Recovery after Spinal Cord Injury in Larval Zebrafish with 4-Aminopyridine

Poster Number: 87

Theme: Regeneration

Presenting Author: **Natalie Clark, BS** - Brody School of Medicine, East Carolina University

Co-Author(s): Karen Mruk, PhD – Assistant Professor, Pharmacology & Toxicology, Brody School of Medicine, East Carolina University

Abstract: A complete spinal cord injury (SCI) in mammals is a devastating injury due to the loss of sensory and motor function. Zebrafish are pro-regenerative, allowing for axonal regeneration and functional recovery within the injury site. 4-Aminopyridine (4-AP) is a clinically used therapeutic that is indicated for use in MS patients. 4-AP inhibits voltage-gated potassium channels (Kv) allowing for increasing action potentials across demyelinated axons. Given the promise of this small molecule, our lab sought to investigate whether 4-AP could be a useful therapeutic for SCI using a larval SCI model. Using live imaging, we show that 4-AP can be dosed continuously in larval zebrafish at low doses but does exert some toxic effects at higher doses. In addition, 4-AP increases swim behavior after SCI in larval fish. Using transgenic lines, we show that this increase in recovery is due to enhanced cellular bridging across the site of injury. This data suggests that 4-AP is a promising new molecule for future SCI studies.

The Effects of Estrogen on Tail Fin Regeneration in Embryonic Zebrafish (Danio rerio)

Poster Number: 88

Theme: Regeneration

Presenting Author: **Alexander Skibinski** - Roanoke College

Co-Author(s): Jacob Barrett – Roanoke College; Lorenzo Camobreco – Roanoke College; Latavia Brooks – Roanoke College; Austin Parker – Roanoke College; Olivia Brichter – Roanoke College; Allyson Herriges – Roanoke College; Christopher Lassiter – Roanoke College

Abstract: 17-beta-estradiol (E2) is a common steroid hormone that plays a role in sexual development, metabolism, and regeneration. The effects of estrogenic compounds on the regeneration process in zebrafish has not been clearly described. To elucidate the effect of estrogen on regeneration, the caudal fin of zebrafish embryos will be amputated 3 days post fertilization and treated with three concentrations of E2. Exposure to E2 in concentrations as small as 100 nM resulted in significantly decreased regenerated tail length and area. Treatment with 1000 nM (1 uM) resulted in incomplete tail regeneration, with a significant decrease in the proportion of embryos possessing normal bi-lobed morphology. The inhibitory effect observed in this paper is of concern with large amounts of estrogen being displaced into the environment on a global scale. The expression of selected genes associated with regeneration signaling networks will be monitored using RT-qPCR. Future work will implement treatments of ICI, an estrogen receptor antagonist that disrupts the function of all estrogen in an organism. Funding provided by Roanoke College Biology Department, Summer Scholars, and Research Fellows programs.

Inflammatory molecules and microglia mediate retinal regeneration after acute or chronic damage of adult zebrafish

Poster Number: 89

Theme: Regeneration

Presenting Author: **Maria Iribarne** - University of Notre Dame

Co-Author(s): David HYDE – PI, University of Notre Dame

Abstract: The zebrafish retina possesses remarkable regeneration abilities, unlike mammals. Here, we evaluate how the immune system contributes to the regeneration process in the adult retina after acute or chronic damage. We employed three distinct injury models: (1) AB wild-type fish following NMDA-induced damage, (2) chronic cone photoreceptor-specific degeneration in the gold rush (gosh) mutant, and (3) rod degeneration in the Tg[XOPS:mCFP] transgenic line. The two chronic injury models were also subjected to an acute NMDA-induced damage. Fish were treated with dexamethasone to inhibit inflammatory cells or morpholino-mediated gene knockdown to inhibit specific inflammatory cytokines. Immunohistochemistry of cryosections and flat mount preparations were used to monitor the inflammatory and proliferative response. All retinal injuries increased reactive inflammatory cells. NMDA-treated wild-type retinas and the gosh mutant showed Müller glia proliferation, while Tg[XOPS:mCFP] retinas exhibited proliferating ONL cells but lacked Müller glia proliferation. Dexamethasone treatment reduced reactive inflammatory cells and INL proliferation, except in Tg[XOPS:mCFP] fish. Both the gosh mutant and Tg[XOPS:mCFP] responded to acute NMDA insult with increased inflammatory cells and Müller glia proliferation. Knockdown of tnfα, il1β, or il10 reduced Müller glia proliferation in NMDA-injured wild-type, gosh, and Tg[XOPS:mCFP] retinas, though il1β knockdown had minimal effect in the gosh mutant. Our findings show that acute retinal damage in chronic degeneration mutants triggers regeneration alongside an inflammatory response. This immune response is essential, as its inhibition or cytokine knockdown impairs Müller glia proliferation. Notably, inflammation levels and regenerative cell types differ between acute and chronic injuries, potentially explaining variations in Müller glia proliferation. Understanding these differences is key to developing strategies for mammalian tissue repair.

