5 - Neuronal Spock1 Induces Blood-Brain Barrier Functional Development

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The brain requires a tightly controlled homeostatic environment for proper neuronal function, and this is achieved via the blood-brain barrier (BBB). The BBB is made up by the vascular endothelial cells in the brain with uniquely restrictive properties, including tight junctions and reduced transcytosis, that are induced during embryonic development. During the course of our previous studies investigating the developmental timeline of the zebrafish BBB, we uncovered a spontaneous mutant with regional barrier leakage in the forebrain and midbrain. We mapped this leaky mutant to the neuronally secreted proteoglycan Spock1 and demonstrated that the spock1 mutants display increased gelatinase activity specifically in the leaky brain regions. Using cell transplantation, we determined that Spock1 controls vascular properties within a 10-20 um range. To determine the subcellular mechanism underlying the mutant leakage, we performed electron microscopy analyses and observed an increase in clathrin-independent transcytosis in mutant vessels and a decreased vascular basement membrane. To further resolve how a neuronal signal regulates the BBB, we turned to scRNA-sequencing of larval mutant and wild type brains. Surprisingly, the only cells with a differenetial gene expression profile in the mutant brains were the vascular endothelial cells and their support cells, pericytes. These analyses revealed a decrease in vascular expression of the cell adhesion molecule mcamb (CD146), which is known to be required for BBB function, specifically in the leaky regions. Strikingly, a single exogenous dose of recombinant human SPOCK1 decreased mutant leakage and gelatinase activity throughout the brain while restoring vascular expression of mcamb. Finally, we demonstrated that Spock1 plays a conserved role in inducing BBB functional development, as embryonic Spock1 knockout mice also have increased BBB leakage. Taken together, we have identified the first neuronal signal that regulates the brain extracellular environment to induce BBB functional differentiation.

6 - Heading towards an in vivo predictive test for personalized ovarian cancer treatment: application of novel therapies in zebrafish patient derived xenografts

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Generally ovarian cancer is diagnosed at an advanced stage, resulting in a poor prognosis. Standard chemotherapy is applied to all epithelial ovarian cancers, but specific subtypes do not respond well and are often excluded from clinical trials. To improve treatment, an *in vivo* predictive test for treatment response is warranted. Very promising are zebrafish patient derived xenografts (zPDX) platforms which are fast and cost-effective.

The aim was to optimize xenograft protocols using cell lines and to compare responses *in vivo*. Fluorescently labeled tumor cells are injected into the perivitelline space of 2dpf embryos. At 1dpi xenografts are scored for presence of tumor cells and tumor size. Then they are randomly distributed into treatment groups and followed for 5 days. Subsequently xenografts are euthanized and stained for apoptosis and proliferation markers to score tumor responses by confocal microscopy on single cell level.

We focused on M28/2, a low-grade ovarian cancer cell line with a *KRAS* variant and sensitive to MEK-inhibitor trametinib (De Thaye et al.). We could observe compact tumor masses *in vivo* and Ki67 staining showed clear proliferation. Upon treatment with trametinib higher caspase activity and pyknotic nuclei were observed, providing evidence that the same sensitivity is obtained as *in vitro* and mouse PDX. Our first results show that therapy responses in zebrafish engraftment experiments correspond to known sensitivities of the cell lines observed in *in vitro* assays and *in vivo* in mouse model. These protocols will now be used to establish zebrafish PDX models from ovarian cancer tumors. Conventional treatments will be applied to see if tumor response in zPDX is similar as in the patients, whether there is resistance or sensitivity. In parallel we will use targeted treatments based on the molecular background of the tumors, aiming for improved responses, and thus a better outcome for the patients.

7 - A tapt1 knockout zebrafish line with aberrant lens development and impaired vision models human pediatric cataract

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Mutations in the gene coding for human trans-membrane anterior-posterior transformation protein 1 (*TAPT1*) were previously reported to cause a complex lethal Osteochondrodysplasia characterized by undermineralization of the skeleton, several fractures and congenital anomalies. In another report, a *TAPT1* mutation was identified in a patient with pediatric cataract (posterior lenticonus cataract), although without any evidence of skeletal involvement. This indicates a broad phenotypic spectrum for *TAPT1* mutations, where pleiotropic and severity differences most likely depend on the type of mutation.

In this study, we report a patient with Osteogenesis imperfecta (osteopenia, multiple fractures, bowing of long bones), several dysmorphic features and bilateral cataract. The patient carries a homozygous 2-bp deletion (c.185_186del, p.(Arg62ProfsTer15)) in exon 1 of the *TAPT1* gene, resulting in the first frameshift mutation reported to date. To gain insights into the pathogenetic mechanisms caused by loss of *TAPT1* and to investigate the resulting phenotypic spectrum, a CRISPR/Cas9 knock-out (KO) zebrafish model was created. The zebrafish *tapt1a*$^{-/-}$;*tapt1b*$^{-/-}$ mutant has an aberrant eye phenotype, with a small and fibrotic lens, dysregulated retinal layers and severely impaired vision. A marked increase in pigmentation of the eye and skin was noted. Zebrafish KO mutants showed a major increase in the locomotor activity during light-dark transmission in a visual motor response test (VMR). The cartilaginous and mineralised structures did not show any differences between mutants and wild type siblings. Finally, RNAseq analysis revealed a significant downregulation of crystallin gene expression and the phototransduction pathway, and increased inflammation and extracellular matrix production, corresponding to the observed lens abnormalities.

In conclusion, our study reports on the first patient and corresponding zebrafish model with complete loss of *TAPT1*. Morphological and functional phenotyping showed that this zebrafish model recapitulates human pediatric cataract, while transcriptomic analysis revealed the underlying pathogenetic mechanisms induced by loss of *TAPT1* gene.

8 - The establishment of the first reported zebrafish model for thoracic aortic dissection and rupture

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About 0,16 percent of the Western population suffers from thoracic aortic aneurysms and dissections (TAAD). Weakening of the vessel wall of the thoracic aorta increases the risk for aortic dissection and rupture, which associates with a high mortality rate. Current treatment options in TAAD are limited to a pharmacological reduction of hemodynamic stress and surgical repair at a critical diameter. Despite the availability of different mouse models for TAAD, the underlying molecular mechanisms remain elusive. We therefore developed a zebrafish model for aortic dissection/rupture. For this purpose, we targeted 2 genes involved in angiogenesis, *SMAD3* and *SMAD6*. In humans, loss of function (LOF) of *SMAD3* results in TAAD, arterial tortuosity and early onset osteoarthritis. *SMAD6* LOF mutations increase the risk for a bicuspid aortic valve and TAAD. In zebrafish, both *SMAD3* and *SMAD6* have 2 paralogues. Using CRISPR/Cas9 gene editing technology, we developed a quadruple knockout (KO): *smad3a*$^{-/-}$;*smad3b*$^{-/-}$;*smad6a*$^{-/-}$;*smad6b*$^{-/-}$. At 5 days post fertilization, quadruple KO embryos showed asymmetrical branching of the aortic arches. Survival of adult quadruple KO zebrafish was severely decreased and all quadruple mutants died before the age of one year. A stress-inducing protocol caused sudden death in 60% of the mutant zebrafish. Histochemical investigation of consecutive sections of the ventral aorta in quadruple mutants stained for elastin showed medial elastolysis, intramural hematomas, aortic dissections and ruptures, which was further supported by 3D reconstructions. These observations indicate that we successfully developed the first ever reported zebrafish model for aortic dissection/rupture. This model will be highly valuable to better understand the pathogenic processes underlying TAAD and to evaluate potential therapeutic compounds.