Ccn2a acts downstream of cx43 to influence joint formation during fin regeneration

Poster Number: 90

Theme: Regeneration

Presenting Author: **Victoria Hyland** - Lehigh University

Co-Author(s): M. Kathryn Iovine – Lehigh University

Abstract: Understanding how skeletal patterning is regulated during regeneration is crucial for uncovering mechanisms of joint formation. Connexin43 (Cx43) is known to influence this process by inhibiting evx1 expression and delaying joint formation. The identification of molecular players acting downstream of Cx43 could provide insights into how the differentiation of joint-forming cells is regulated. Here, we identify cellular communication network factor 2 (ccn2a) as a key player in this pathway. Our data suggest that ccn2a acts downstream of Cx43, similarly suppressing evx1 to regulate joint formation. Pharmacological inhibition of β-catenin indicates that ccn2a is likely under β-catenin control, while additional evidence points to Yap signaling as another regulatory input for ccn2a in joint formation. Given that CCN2 (the mammalian homolog of ccn2a) has been implicated in osteoarthritis progression by promoting extracellular matrix remodeling and chondrocyte differentiation1.This raises exciting questions about potential conserved roles for ccn2a in joint maintenance and disease. By studying ccn2a in zebrafish, we can gain new insights into the molecular mechanisms driving skeletal patterning that may ultimately inform our understanding of skeletal disorders.

Regenerative capacity of different lateral line progenitor cell types

Poster Number: 91

Theme: Regeneration

Presenting Author: **Jason Meyers, PhD** - Colgate University

Co-Author(s): Hadley Johnson – Colgate University; Benjy Schneider – Colgate University; David Maynard – Colgate University; Josie Ward – Colgate University; Eva Wiener – Colgate University

Abstract: In the mammalian inner ear, loss of the mechanosensory hair cells leads to permanent sensory deficit, as these cells do not regenerate. However, in other vertebrates, including fish, hair cells can be readily regenerated as the adjacent non-sensory supporting cells dedifferentiate, returning to the cell cycle. In addition to inner ear hair cells, fish have a mechanosensory lateral line system comprised of organs called neuromasts along the head and trunk. Each neuromast has a central cluster of hair cells and supporting cells surrounded by an outer layer of mantle cells, with a string of interneuromast cells between them. Although supporting cells give rise to replacement hair cells, mantle cells and interneuromast cells have been hypothesized to be quiescent progenitors that can contribute to regeneration following more significant damage. However, the mechanisms underlying this capacity have not been well studied. Utilizing a novel laser photoablation technique to make patterned lesions, we have examined the capacity of these different progenitor pools to give rise to replacement cells. Ablation of entire neuromasts, leaving only interneuromast cells leads to a complete lack of

regeneration, even when Erbb signaling is blocked with AG1478, suggesting that these cells do not have a significant regenerative capacity post-embryonically. Similarly, following ablation of all interneuromast cells, they are not regenerated. Upon ablation of support cells and hair cells, the mantle cells quickly regenerate replacement support cells and hair cells within 48 hours. This regeneration is dependent on Wnt and FGF signaling, with the mantle cells undergoing an epithelial-to-mesenchymal transition following damage and then re-entering the cell cycle. We also find that supporting cells readily regenerate mantle cells when they are lost, in a notch-dependent process. Together these data point to important pathways controlling multipotent progenitors that provide insight into methods for triggering regeneration of mammalian hair cells.

Reconstruction of the peripheral nervous system during fin regeneration

Poster Number: 92

Theme: Regeneration

Presenting Author: **Steffani Manna** - UW-Madison

Co-Author(s): Daniel Osorio-Méndez – UW-Madison; Anjelica Rodriguez-Parks – UW-Madison; Adib Mohd Azilan – UW-Madison; Junsu Kang – UW-Madison

Abstract: Appendage regeneration varies widely across species. While adult mammals are restricted to regenerating digit tips, teleost fish and urodele amphibians can regenerate fully amputated appendages. An essential event for successful appendage regeneration is early innervation of the wound area. Although the importance of nerve dependency in regeneration is well established, the mechanisms governing peripheral nervous system regeneration remain largely unexplored. Here, we utilize zebrafish fins, combined with transgenic animals and imaging analysis, to investigate how nerves regenerate during appendage regeneration. We first examined neuroanatomy of fin tissues and identified two distinct peripheral nerve types: epidermal and mesenchymal nerves. Following amputation, mesenchymal nerves broadly and extensively innervate the entire blastema cells. Notably, once the blastema forms, mesenchymal nerves align with osteoblast progenitors at the peripheral regions of blastema, leaving the central blastema devoid of neurons. We hypothesize that osteoblast progenitors and central blastema cells secrete axon attractants and repellents, respectively. Our transcriptomic analysis has determined candidate axon guidance molecules, which will be further evaluated using transgenic approaches and live imaging. Identifying these guidance molecules will provide critical insights into the mechanisms underlying peripheral nerve regeneration and its role in appendage regeneration.

Transcriptomics to identify the mechanisms that mediate axon regeneration

Poster Number: 93

Theme: Regeneration

Presenting Author: **Alexis Cramer** - Dartmouth Geisel School of Medicine

Co-Author(s): Lauren Walker, PhD – Principal Investigator, Department of Molecular and Systems Biology, Dartmouth Geisel School of Medicine

Abstract: Regenerating axons in the peripheral nervous system must extend over long distances to reconnect with their original synaptic targets for functional recovery. Re-establishing a complex trajectory and then selecting the appropriate target, long after this circuitry was established during development, represents a unique challenge to the axon and requires unknown cellular and molecular cues. We established the motor innervation of the larval zebrafish pectoral fin, equivalent to tetrapod forelimbs, as a vertebrate model system in which to study this process. Using a laser, we transect these motor nerves and find that regenerating axons regrow robustly and navigate to their original muscle domains with high fidelity that restores functionality. Here, we report recent work in establishing protocols to perform single-nuclei transcriptomics in pectoral fins after axon injury. We will leverage this genetically-tractable and highly-regenerative system to identify the cells and signals that mediate target-selective axon regeneration.

Projecting Trouble: miR-10 Loss Unravels The Healing Edge

Poster Number: 94

Theme: Regeneration

Presenting Author: **Jacquelyn Jacobs** - Northern Michigan University

Co-Author(s): Hosanna Brindle – Northern Michigan University; Autumn Jennings – Northern Michigan University; Jaelyn Kriegl – Northern Michigan University; Ahna Larson – Northern Michigan University; Danny LeBert – Northern Michigan University; Cameron Macklem – Northern Michigan University

Abstract: Wound healing is a complex regenerative process involving immune regulation, and wound projection formation. Prior studies from the laboratory has identified microRNA-10 (miR-10) as being overexpressed at the site of caudal-fin amputation in the larval zebrafish (Danio rerio). Here, we show that miR-10 expression is required for optimal wound healing outcome. Further, we investigate the cellular mechanisms underlying impaired wound healing following miR-10 knockdown, with a focus on the innate immune response, and wound healing projection formation. Transient morpholino suppression of miR-10 expression results in defective fin regeneration, a phenotype we successfully rescued via exogenous miR-10 oligonucleotide injection. Interestingly, a significant reduction in wound healing projections was also observed. Because of this, we sought to investigate a potential connection to Vimentin, which has been shown to regulate collagen remodeling within healing projections. Confocal imaging revealed a reduction in vimentin expression at the wound margin 20 hours post-injury in miR-10-deficient larvae, indicating that miR-10 may influence cytoskeletal remodeling and projection formation essential for tissue closure, though more validation is needed. Parallel analysis of innate immune cell dynamics showed a significant decrease in both neutrophil and macrophage recruitment to the wound site across multiple time points (1, 6, and 24 hours post-injury). Live tracking confirmed reduced recruitment in miR-10-deficient animals, but analysis of cell motility did not indicate significant defects in cell velocity to the wound, or movement once at the wound site. These data suggest that miR-10 contributes to effective wound repair through dual roles: regulating the innate immune response, and by promoting the formation of wound healing projections, potentially indirectly through the regulation of Vimentin. Together, these findings position miR-10 as a novel modulator of the regenerative microenvironment in larval zebrafish and a potential target for regenerative medicine strategies.