9 - Teneurin trans-axonal signaling prunes topographically missorted axons.

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Building precise neural circuits necessitates the elimination of axonal projections that have inaccurately formed during development. Although axonal pruning is a highly selective process, how it is initiated and controlled in vivo remains largely unknown. Here, we show that trans-axonal signaling mediated by the cell surface molecules Glypican-3, Teneurin-3 and Latrophilin-3 prunes misrouted retinal axons in the visual system. Retinotopic neuron transplantations revealed that pioneer ventral retinal axons that elongate first along the optic tract instruct the pruning of dorsal axons that missort in that region. Glypican-3 and Teneurin-3 are both selectively expressed by ventral retinal ganglion cells and cooperate for correcting missorted dorsal axons. The adhesion G-protein coupled receptor Latrophilin-3 also participates in the elimination of topographic sorting errors. Altogether, our findings reveal an unsuspected function for Glypican-3, Teneurin-3 and Latrophilin-3 in topographic tract organization and demonstrate that axonal pruning can be initiated by signaling among axons themselves.

10 - Crispant screening in zebrafish as a promising approach for rapid functional screening of osteoporosis candidate genes and known genes for Osteogenesis Imperfecta.

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Background/introduction: Genome-wide association studies (GWAS) have improved our understanding of the genetic architecture of common complex diseases such as osteoporosis. Nevertheless, to attribute functional skeletal contributions of candidate genes to osteoporosis-related traits, there is a need for efficient and cost-effective *in vivo* functional testing.

Purpose/Methods: We aimed to conduct a pilot-study (ECD19/09) for rapid *in vivo* functional validation of candidate genes for skeletal disorders, using the zebrafish model system. We used a so-called crispant screening approach, based on CRISPR/Cas9 technology, in order to phenotype directly in first-generation (FO) mosaic founder zebrafish (crispants). The short generation time of crispants (<3 months) enables the screening of a large set of genes in a short period of time. Recently we showed that crispants for the osteoporosis gene *lrp5* phenotypically and molecularly resemble knockouts.

Results: We selected a panel of 10 genes, consisting of candidate genes for osteoporosis and known genes for the rare skeletal disorder osteogenesis imperfecta. CRISPR/Cas9 components targeting the gene of interest, were micro-injected in zebrafish embryos with an osteoblast-specific Tg(osx:Kaede) transgenic background. NGS amplicon sequencing revealed out-of-frame efficiencies higher than 70%, indicating a high fraction of knock-out alleles for the 10 genes and thus resembling a stable knock-out model. Skeletal phenotyping was performed through fluorescence microscopy, alizarin red bone staining and micro-CT analysis. Crispants for *creb3l1* and *sost* show skeletal abnormalities, such as vertebral fusions, callus formation in the vertebral arches and ectopic mineralization at 90 dpf. These investigations are also ongoing for the remaining genes.

Conclusion: Taken together, we showed that crispant screening in zebrafish is a promising approach for rapid functional screening of candidate genes for skeletal diseases. Moreover, the crispants have the potential to provide new insights into the role of these genes in skeletal biology and can be used as a tool for osteogenic compound screening.

11 - JC-10 probe as a novel and sensitive method for investigating cell stress and mitochondrial membrane potential in zebrafish embryos

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Background: A sensitive method to investigate cellular stress and cytotoxicity is based on measuring mitochondrial membrane potential. Recently, JC-10, was developed to measure mitochondrial membrane potential *in vitro* and used as an indicator for cytotoxicity. Yet, JC-10 has never been used *in vivo* (whole organism). In normal cells, JC-10 concentrates in the mitochondrial matrix, where it forms red fluorescent aggregates. However, in apoptotic/necrotic cells, JC-10 diffuses out of the mitochondria, changes to monomeric form, and stains cells in green. Here, we aimed to develop and optimize a JC-10 assay to measure cytotoxicity in zebrafish embryo. We also investigated the effectiveness of JC-10 assay by comparing it to common cytotoxicity assays. **Methods:** Zebrafish embryos were exposed to a toxic surfactant AEO-7 at no observed effect concentration (6.4 µg/L), and then cytotoxicity was measured using (i) JC-10 mitochondrial assay, (ii) acridine orange (AO), (iii) TUNEL assay, and (iv) measuring the level of Hsp70 by western blotting. **Results:** As compared to the negative control, embryos treated with NOEC of AEO-7 did not show significant cytotoxicity when assessed by AO, TUNEL or western blotting. However, when JC-10 was used under the same experimental conditions, a significant increase of green:red fluorescent ratio signal was detected in the AEO-7 treated embryos, indicating mitochondrial damage and cellular cytotoxicity. Noteworthy, the observed green: red ratio increase was dose dependent, suggesting specificity of the JC-10 assay. **Conclusion:** JC-10 is a sensitive *in vivo* method, thus, can be used as surrogate assay to measure cytotoxicity in whole zebrafish embryos.

12 - Spatial transcriptomics reveals a novel role for cilia at the tumor-microenvironment interface

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As tumors grow, they interact with different cells and tissues neighboring the tumor, but it is unclear how these interactions influence tumor cell behavior and progression. To investigate this, we applied spatial transcriptomics, single-cell RNA-seq, and single-nucleus RNA-seq to a whole-animal zebrafish model of melanoma. Using spatial transcriptomics, we identified a unique “interface” cell state localized to where the tumor contacts neighboring tissues. We used single-cell and single-nucleus RNA-seq to find that the interface is composed of specialized tumor and microenvironment cells that upregulate a common set of cilia genes, and used in vivo confocal microscopy to show that cilia proteins are enriched specifically where tumors contact other tissues. These results indicate that physical interactions between tumor and non-tumor cells cause both cell types to adopt a unique cilia-enriched cell state, suggesting a novel role for cilia in mediating cell-cell interactions during invasion. We further discovered that cilia gene expression is regulated by ETS-family transcription factors, which normally act to suppress cilia genes outside of the interface. We also found evidence of a cilia-enriched interface in human patient samples, suggesting it is a conserved feature of human melanoma. Indeed, using human cells we found that cilia are required for melanoma invasion *in vitro*. In addition to investigating the mechanism by which cilia promote tumor invasion, we are currently studying how the interface cell state is established, and have uncovered a potential role for the chromatin modifier HMGB2 as a master regulator of the interface cell identity. Together, our results reveal a novel role for cilia at the tumor boundary, and demonstrate the power of spatially resolved transcriptomics in uncovering the biology underlying cell-cell interactions *in vivo*.

13 - A FRET-Based Zebrafish Model for Visualization of Natural Killer Cell-Mediated Killing of Cancer Cells

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Tumor immunotherapy has been an important advancement in cancer treatment in recent years. Compared with T cell-based therapy, natural killer (NK) cell-based therapy does not require human leukocyte antigen matching and has fewer side effects; thus, NK cell therapy has gradually attracted the attention of researchers and clinicians. Reliable and effective animal models are essential for evaluating the effects of NK cell therapy. NK cells kill cancer cells mainly through apoptosis. In this study, we established a fluorescence resonance energy transfer (FRET)-based zebrafish tumor model for the real-time visualization of the killing effects of NK cells at single-cell resolution. In our model, cancer cells changed from green to blue when undergoing apoptosis induced by NK cells. This FRET-based zebrafish tumor model can serve as a powerful *in vivo* tool that can facilitate the development of NK cell-based therapy. More importantly, cancer cells from cancer patients can be labeled with our apoptotic biosensor and then transplanted into zebrafish to evaluate the sensitivity of the cancer cells to NK cells to help clinicians make treatment plans that can benefit patients.

14 - Generation of a relevant zebrafish model for Marfan Syndrome.

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Background: Marfan syndrome (MFS) is a pleiotropic connective tissue diosorder, mostly caused by defects in the Fibrillin-1 gene (*FBN1*). The most life-threatening complication of MFS patients is their high predisposition to develop aneurysms and dissections of the ascending aorta. Although the prognosis of MFS patients has improved in recent years, there is still a lack of disease specific treatments besides surgery which is attributable to an insufficient comprehension of the underlying mechanisms.

Methods: Therefore, we aimed to generate a zebrafish model to study the mechanisms relating fibrillin defects to the cardiovascular system. The CRISPR/Cas9 system was used to induce indel mutations in the 3 fibrillin genes in Tg(kdrl:GFP) zebrafish (fbn1, fbn2a and fbn2b) to visualize the inner layer of the cardiovascular system.

Results: We found that zebrafish lacking fbn1 and/or fbn2a do not show any cardiovascular phenotype during development, besides a mild dilated cardiomyopathy in adult homozygous fbn1 mutant zebrafish. Interestingly, approximately 50% of homozygous fbn2b mutant (fbn2b-/-) zebrafish embryo's show endocardial detachment, vascular embolism and premature mortality at 7-9dpf. Interestingly, the remaining fbn2b-/- zebrafish survive, but during larval stages develop a dilation of the bulbus arteriosus. This structure is considered similar to the aortic root in humans, which is the predominant location of aneurysm formation in MFS. In addition, the caudal vein of all fbn2b-/- embryos develops abnormally as a cavernous structure lacking vessel integrity. This phenotype resolves in embryos retaining normal blood flow. We found that pharmacological inhibition of blood flow led to a more severe caudal vein phenotype in fbn2b-/- embryos than in wild-type controls. These data indicate that fbn2b-/- zebrafish can be a relevant model to explore the mechanisms leading from fibrillin deficiency to the cardiovascular symptoms observed in MFS. Our ongoing work suggests that there is an interplay between fibrillin deficiency and biomechanical signaling.

15 - Hemato-vascular specification requires arnt1 and arnt2 genes in zebrafish embryos

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During embryonic development, a subset of cells in the mesoderm germ layer are specified as hemato-vascular progenitor cells, which then differentiate into endothelial cells and hematopoietic stem and progenitor cells. In zebrafish, the transcription factor *npas4l*, also known as *cloche*, is required for the specification of hemato-vascular progenitor cells. However, it is unclear if *npas4l* is the sole factor at the top of the hemato-vascular specification cascade. Here we show that *arnt1* and *arnt2* genes are required for hemato-vascular specification. We found that *arnt1;arnt2* double homozygous mutant zebrafish embryos (herein called *arnt1/2* mutants), but not *arnt1* or *arnt2* single mutants, lack blood cells and most vascular endothelial cells. *arnt1/2* mutants have reduced or absent expression of *etv2* and *tal1*, the earliest known endothelial and hematopoietic transcription factor genes. *npas4l* and *arnt* genes are PAS domain-containing bHLH transcription factors that function as dimers. We found that Npas4l binds both Arnt1 and Arnt2 proteins *in vitro*, consistent with the idea that PAS domain-containing bHLH transcription factors act in a multimeric complex to regulate gene expression. Our results demonstrate that *npas4l*, *arnt1* and *arnt2* act together as master regulators of endothelial and hematopoietic cell fate. Our results also demonstrate that *arnt1* and *arnt2* act redundantly in a transcriptional complex containing *npas4l*, but do not act redundantly when interacting with another PAS domain-containing bHLH transcription factor, the aryl hydrocarbon receptor. Altogether, our data enhance our understanding of hemato-vascular specification and the function of PAS domain-containing bHLH transcription factors.

16 - Activity dependent modulation of adult spinal neurogenesis

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Physical exercise dynamically modulates proliferation and neurogenesis in the adult zebrafish spinal cord. Here, we demonstrate a mutually antagonistic interplay between cholinergic and GABAergic neurotransmission in modulating neural stem/progenitor cells (NSPCs) activity. We also show that locomotor V2a interneurons play a critical role in providing direct cholinergic synaptic input on NSPCs. On the contrary, we found that GABA acts in a non-synaptic fashion, maintaining the NSPCs quiescent. Successful activation of the spinal NSPCs requires increased cholinergic neurotransmission and reduction of the $GABA_A$ receptors. Pharmacological manipulation of the NSPC cholinergic and GABAergic receptors after the injury, bolsters the spinal cord neurogenesis, regeneration, and functional recovery. Collectively, our data provide an entry model for locomotor networks' activity-dependent neurogenesis during homeostasis and regeneration in the adult zebrafish spinal cord.

17 - Zebrafish fin regeneration involves generic and regeneration-specific responses of osteoblasts to trauma

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Successful regeneration requires the coordinated execution of multiple cellular responses to injury. In amputated zebrafish fins, mature osteoblasts dedifferentiate, migrate towards the injury and form proliferative osteogenic blastema cells. We show that osteoblast migration is preceded by cell elongation and alignment along the proximodistal axis, which require actomyosin, but not microtubule turnover. Surprisingly, osteoblast dedifferentiation and migration can be uncoupled. Using pharmacological and genetic interventions, we found that NF-kB and retinoic acid signalling regulate dedifferentiation without affecting migration, while the complement system and actomyosin dynamics are required for migration but not dedifferentiation. Furthermore, by removing bone at two locations within a fin ray, we established a trauma model containing two injury sites. We found that osteoblasts dedifferentiate at and migrate towards both sites, while accumulation of osteogenic progenitor cells and regenerative bone formation only occur at the distal-facing injury. Together, these data indicate that osteoblast dedifferentiation and migration represent generic injury responses that are differentially regulated and can occur independently of each other and of regenerative growth. Successful bone regeneration appears to involve the coordinated execution of generic and regeneration-specific responses of osteoblast to trauma.

18 - Elucidating the mechanisms controlling the development and regeneration of endothelial and vascular mural cells in zebrafish fin blood vessels

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Vascular networks are comprised of endothelial cells and mural cells, which include pericytes and smooth muscle cells. We report here on the development and regeneration of these three cell types in the zebrafish fin, with an emphasis on the mural cell populations. Mural cells colonizing arteries proximal to the body wrapped around them, while those in more distal regions extended protrusions along the proximo-distal vascular axis. Both cell populations expressed platelet-derived growth factor receptor beta (pdgfrb) and the smooth muscle cell marker myosin heavy chain 11a (myh11a). Most wrapping cells in proximal locations additionally expressed acta2. Loss of Pdgfrb signalling specifically decreased mural cell numbers at the vascular front. Using lineage tracing, we demonstrate that precursor cells located in periarterial regions and expressing Pgdfrb can give rise to mural cells. Studying tissue regeneration, we did not find evidence that newly formed mural cells were derived from pre-existing ones. Together, our findings reveal conserved roles for Pdgfrb signalling in development and regeneration and suggest a limited capacity of mural cells to self-renew or contribute to other cell types during tissue regeneration.