A meteoric power to repair: Investigating the role of meteorin and meteorin-like during spinal cord regeneration in zebrafish.

Poster Number: 95

Theme: Regeneration

Presenting Author: **Lillian Mearsheimer** - Smith College

Co-Author(s): Narendra Pathak, PhD – Smith College; Michael Barresi, PhD – Smith College

Abstract: Radial glia are the primary neural stem cells during embryonic development, with the potential to give rise to both neuronal and glial cells. Radial glia also serve as a scaffold for migrating progenitor cells and axons during neurogenesis and commissure formation. We have demonstrated that radial glia are essential for central nervous system (CNS) development in zebrafish. Radial glia are also influenced by a variety of signals that serve to control their proliferation and differentiation behaviors. Previous work in the Barresi Lab has focused on elucidating the role of the putative secreted proteins Meteorin (Metrn) and its paralog Meteorin-like (Metrnl) as radial glia regulators during embryonic development. Given the important role radial glia play during regeneration in zebrafish, we sought to determine whether metrn/metrnl may respond to spinal cord injury and support its regeneration. To test this, we transected the spinal cords of 2-day-old larval zebrafish and characterized the expression of metrn/metrnl 10 hours post-injury by hybridization chain reaction. Remarkably, metrnl was upregulated at the site of injury. We have optimized our spinal cord injury experimental design and are now seeking to determine the spatiotemporal and cell types that are expressing metrn/metrnl during regeneration. Furthermore, based on their expression domains and known regulators of regeneration, we are evaluating whether Metrn/Metrnl functions downstream of the Hedgehog signaling pathway. In support of this, we have observed downregulation of both metrn and metrnl following cyclopamine treatment during development and are now testing to see if this response similarly occurs during regeneration. This research holds the potential to reveal the importance of two novel secreted proteins in the regulation of radial glial development during embryogenesis and regeneration alike. This work was supported by the NSF (ABR 1656310) to MJBarresi and by Smith College (McKinley Fellowship).

Elucidating the role of serotonin receptors in larval zebrafish optic nerve regeneration

Poster Number: 96

Theme: Regeneration

Presenting Author: **Shrobona Guha, PhD** - University of Pennsylvania

Co-Author(s): Melissa Baxter – University of Pennsylvania; Kristian Santiago – University of Pennsylvania; Michael Granato – University of Pennsylvania

Abstract: Regeneration in most mammals is plagued by inhibitory factors, which have made it difficult to study pro-regenerative factors and systems. The larval zebrafish, however, has the capability to regenerate its central nervous system robustly. In this study, we employ the use of optic nerve transection assay to study regeneration in the larval zebrafish. In a recent small-molecule screen

performed in the lab, serotonin signaling regulators were identified to inhibit ON regeneration. Now, we have employed the use of CRISPR-Cas9 to genetically knock out the serotonin receptor HTR1B, one of the most highly expressed serotonin receptors, to better understand regeneration. In the mutant animals, the regenerating optic nerve has increased ectopic axonal growth after the optic chiasm. We also use the small molecule to study the effects of serotonin modulation in the absence of the receptor during regeneration. Our results show the importance of serotonin as an axonal guidance molecule in the process of optic nerve regeneration.