19 - The Effects of Estrogen on Tail Fin Regeneration in Embryonic Zebrafish (Danio rerio)

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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.

20 - Transcriptional control of pretectal neural circuit development and function by GS homeobox 1

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Transcription factors are regionally expressed during neurodevelopment to regulate initial cell identity, migration, and connectivity. Appropriate assembly of neural circuits is imperative for normal brain function, and these processes can be readily studied in the zebrafish visual system. Retinal ganglion cell (RGC) axons project from the eye and terminate in ten arborization fields (AFs) in the optic tectum (TeO) and pretectum (Pr). Pretectal AFs 1-9 mediate distinct behaviors, yet mechanisms for RGC axon synaptogenesis in the Pr remain to be as fully explored as they have been in AF10 in the TeO. *GS homeobox 1* (*gsx1*) is expressed in the Pr and TeO in zebrafish and in superior colliculus precursors in mice. Given its expression, we sought to determine if *gsx1* plays an important role in the differentiation of visual neural circuits. Using transgenic lines, immunohistochemistry, and imaging, we observed that *gsx1* mutants lack vesicular glutamate transporter, *vglut2a*, expression but have the same number of neurons as wildtypes in the Pr. In addition, *gsx1* mutants have disrupted AF formation, including loss of AF7. Prey capture was significantly decreased in *gsx1* mutants, consistent with known AF7 function. *gsx1* mutants have normal optic nerve and optic chiasm formation and maintain proper RGC layer retinal morphology. Thus, we concluded that *gsx1* affects vision downstream of the eye and forebrain. In wildtypes, AF7 is surrounded by *vglut2a*-positive neurons, and laser ablation of these neurons early in development results in failure to form AF7 by later larval stages. These results strongly suggest that AF7 termination is dependent on glutamatergic neuron activity or specific cues produced by Pr *vglut2a*-positive neurons. This work has led us to identify for the first time multiple roles for Gsx1 in the development and function of visual neural circuits and elucidates novel, testable cellular mechanisms contributing to RGC axon synaptogenesis.

21 - Chemokine-biased robust self-organizing polarization of migrating cells in vivo

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To study the mechanisms controlling front-rear polarity in migrating cells, we used zebrafish primordial germ cells (PGCs) as an *in vivo* model. We find that polarity of bleb-driven migrating cells can be initiated at the cell front, as manifested by actin accumulation at the future leading edge and myosin-dependent retrograde actin flow toward the other side of the cell. In such cases, the definition of the cell front, from which bleb-inhibiting proteins such as Ezrin are depleted, precedes the establishment of the cell rear, where those proteins accumulate. Conversely, following cell division, the accumulation of Ezrin at the cleavage plane is the first sign for cell polarity and this aspect of the cell becomes the cell back. Together, the antagonistic interactions between the cell front and back lead to a robust polarization of the cell. Furthermore, we show that chemokine signaling can bias the establishment of the front-rear axis of the cell, thereby guiding the migrating cells toward sites of higher levels of the attractant. We compare these results to a theoretical model according to which a critical value of actin treadmilling flow can initiate a positive feedback loop that leads to the generation of the front-rear axis and to stable cell polarization. Together, our in vivo findings and the mathematical model, provide an explanation for the observed nonoriented migration of primordial germ cells in the absence of the guidance cue, as well as for the directed migration toward the region where the gonad develops.

22 - A robust and tunable system for targeted cell ablation in developing embryos

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Cell ablation is a key method in developmental biology, tissue regeneration, and tissue homeostasis research fields. Eliminating specific cell populations allows for characterizing interactions that control cell differentiation, death, behavior, and spatial organization of cells. However, current methodologies for inducing cell death suffer from relatively slow kinetics, making them unsuitable for analyzing rapid events and following primary and immediate consequences of the ablation. To address this, we present a cell ablation system that is based on bacterial toxin/anti-toxin proteins and enables rapid and cell-autonomous elimination of specific cell types in live zebrafish embryos. We compare this method with the available alternatives and demonstrate its function in developing organs. A unique feature of this system is that it uses an antitoxin, which allows for controlling the degree and timing of ablation and the resulting phenotypes. The transgenic fish generated in this work represent an extremely convenient and robust tool for ablating of various cells types and tissues in live zebrafish, and this general approach is applicable to other model organisms as

demonstrated for *Drosophila*

23 - Examination of hydrogen gas (H2) and electromagnetic field (EMF) treatment on growth and tissue regeneration in zebrafish embryos

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While the global COVID-19 pandemic caused massive fatality mainly due to respiratory failure, the inhalation of hydrogen and oxygen gas mixtures ($2H_2 + O_2$) showed beneficial effects in clinical trials against COVID-19 symptoms. In an earlier laboratory experiment, Ohsawa et al. showed that oxidative stress damage, that had been induced by brain ischemia-reperfusion, was suppressed by H_2-therapy in rats. Reactive oxygen species (ROS) like OH* and ONOO* generally cause oxidative stress and tissue inflammation, though certain ROS (such as NO*) act as signaling molecules to regulate cell growth. Given that molecular H_2 has a chemical reactivity that tends to quench OH* and ONOO*, while leaving NO* unscathed, a pathway for the beneficial effects of H_2-therapy has been proposed.

Generating H_2 by electrolysis, with and without accompanying O_2, here we have investigated the effect of molecular hydrogen on growth and tissue repair in zebrafish embryos. The larvae were incubated on 3 consecutive days in H_2-containing aqueous medium to allow transcutaneous gas exchange for 3 hours at a time. Starting with intact-fins as well as partially amputated-fins, respectively, the regular development and regeneration of these fins were evaluated by measuring fin-area growth rate and neutrophil count at the injured site. Our preliminary study shows that molecular hydrogen accelerates both uninjured-fin development as well as amputated-fin regeneration. This finding indicates that hydrogen may serve as a potential therapeutic supplement for wound healing and skin repair, by acting as an effective antioxidant and suppressing oxidative damage.

As an alternative treatment modality, a “health-promoting” electromagnetic wave generator was also used to study tissue growth. The 144 MHz oscillating electromagnetic field could potentially dissociate H_2O into H_2 and O_2 or generate some free radicals ($2H_2O \rightarrow H_2 + 2OH^*$), hence these molecular products are also expected to have an effect on tissue growth and regeneration.