Investigation of the Cellular Dynamics and Molecular Drivers of Cutaneous Wound Healing in Zebrafish

Poster Number: 97

Theme: Regeneration

Presenting Author: **Leah Greenspan, Post-doctoral** - National Institutes of Health

Co-Author(s): Keith Ameyaw – National Institutes of Health; Daniel Castranova – National Institutes of Health; Gennady Margolin – National Institutes of Health; Caleb Mertus – National Institutes of Health; Van Pham – National Institutes of Health; Brant Weinstein – National Institutes of Health

Abstract: Two percent of the US population is plagued by open chronic wounds, with delayed vascular reperfusion being a major contributor to defects in wound closure. This delay often occurs in aged or diabetic adults, but the reasons for this remain unclear. Mammalian models have revealed cell types and signals important for wound healing, but the lack of high-resolution, real-time imaging of the healing process in these models makes it difficult to study transient events. Zebrafish are an ideal model for visualization and experimental dissection of cutaneous wound healing in a living animal, with many transgenic lines available that mark relevant cell populations and powerful methods for imaging these lines. We have established an innovative zebrafish cutaneous wound model using a rotary tool combined with cellular-level long-term confocal imaging of wounds in living adult fish. In younger animals, skin re-epithelialization and neutrophil recruitment initiate within hours after injury, peaking in one day, while macrophage activity and vessel regrowth increase between 1-4 days post injury, and vessel re-patterning takes many additional months. However, we find that these cellular dynamics seem to be altered in aging adult animals. We have also devised novel tools to profile the endothelial translatome using TRAP-RNAseq of “AngioTag” transgenic fish and have uncovered common endothelial signatures between adult organs and unique endothelial genes within the vasculature of each organ. We are now using these tools to explore changes in endothelial gene expression during the wound healing process. Together these studies will uncover the mechanisms that restore vascular networks after cutaneous injury, providing potential new therapeutic targets.

A clear vision: Elucidating in vivo cellular and molecular mechanisms guiding optic nerve regeneration

Poster Number: 98

Theme: Regeneration

Presenting Author: **Beth Harvey** - University of Pennsylvania Perelman School of Medicine

Co-Author(s): Melissa Baxter – University of Pennsylvania; Alexis Garcia – University of Pennsylvania; Michael Granato – University of Pennsylvania

Abstract: Injury to RGC axons of the optic nerve causes blindness due to the poor capacity of the mammalian CNS to regenerate. Studies have identified RGC intrinsic signaling pathways that improve RGC survival and increase long range axonal growth after injury. However, enhancing axonal growth by manipulating RGC intrinsic pathways results in aberrant regrowth before and at the optic chiasm during the initial stages of regeneration. Thus, there is a critical need to identify molecular and cellular mechanisms that guide RGC axons as they regrow during regeneration. We developed a novel optic nerve transection assay in the larval zebrafish with which we perform live cell imaging to observe axonal regeneration and cellular dynamics in vivo. Using this assay, we identified the glycosyltransferase Lh3 to be required during the process of regeneration to direct regrowing RGC axons toward the midline. Moreover, we find that mutants in collagen 18a1 (col18a1), a putative Lh3 substrate, display RGC axonal misguidance phenotypes similar to lh3 mutants, suggesting that Lh3 may act through Col18a1 during regeneration. Finally, we show that transgenic Lh3 expression in sox10+ presumptive olig2+ oligodendrocytes located near the optic chiasm restores directed axonal growth. Combined these data identify Lh3 and Col18a1 as part of a glial derived molecular pathway critical for guiding in vivo regenerating RGC axons toward and across the optic chiasm. Future experiments will further define the role of oligodendrocytes in directing RGC axon regeneration and determine the molecular mechanisms by which Col18a1, potentially acting through Wnt signaling, directs RGC axonal regeneration. Additional work will also define the regenerative capacities of the different RGC subclasses using backfilling and sparse labeling regenerating RGCs. These results will contribute impactful insight for the entire optic nerve regeneration field into previously understudied mechanisms that guide regrowing RGC axons during optic nerve regeneration.

Dissecting the neural stem cell state during zebrafish spinal cord regeneration

Poster Number: 99

Theme: Regeneration

Presenting Author: **Sarah Lusk, PhD** - Oregon Health & Science University

Co-Author(s): Bret Pearson – Oregon Health & Science University

Abstract: The regenerative capacity of the central nervous system (CNS) is severely limited in humans and other mammals, such that neural injury is often irreparable. To potentially overcome CNS damage, new neurons are ultimately made from stem cells, yet the genetic programs that stem cells use to regenerate lost neurons remain largely unknown. Using zebrafish, a powerful genetic model of vertebrate CNS regeneration, I seek to identify the factors that define the neural stem cells (NSCs) in the spinal cord and are activated in response to neural injury. We generated a single-cell RNA-sequencing atlas of spinal cord regeneration in larval zebrafish, capturing the first seven days post-injury. From this dataset, I subclustered neural lineages and identified injury-enriched neural cell clusters. I find that canonical stem cell markers define multiple cell populations, indicative of molecular heterogeneity in zebrafish spinal NSCs. Using multicolor lineage tracing and live imaging approaches, I can assay lineage decisions during spinal cord regeneration to determine if subpopulations of NSCs produce distinct, functional cell lineages. I have selected an initial candidate, musashi1, as a potential regenerative NSC