24 - Yap regulates an SGK1/mTORC1/SREBP-dependent lipogenic program to support oncogenic liver growth

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Liver cancer remains one of the most lethal cancers worldwide. Although liver cancer is a heterogeneous disease at the genetic level, one of the unifying features is the deregulation of transcription factors. Yes-associated protein (Yap) is an oncogenic transcription factor and nuclear effector of the Hippo pathway. Emerging evidence suggests that Yap reprograms cellular metabolism to meet the anabolic demands of growth, although the mechanisms involved are poorly understood. This study aimed to determine the role that Yap plays in regulating lipid metabolism in liver growth. We took advantage of a larval zebrafish model in which a hyperactivated form of Yap is specifically expressed in hepatocytes (*lf*:Yap^{S87A}). We found that the oncogenic expression of Yap was sufficient to stimulate *de novo* lipogenesis (DNL) and induce lipid droplet formation in hepatocytes. Furthermore, transcriptomic analyses of the liver tissue revealed that Yap caused an increase in the expression of Sterol regulatory-element binding proteins (SREBP) target genes responsible for DNL. Given that the maturation of SREBP is dependent on the mTORC1 pathway, we examined the effect of rapamycin treatment, and found that it suppressed Yap-dependent DNL and hepatomegaly. Mechanistically, we found that the Yap target gene, serum/glucocorticoid regulated kinase 1 (SGK1), was required to induce mTORC1-dependent hepatomegaly. To determine whether DNL was required for Yap- dependent oncogenic growth, we targeted fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD). We observed that both genetic and pharmacological loss of FAS or SCD function suppressed Yap-dependent hepatomegaly, whilst having no effect on normal liver growth. Together, these findings suggest that Yap-driven oncogenic growth is conditionally dependent upon the SGK1/mTORC1/SREBP-mediated stimulation of DNL. Consequently, our results provide a rationale for examining the clinical efficacy of DNL inhibitors to combat liver cancer.

25 - Evidence of non-canonical STAT5 functionality in zebrafish

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Signal transducer and activator of transcription (STAT) proteins are the key transcription factors facilitating the effects of cytokines. One of these, STAT5, is known to be involved in the regulation of blood and immune cell development and function, growth, and metabolism. This has assumed to be via its so-called “canonical” functionality, whereby cytokines trigger tyrosine phosphorylation of inactive cytoplasmic STAT monomers allowing their dimerization though reciprocal interactions between the phosphotyrosine-containing motifs on one STAT with the SH2 domain on another and subsequent nuclear translocation where they can influence transcription. However, a growing literature has suggested that STATs can also exert function via alternative “non-canonical” functionalities. However, understanding of canonical versus non-canonical functionalities in the context of normal development remains limited.

CRISPR-Cas9 based genome editing was utilised to target the SH2 domain, phospho-tyrosine-containing motif (PTM) and transactivation domain (TAD) in zebrafish Stat5.1[AW1] each of which should ablate canonical functionality. In addition, Stat5.1 knockout were also generated targeting N-terminal domain where all Stat5.1 functional aspects are deficient.

These Stat5.1 mutants were subsequently assessed for immune cell development and growth, key parameters in which Stat5.1 contributes. In comparison to wild-type zebrafish, the SH2, PTM and TAD mutants all showed a significant reduction in the number of both progenitor and mature T cells, with the body size also significantly smaller. However, compared to Stat5.1 knockout zebrafish, there were no difference in progenitor T cells, although the number of mature T cells was elevated. In addition, body size was significantly smaller in SH2 and PTM mutants. Collectively this suggests that canonical STAT5 functionality plays an important role in both T cell development and growth, but non-canonical functionalities also contribute to T cell maturation and growth via different mechanisms.

Key Words: STAT5, non-canonical, CRISPR-cas9, Immune-regulation

26 - Single-Cell Analysis of Mechanisms Coupling Brain and Bone Growth

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Proper skull shape and size requires coupling of brain growth to the growth of the overlying skull bones. Cranial sutures separate neighboring skull bones and supply skeletal stem cells that grow skull bones, but the mechanisms that regulate bone growth to meet the demands of the growing brain are poorly understood. We integrate novel zebrafish reporter lines, single-cell transcriptomes, and genetic manipulations to uncover temporally distinct mechanisms controlling bone growth. Employing a novel photoconvertible osteoblast reporter, we observe a rapid de novo osteoblast differentiation during initial bone growth that decelerates after cranial suture formation. Single-cell transcriptomes of skullcaps before and after cranial suture formation reveals signatures of osteoblast progenitors and meningeal cells associated with the underlying brain. Meninges have been proposed as an important regulator of cranial suture biology but their precise contributions to skull growth have remained untested. We find that ablating *foxc1b*+ meningeal cells prior to skull bone formation results in much reduced osteoblast differentiation and abnormal skulls, implicating the brain-associated meninges in promoting skull growth. During later development, we find upregulation of several BMP antagonists as sutures form. Consistent with this observation, expression of a *grem1a*:nlsEOS reporter coincides with suture formation. In *twist1b*; *tcf12* mutants that display accelerated osteoblast differentiation and suture fusion, *grem1a*:nlsEOS+ cells are lost, supporting a potential role for *grem1a*+ cells in establishing a niche that slows osteoblast differentiation as sutures form. Together, these data highlight novel cellular and transcriptional events that coordinate brain and skull growth and may be decoupled in birth defects affecting the skull.

27 - A conditional Tnnt2a-degron line enables temporal control of cardiac contraction

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Cardiomyopathies are diseases affecting the heart muscle, which ensures cardiac contractions and blood flow throughout the organism. The genetic causes of cardiomyopathies include mutations in the gene encoding cardiac troponin T (*TNNT2*), a sarcomeric protein. Zebrafish mutant for *tnnt2a* displays a cardiomyopathy-related phenotype with a loss of cardiac contractions, resulting in morphological defects such as cardiac chamber enlargement, and absence of valve and trabeculae. Here, we describe a novel system to control cardiac contractions in zebrafish by driving Tnnt2a protein for proteasomal degradation. We generated a Tnnt2a-eGFP fusion line in the endogenous *tnnt2a* locus using a Crispr/Cas9 knock-in approach. We find that the fusion protein integrates into the cardiac sarcomere, does not affect heart function, and recapitulates the endogenous *tnnt2a* expression pattern. We then use the eGFP as a degron domain to degrade the Tnnt2a protein using a cardiomyocyte-specific expression of the zGrad system. Using this system, we show that degradation of Tnnt2a-eGFP results in strong cardiac contraction defects that recapitulate the *tnnt2a* mutant phenotype. We also describe another transgenic line that allows temporal control of zGrad expression and Tnnt2a-eGFP degradation in cardiomyocytes. Overall, this model will help us better understand how blood flow and cardiac contractions shape the heart, and will provide the community with a novel genetic tool for studying sarcomeric defects and related cardiomyopathies.

28 - Hindbrain rhombomeres do not form in an even/odd pattern, but derive from early multi-lineage progenitor domains

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A key goal of developmental neurobiology is to understand how specification of distinct neural cell types from progenitor cells is controlled in space and time to ensure appropriate circuit formation. Transient 'patterning' of the embryonic nervous system into neuromeres – compartments that ensure separation of distinct cell populations – is one mechanism to achieve correct positioning of progenitors The embryonic hindbrain is divided into rhombomeres (r) and serves as a model for neuromere formation, but the mechanistic basis of rhombomere formation remains unclear. The prevailing model posits that rhombomeres form in an even/odd pattern, such that even-numbered rhombomeres share a genetic program distinct from that operating in odd-numbered ones. This model is largely based on interpretation of gene expression patterns, but the number of genes analyzed is limited, and the model is at odds with embryonic brain morphology. Proper testing of this model has been hampered by the lack of comprehensive molecular analyses of early rhombomere formation. We have now used combined single nucleus RNA-seq/ATAC-seq (scMultiome) to define the complete transcriptional and chromatin state of the zebrafish hindbrain primordium from the onset of neurulation until rhombomeres are formed. We do not find support for the even/odd model. Instead, our data reveal that the hindbrain primordium is initially divided into three "primary hindbrain progenitor domains" (PHPDs) that correspond to future r1/r2/r3, r4 and r5/r6, respectively. These domains appear to contain multi-lineage progenitors that undergo further refinement into the mature rhombomeres. Our identification of PHPDs is consistent with long-standing visual observations in many species, including human embryos, and is important for efforts at understanding and modeling neurodevelopmental disorders, as well as for the eventual implementation of restorative or replacement strategies as clinical treatments.

29 - From disease genes to behavioural screens using zebrafish F0 knockouts

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Hundreds of human genes are associated with neurological diseases. To yield disease-modifying therapies, we must now discover the biological processes in which these genes are involved. Larval zebrafish are a promising model for this purpose, but generating homozygous knockout larvae for a single gene often takes six months and more than a hundred adult animals. This bottleneck makes it almost inconceivable to systematically screen disease-associated genes in a vertebrate. A possible solution in zebrafish is the F0 knockout route, as it allows the injected embryos to be directly used in experiments. While previous “crispant” methods had success for morphological phenotypes, they cannot be confidently used to study behavioural phenotypes due to incomplete phenotypic penetrance. We developed a highly effective CRISPR-Cas9 method capable of converting > 90% of injected embryos directly into F0 biallelic knockouts. We demonstrate that F0 knockouts reliably recapitulate complex mutant phenotypes, such as altered molecular rhythms of the circadian clock or multi-parameter day-night locomotor behaviours. The technique is sufficiently robust to knockout multiple genes in the same animal, for example to create the transparent triple knockout *crystal* fish for imaging. Our F0 knockout method effectively cuts the experimental time from gene to behavioural phenotype in zebrafish from months to one week. We are now applying the approach to screen the behavioural phenotypes of larvae carrying mutations in genes associated with Alzheimer’s disease. Our work thus far has found that knockout of several Alzheimer’s disease genes leads to altered sleep/wake architecture behaviours already in zebrafish larvae, suggesting that perturbations in sleep architecture across a lifetime may contribute to disease initiation. The F0 knockout method can now be employed to screen tens or hundreds of genes associated with other conditions, such as schizophrenia or epilepsy.

30 - Structural and molecular characterization of EVL-deep cell interactions during zebrafish development

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During zebrafish epiboly, the blastoderm, comprised of the epithelial enveloping layer (EVL) and mesenchymal deep cells, thins and spreads vegetally to enclose the yolk cell. The EVL and deep cells interact dynamically during gastrulation but do not intermix. The significance of these interactions and their importance for epiboly is currently unknown.

The EVL-deep cell interface is analogous to Brachet's cleft in frog where ectoderm and mesoderm cells establish a boundary through cycles of cell adhesion and repulsion facilitated by EphrinB1 signalling. Brachet's cleft is essential for normal morphogenesis as the mesoderm uses the basal surface of the ectoderm as a migratory substrate. We hypothesize that EVL-deep cell dynamics might similarly be required to facilitate epiboly movements and could be mediated by Eph/Ephrin signalling.

Consistent with this idea, ectopic expression of dominant negative EphrinB1 ligand resulted in prolonged EVL-deep cell contacts compared to controls where dynamic and transient interactions are observed. To further investigate the function of EphrinB1 and its cognate receptor, EphB3b, ATG morpholino knock-downs were done. Knock down of each gene produced mild epiboly delays; whereas, double knock down resulted in significant epiboly delay. Live imaging revealed prolonged physical contacts between EVL and deep cells in double *ephrinb1/ephb3b* morphants. These results suggest that the repulsive signal that facilitates tissue boundary formation is lost in double knock down morphants and the cell-cell dynamics and epiboly movements are altered.

The current focus is to investigate the spatiotemporal changes in cytoskeletal and adhesive molecules that lead to prolonged EVL-deep cell interactions. I have also used CRISPR genome editing to generate *ephrinb1* and *ephb3b* mutant founder fish to be characterized in future work. My studies will bring new insights into EVL-deep cell interactions and their contribution to tissue morphogenesis during early development.

31 - CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos

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Early embryonic development is driven exclusively by maternal gene products deposited into the oocyte. Although critical in establishing early developmental programs, maternal gene functions have remained elusive due to a paucity of techniques for their systematic disruption and assessment. CRISPR-Cas13 systems have recently been employed to degrade RNA in yeast, plants, and mammalian cell lines. However, no systematic study of the potential of Cas13 has been carried out in an animal system. Here, we show that CRISPR-RfxCas13d (CasRx) is an effective, and precise system to deplete specific mRNA transcripts in zebrafish embryos. We demonstrate that zygotically expressed and maternally provided transcripts are efficiently targeted, resulting in up to 80-90% decrease in transcript levels by 2 hours post injections (HPI) and recapitulation of well-known embryonic phenotypes. Also, our maternal RNA screening using the CRISPR-RfxCas13d system shows that maternal RNAs are required for the regulation of early embryonic development in zebrafish. Moreover, we show that this system can be used in medaka, killifish, and mouse embryos. Altogether, our results demonstrate that CRISPR-RfxCas13d is an efficient and rapid knockdown platform to interrogate the functions of different kinds of RNA in animal embryos.

32 - Shared and tissue-specific molecular states during zebrafish development

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A fundamental question in developmental biology is to understand the transcriptional events that transform a fertilized egg into the numerous distinct cell types present in an animal. To profile the molecular cell types during vertebrate development, we sequenced ~450,000 single cell transcriptomes across closely spaced stages spanning zygotic genome activation to larval stages (3–120 hours post-fertilization). By analyzing these data together and per tissue, we generated a detailed catalog of >400 cell states in zebrafish development and characterized the developmental trajectory of transcriptional changes that occur during the differentiation of several cell types. Cross-tissue comparisons revealed developmental gene expression programs shared between functionally distinct tissues. We also detected several cell types with shared functional gene expression programs that recurred across multiple tissues (e.g., ionocytes, lysosome-rich cells). We found unexpected heterogeneity within some cell types, such as hepatocytes and surfactant-producing cells. Moreover, this approach enabled detailed classification of poorly understood cell types, including discovery and subtype-specific marker identification of 7 distinct smooth muscle and 3 distinct pericyte molecular cell types, thereby alleviating a major challenge of mural-cell research. Finally, this approach uncovered a zebrafish homolog of a recently discovered human intestinal enterocyte population (*best4*$^{+}$*/otop2*$^{+}$) that is potentially linked to human disease. Their developmental origins and specification programs remain unexplored, so we used trajectory analysis to identify the cascade of gene expression events leading to these cells and predict candidate regulators that govern their specification. Our goal is to provide this atlas as a public resource that can reveal specialized and shared cross-tissue insights during zebrafish development.