factor, and I am using novel transgenic approaches to probe its cellular identity and function as a stem-cell factor in spinal cord regeneration. The findings generated from this research will help elucidate the complicated biology of adult stem cell lineage development and, in turn, impact understanding of how the neural stem cell state can instruct proper neurogenesis following injury.

Wound Heal and Chill: A Comparative Study of Zebrafish and Burbot Wound Healing

Poster Number: 100

Theme: Regeneration

Presenting Author: **Hosanna Brindle** - Northern Michigan University

Co-Author(s): Chase Stahl – Northern Michigan University; Autumn Jennings – Northern Michigan University; Ahna Larson – Northern Michigan University; Ashlynn Muellenberg – Northern Michigan University; Haylee Vickers – Northern Michigan University

Abstract: Wound healing and regeneration are critical biological processes with profound implications for human health, particularly in reducing scar formation and restoring full tissue functionality. The zebrafish (Danio rerio) is a well-established model for studying epithelial wound closure, which occurs via a rapid, actomyosin-driven purse-string mechanism. However, the influence of cold environmental temperature on this regenerative process, in a living organism, remains underexplored. To investigate the impact of cold temperature on wound repair, we examined larval burbot (Lota lota), a cold-water species native to Lake Superior that thrives at 4°C and exhibits optical transparency during early developmental stages along with developmental patterning similar to larval zebrafish. We first examined the ability of the larval burbot to heal caudal-fin amputations within their environmental temperature range. We the used phalloidin staining to visualize filamentous actin, to provide the first direct evidence of purse-string wound closure in larval burbot. Remarkably, despite the drastic temperature difference, burbot exhibit wound closure rates comparable, though slightly slower, to larval zebrafish, suggesting the presence of highly efficient regenerative mechanisms even in frigid environments. This finding runs counter to in vitro data showing actin polymerization to slow dramatically at 4C. It also suggests the presence of a novel mechanism within the burbot that promotes actin polymerization in this environment, though more investigation is needed to validate. Furthermore, our preliminary data indicate that burbot may serve as an interesting comparative model to study not only epithelial repair but also temperature-sensitive modulation of the innate immune response following injury, as a massive inflammatory response in comparison to development matched zebrafish, as indicted by Sudan Black-b staining, occurs following amputation. Together, this work positions burbot as a novel complementary model to zebrafish for elucidating the molecular basis of cold-temperature regeneration and wound healing.

Tuft-like spike cells are robustly regenerated from the epidermal stem cells during zebrafish fin regeneration

Poster Number: 101

Theme: Regeneration

Presenting Author: **Siyang Cao** - University of Wisconsin - Madison

Co-Author(s): Margaret Keating – University of Colorado Anschutz Medical Campus; Julia O'Hagan – University of Wisconsin - Madison; Anjelica Rodriguez-Parks – University of Wisconsin - Madison; Tanner Robertson – University of Wisconsin - Madison; Yiran Hou – University of Wisconsin - Madison; Anna Huttenlocher – University of Wisconsin - Madison; Junsu Kang – University of Wisconsin - Madison

Abstract: Specialized epithelial cells play critical roles in tissue regeneration, sensory perception, and immune modulation. In the zebrafish caudal fin, we identified a previously undescribed epithelial cell population marked by avil expression. Our scRNA-seq analysis revealed that this avil+ cluster exhibit enrichment of tuft cell markers, indicating tuft-like identity. Using the avil reporter line, our imaging analysis demonstrated that these cells exhibit a unique morphology and spatial organization in the fin epithelium. Notably, avil+ cells are large, span multiple epithelial layers, and feature a distinct apical spike projection penetrating the superficial epithelial surface. These avil+ spike cells are robustly replenished following fin amputation. Daily time-lapse imaging analysis uncovered that they undergo morphological transitions through distinct forms, including spike, sphere, and elongated shapes, suggesting their dynamic features during regeneration. EdU pulse-chase experiment showed that avil^{+} spike cells arise from epithelial stem/progenitor population rather than through self-renewal. Furthermore, our scRNA-seq analysis and pharmacological assays identified the aldh1a3 signaling pathway as a potential regulator of spike cell regeneration. Together, our study discover novel, dynamically regulated tuft-like epithelial spike cells in zebrafish skin, providing an excellent model for imaging-based investigation of tuft cells biology.