33 - CRISPR-STAT based screening method for generation of knock-in zebrafish models using single-stranded oligodeoxynucleotide donors

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Genome editing using CRISPR/Cas9 has become a powerful tool in zebrafish to generate targeted gene knockouts models. However, its use for targeted knock-in remains challenging due to inefficient homology directed repair (HDR) pathway in zebrafish, highlighting the need for efficient and cost-effective screening methods. Here, we present our CRISPR Somatic Tissue Activity Test (CRISPR-STAT) based screening approach for knock-in using a single-stranded oligodeoxynucleotide donor (ssODN) as a repair template for the targeted insertion of epitope tags, or single nucleotide changes to recapitulate pathogenic human alleles. Our pipeline consists of 3 phases: design, somatic screening, and germline screening. The design phase consists of CRISPR activity validation and designing the ssODN based on the optimal sgRNA. Somatic screening is done with the CRISPR-STAT protocol to assess for enrichment of the KI allele in injected embryos followed by validation by TOPO cloning. Germline screening is performed by prescreening F0 adults for the fish most likely to transmit to the germline followed by screening progeny of those fish. As proof-of-principle, we present data for the insertion of a FLAG tag at the *tcnba* locus and an HA tag at the *gata2b* locus. We took advantage of the expected change in size of the PCR product following insertion of the epitope tag. For point mutations, we combined CRISPR-STAT with restriction fragment length polymorphism analysis to distinguish the fish with the knock-in allele. We targeted the *gba* gene to generate a point mutation observed in Gaucher disease. While germline transmission rates were low (1-5%) combining our screening methods with prioritization of founder fish by fin biopsies allowed us to establish stable knock-in lines by screening 12 or less fish per gene. We believe that our methods to generate efficient knock-in fish models using CRISPR/Cas9 and ssODNs will benefit the zebrafish community.

34 - Transcriptomic profiling of hypothalamic feeding networks reveals neuropeptide diversity, conserved gene expression and potential mutual interactions

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We previously identified two ventral hypothalamic loci in zebrafish that are bidirectionally active across hunger, voracious feeding and satiety, and which contribute towards the homeostatic control of feeding (Wee et al, *eLife*, 2019). However, the precise cellular identities of these circuits, as well as potential mechanisms of interactions between them were still unknown. We have now conducted transcriptomic profiling of these loci using the same transgenic reporter lines previously used for functional analyses, and identified conserved neuropeptides and other genes expressed in these neighboring regions. Fluorescent *in situ* hybridization of differentially expressed neuropeptides reveals dynamic gene expression and activity patterns across hunger and satiety states. The role of these neuropeptides in feeding was also examined using pharmacological studies. Further, the presence of neuromodulator-receptor pairs has uncovered opportunities for cross-talk. Overall, this new data has advanced our previous model of how interacting hypothalamic loci may regulate energy balance in the vertebrate brain.

35 - Effects of kinesin light chain 2 (klc2) loss of function and overexpression on neuronal development

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Development of highly elaborate and polarized neuronal morphology requires precise regulation of cellular cargo transport by motor proteins such as kinesin-1. The mechanisms by which kinesin-1 selects specific cargos are not well understood, however the cargo-binding kinesin light chain (KLC) subunits are likely involved in specificity. The developmental processes mediated by individual KLCs in neurons are poorly understood, despite the fact that mutation in human *klc* genes cause disease with very early onset. A mutation in *klc2* that results in *klc2* overexpression causes human SPOAN syndrome, which is characterized by spastic paraplegia with onset in infancy. Zebrafish *klc2* is expressed broadly in the embryonic brain and spinal cord. We are modeling SPOAN syndrome by overexpressing *klc2* in neurons and analyzing effects on axon development and maintenance. In addition, we are using loss of function approaches, including *klc2* crispant and mutant analysis, to determine KLC2 functions in neuronal development. We generated a *klc2* mutant allele with a 7 aa deletion in the KLC2 cargo-binding TPR domain. Mutant embryos and G0 crispants both show increased degeneration of somatosensory axons at 24 hpf, suggesting KLC2 is important for axon maintenance at developmental stages. We currently are using live imaging approaches to further characterize the dynamics of degeneration and the effects of KLC2 manipulation on axon growth and guidance.

36 - (Some) of the nasty effects caused by a chronic exposure to a dirty cocktail

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Persistent Organic Pollutants (POPs) have been widely described as major health threats. Most of the research on them has focused on single compounds, while the environment and individuals are continuously exposed to a variety of substances. Here, we tested a synthetic version of a POP mixture consisting of 29 compounds (Total Mix) that is based on environmentally relevant concentrations found in the blood of Scandinavian people. In addition, we tested sub-mixtures of each separate class of compounds (perfluorinated, chlorinated, and brominated compounds) of such Total Mix. Zebrafish larvae were exposed to seven different mixtures for 4 days and then monitored for their movement behavior, heart rate, cartilage and bones deformations, inflation of swim bladder, and gene expression. Their responses varied significantly depending on the mixture used. The most remarkable effects were seen in those fish treated with the Total Mix, suggesting a distinctive synergistic effect. The perfluorinated-containing class of compounds induced the most remarkable defects in all the assessed endpoints. Our results add up to the already existing catalogue of toxic effects described for POPs, paving the way to a much deeper understanding on how POP mixtures may affect wildlife and humans.

37 - Genetic dissection of angiogenic signaling during mycobacterial infection

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The zebrafish has long served as an important model for understanding vascular development and pathology. One of the hallmark features of the human disease tuberculosis is the presence of extensive webs of pathological neovasculature in the proximity of the infectious focus, although the functional consequences of this vasculature are only recently beginning to be understood. We applied the zebrafish-*Mycobacterium marinum* infection model to study the conserved pathological angiogenic response to mycobacterial infection. We previously identified a specific bacterial cell wall lipid, trehalose 6-6'-dimycolate (TDM), that is sufficient to induce a potent angiogenic response. However, the signaling pathways engaged in host immune cells to drive this process remain poorly understood. Through the application of genetic approaches and novel transgenic tools, we found that macrophage-specific NFAT activation and transcription is required for a robust angiogenic response both to TDM and live mycobacteria. We found that this NFAT-dependent response is conserved in human cells exposed to *Mycobacterium tuberculosis.* This work highlights a widely unappreciated role for macrophage-specific NFAT signaling in mycobacterial pathogenesis and may provide new leads for anti-tuberculosis therapies.

38 - Characterization of functionalized carbon dots and their interactions with bone: toward the development of bone-specific nanocarriers for drug delivery

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Osteoporosis treatments focus on preventing bone erosion instead of restoring bone mass because drugs that could promote cell proliferation in bone could promote cancer in other tissues. Thus, methods that can bypass these adverse effects by directly delivering drugs to bones are highly sought after. Carbon nanodots (C-dots) have emerged as novel therapeutic and diagnostic biomaterials due to their unique physicochemical properties such as small size (<10 nm), high carbon content, robust photostability and bright fluorescence. Our previous work has shown that particular preparations of C-dots bind to high affinity and specificity to larvae and adult zebrafish bones undergoing homeostatic turnover, repair and regeneration. Here we report further experiments characterizing C-dot's physicochemical properties and show their potential as drug-nanocarriers. We have derivatized C-dots in the presence of different concentrations of the infrared fluorophore Cy5, and characterized their bone-binding properties, immunogenicity and bio-tolerance. Spectrophotometric analysis confirmed that Cy5 fluorescence at 650nm increased proportionally to the level of Cy5 bound to the C-dots. Cell culture assays using mouse macrophages indicate that unconjugated and Cy5-conjugated C-dots do not trigger inflammation. Finally, similar to controls, Cy5-labeled C-dots injected into adult zebrafish undergoing caudal fin regeneration were observed to bind to bones, at sites of appositional growth, without interfering with bone regeneration and growth processes. We did observe, however, a dose-dependent decrease in C-dots deposition in bones as the amount of conjugated Cy5 increased. Together, these results support the further development of C-dots as therapeutic agents for the highly specific delivery of drugs to bones. **(Funding support NIH-NIAMS R21AR072226 and NSF-DMR 1809419)**