Hedgehog Signaling in Zebrafish Liver Development and Regeneration

Poster Number: 102

Theme: Regeneration

Presenting Author: **Mingkai Zhu, PhD** - Massachusetts General Hospital

Co-Author(s): Wolfram Goessling – Massachusetts General Hospital

Abstract: The Hedgehog signaling pathway is known to be essential for organogenesis during embryonic development. In mouse livers, Hedgehog signaling regulates the proliferation and differentiation of liver progenitor cells (LPCs) during development. In addition, Hedgehog activation has been found in liver cells upon injury, and it was required for liver regeneration following partial hepatectomy. As for zebrafish, previous studies have shown that mutation in the Hedgehog signaling pathway can cause severe malformation in zebrafish embryos. However, conflicting results have been reported regarding whether Hedgehog deficiency impedes liver development in zebrafish. In the current study, we investigated the roles played by Hedgehog signaling in zebrafish liver development and LPC-mediated liver regeneration. By chemically altering the activity of Hedgehog signaling during embryonic development, we found that Hedgehog signaling inhibition promoted liver growth in zebrafish larvae. The increased liver volume in the larvae was associated with increased liver cell proliferation. By contrast, Hedgehog activation was required for LPC-mediated liver regeneration following metronidazole (mtz)-mediated hepatocyte ablation. We found that Hedgehog signaling was activated during liver regeneration based on the expression of the fluorescent reporter and the downstream target genes. Furthermore, inhibiting Hedgehog signaling following hepatocyte ablation attenuated liver

regeneration, whereas promoting Hedgehog activation facilitated it. Together, these results provide new insights into the potentially different roles played by the Hedgehog signaling pathway between liver development and liver regeneration.

Interrogating signaling and growth dynamics in regenerating zebrafish scales

Poster Number: 103

Theme: Regeneration

Presenting Author: **Sushant Bangru** - Morgridge Institute for Research

Co-Author(s): Lucas Garcia – Duke University; Kazunori Ando – Morgridge Institute for Research; Rebecca Myatt – Morgridge Institute for Research; Pierre Gillotay – Morgridge Institute for Research; Stefano Di Talia – Duke University; Kenneth Poss – Morgridge Institute for Research

Abstract: Bone regeneration in mammals is primarily limited to fracture repair and digit tip regeneration, a process driven by osteoblasts transiently re-entering the cell cycle to rebuild damaged tissue. However, severe injuries such as limb amputations exceed mammalian regenerative capabilities, due in part to restricted plasticity of adult osteoblasts and an absence of sustained developmental signaling. In contrast, zebrafish can regenerate complex skeletal structures, including fins, jaws, and dermal scales. Zebrafish scales—flat, disc-shaped dermal bones—rapidly regenerate within 2 weeks of removal, restoring size, shape, and position. Their optical transparency, genetic tractability, and regenerative efficiency make scales a powerful model of regeneration. Tissue regeneration involves precise spatial and temporal coordination of extracellular signals and intracellular gene regulation across substantial distances. Recent work has revealed traveling waves of ERK activity that propagate through regenerating scale osteoblasts to spatiotemporally control growth. Here, we imaged ERK wave propagation in zebrafish mutants exhibiting altered scale size and geometry. Our analysis revealed specific correlations between ERK wave parameters and scale size and geometry. We also generated genetic tools for spatially restricted modulation of ERK signaling, to test effects of local ERK activation. By dissecting mechanisms of ERK wave propagation in regenerating zebrafish scales, we expect to derive new concepts by which chemical waves control tissue regeneration.

Regulation of Retinal Regeneration by the Extracellular Matrix Protein Adam22

Poster Number: 104

Theme: Regeneration

Presenting Author: **Dmitri Serjanov** - University of Notre Dame

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Abstract: Over a billion people are affected by vision loss or blindness worldwide. Unfortunately, such vision loss in humans is often irreversible and progressive due to the limited regenerative capacity of the human retina. Interestingly, the zebrafish retina, which shares anatomic and molecular similarities with human, possesses a remarkable regenerative capacity, making it an attractive model to study the process of regeneration. In response to retinal injury, Müller Glia (MG) residing in the Inner Nuclear Layer (INL) of both human and zebrafish retinas enter a gliotic state. While human MG eventually form a

gliotic scar which prevents regeneration, zebrafish MG can reprogram to exit the gliotic stage and re-enter the cell cycle. Consequently, reprogrammed zebrafish MG divide asymmetrically to produce another MG as well as a neuronal progenitor cell (NPC). The NPCs continue to proliferate and eventually regenerate all lost cell types, as well as vision. This study aims to investigate the role of the extracellular matrix (ECM) in the process of retinal regeneration, focusing on the role of proteins regulating the molecular signaling between the ECM and MG. Specifically, we focused on a member of the disintegrin and metalloprotease (Adam) family, Adam22. Using a morpholino-mediated gene knockdown of Adam22, we demonstrated that it plays a role in regulating MG proliferation, as demonstrated by an accelerated rate of MG cell cycle entry in adam22 morphants. This observation was confirmed via an EdU/BrdU pulse/chase approach, which also suggested an accelerated cell cycle progression in the adam22 morphants. Furthermore, qRT-PCR assay suggested integrin a3b-containing cell surface receptor as the potential partner the function of which Adam22 mediates. Indeed, itga3b morphant retinas appear to phenocopy adam22 morphants. Together, our findings suggest that Adam22 plays an inhibitory role in MG cell cycle entry in the early phases of light-damage-induced retinal damage and regeneration.

Cranial tenocyte diversity in zebrafish and roles for Wnt signaling in embryonic tendon patterning

Poster Number: 105

Theme: Other

Presenting Author: **Thomas Schilling** - University of California, Irvine

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Abstract: Structural requirements generate functional diversity in the same cell types exposed to different environmental cues during development. Tenocytes that generate the extracellular matrix (ECM) of tendons and ligaments form and adapt to mechanical forces depending on the structural demands of the muscles and bones to which they attach. Everyone deals with injuries to these tissues as well as loss of tendon strength with age, yet very little is known about the genetic mechanisms that control their development or maintenance. Transcription factors such as Scleraxis (Scx) and Sox9 directly regulate expression of collagens and other ECM components to achieve the appropriate tendon stiffness or softness depending on force demands. Here we investigate heterogeneity in developing tenocytes and how signals from surrounding cells and ECM regulates their underlying transcriptional signatures. Using scRNA-seq with purified populations of embryonic zebrafish cranial tenocytes we show that they come in different transcriptional flavors depending on tendon type (load-bearing versus soft) as well as their locations within individual tendons (entheses versus myotendinous junction). We also show that canonical Wnt signaling plays a role in these processes specifically promoting an MTJ transcriptional signature. In situ analyses confirm that many of the genes identified mark functionally distinct subsets of tendons or ligaments, in temporal and spatial patterns that correlate with their responses to mechanical forces of muscle contraction.

Swimming against the rapids – Overcoming challenges in establishing a zebrafish research facility in the Philippines

Poster Number: 106

Theme: Other

Presenting Author: **Olivia Erin Buenafe, PhD in Biomedical Sciences (Pharma)** - Ateneo de Manila University

Co-Author(s):

Abstract: Teaching and performing STEM-related research in the Philippines present a unique set of opportunities for growth and frustration for returning Filipino scientists who trained overseas, including those who wish to valorize their doctoral research work and establish new, emerging technologies to which they had been exposed during their years outside the country. This is especially true for zebrafish research scientists who return home and attempt to start their own stable on-going zebrafish facility for continuing their research and train local undergraduate and graduate students in the field. Navigating through labyrinthine government regulations and the highly-competitive local research grant calls by national government agencies limit the extent of what type and level of complexity of zebrafish research can be done. We illustrate how these challenges shaped the kind of research work done using larval zebrafish, from performing toxicological and drug-discovery type assays using selected Philippine ethnomedicinal plants, to establishing a nanotoxicity screening process for locally-synthesized nanomaterials, to exploring the emerging field of toxicometabolomics.