39 - Adaptation to starvation and resilience in cavefish

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Adapting to extreme environments requires drastic changes to an animal's metabolism. Adaptation to the total darkness and food limitation of caves can be particular challenging. The cavefish *Astyanax mexicanus* is a promising research organism to unravel the genetic basis of starvation resilience, as surface and cave morphs of the same species remain interfertile and can be bred outside their natural environments. We have previously shown that cavefish evolved impressive adaptations such as increased appetite, starvation resistance, and altered feeding due to mutations in *mc4r*. In addition, we found that cavefish display elevated blood sugar levels and insulin resistance caused by a mutation in the insulin receptor. In contrast to human patients, carrying the same mutations, cavefish do not display common markers of metabolic diseases or high blood sugar. Furthermore, cavefish develop hypertrophic visceral adipocytes without obvious signs of inflammation due to reduced amounts of pro-inflammatory cytokines. Taken together, our work suggests that cavefish develop these phenotypes as part of their starvation resistance and have evolved resilience phenotypes that allow them to tolerate stark deviations from what would be considered normal physiology in other vertebrates, including humans. This positions cavefish as a promising model to study disease phenotypes from an evolutionary and adaptive perspective.

40 - An Enteroendocrine-vagal sensory pathway that transmit gut bacterial signal to the brain

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The intestine harbors complex and dynamic microbial communities that shape host physiology. However, mechanisms by which the intestine perceives distinct microbial species and relays that information to the brain remain unresolved. Enteroendocrine cells (EECs) are specialized sensory epithelial cells in the intestine that detect nutrients and other chemicals in the intestinal lumen. Recent studies in mice show EECs synapse directly with vagal neurons and that nutritional stimuli in the intestine can activate this neuroepithelial circuit. Despite these central roles of EECs in intestinal nutrient sensing, it remained unknown if EECs sense bacteria and what the downstream effects may be. To test the impact of bacteria on EECs *in vivo*, we developed novel zebrafish genetic models and established high-resolution calcium imaging approaches to record spatial-temporal EEC activity in the whole animal and track EEC function in real-time in vivo. We screened a panel of bacteria and identified the bacterium *Edwardsiella tarda (E. tarda)* significantly elite EEC activity through activating the transient receptor potential ankyrin 1 (Trpa1). Using the bacterial biochemistry approach, we identified novel Tryptophan metabolites secreted by the *E.tarda* bacteria that activate the Trpa1 receptor. Using optogenetic and *in vivo* vagal calcium imaging, we have discovered that intraluminal *E. tarda* bacteria or its tryptophan metabolites directly activate vagal sensory neurons to modulate brain activity through EECs. Using the single-cell RNA sequencing, we further revealed that the Trpa1+EECs specifically enriched in a neuronal peptide that is involved in pathogen defense. Blocking this neuronal peptide signaling inhibits Trpa1+EECs transmit bacterial signals to vagal sensory neurons. Collectively, using new approaches in zebrafish model, our data establish a new molecular pathway by which EECs regulate brain activity through vagal sensory neurons in response to specific microbial signals.

41 - Redeployment of Pluripotency Factor Nr5a2 in Postmigratory Neural Crest Cells Ensures Non-Skeletal Fates in the Jaw

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Organ development depends on the differentiation of diverse cell types with spatial and temporal precision. In the vertebrate jaw, this requires the coordinated induction of cartilage, bone, tendon, and glandular fates from mandibular arch neural crest cells, yet how such fate decisions are spatiotemporally regulated remains unclear. Our single-cell sequencing analysis in zebrafish reveals redeployment of the pluripotency factor *Nr5a2*, an orphan nuclear receptor, within post-migratory mesenchyme of the aboral mandibular arch. In mammals, this aboral domain generates the salivary gland and tendons of the jaw and middle ear. In zebrafish *nr5a2* mutants, aboral *nr5a2*-expressing cells inappropriately differentiate into chondrocytes instead of tenocytes, resulting in an expanded lower jaw skeleton, tendon loss, and disorganized jaw muscles. In mice, conditional loss of *Nr5a2* in neural crest cells results in similar mandibular defects, including abnormal jaw and middle ear skeletons, disorganized tendon and muscle attachments, and salivary gland agenesis. Genetic mosaics and *nr5a2* misexpression experiments in zebrafish show that Nr5a2 functions cell-autonomously in neural crest cells to inhibit chondrocyte and promote tenocyte differentiation. Mechanistically, single-cell multiome analysis reveals that Nr5a2 opens a number of jaw-specific enhancers for genes implicated in maintenance of multipotent perichondrium progenitors and their differentiation into tendon and glandular fates. We propose that redeployment of the pluripotency factor Nr5a2 within the mandibular aboral domain locally prolongs multipotency to promote alternative non-skeletal fates important for proper jaw and middle ear function.

42 - Development of a model of alcoholic liver disease in adult zebrafish

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Liver failure is one of the leading causes of death worldwide. Alcoholic liver disease (ALD) is caused by alcohol abuse, and can ultimately result in liver failure. ALD is marked by distinct stages that include steatosis, an accumulation of lipid droplets within the liver, steatohepatitis, inflammation of the liver, and fibrosis, which is marked by collagen deposition and scarring of the liver. Extended periods of fibrosis can lead to cirrhosis, at which point damage done to the liver is considered irreversible. Hepatic stellate cells (HSCs), a group of pericyte-like cells within the liver that differentiate to a myofibroblast state upon liver injury, have been implicated in collagen deposition and liver scarring.

To better understand the cellular changes underpinning ALD, a physiologically relevant animal model must be developed. Human and rodent models present ethical, financial, and technical limitations, prompting the need to look at other organisms. Here, we used adult zebrafish (*Danio rerio*) to create a model for ALD. We leveraged an existing transgenic line, *TgBAC(hand2:EGFP)*, that allows for the labeling of HSCs in the liver. Transgenic fish were exposed to ethanol in a number of experimental paradigms, following which, a Nile Red lipophilic dye was used to stain live zebrafish liver cells. Hepatocytes of fish that underwent chronic ethanol exposure demonstrated an increase in number, size, and intensity of lipid droplets, indicating the presence of steatosis. Additionally, we used Sirius Red and collagen antibody staining on fixed zebrafish liver tissue to visualize an increase in collagen around the central veins and liver parenchyma of fish that were exposed to ethanol. This suggests that in addition to developing steatosis, ethanol-exposed fish also developed fibrosis. The creation of a compelling model of ALD will motivate further study of the issue through single cell RNA sequencing to determine the cellular mechanisms driving ALD.

43 - Functional restoration of the regenerating adult zebrafish retina

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Zebrafish can regenerate many organs and tissues, including the central nervous system (CNS).Within the CNS-derived neural retina, light lesions cause a loss of photoreceptors and subsequentactivation of Müller glia, the retinal stem cells. Müller glia-derived progenitors differentiate andeventually restore retinal anatomy within four weeks. However, little is known about how lightlesions impair vision functionally, and how well visual function is restored during regenerationin adult animals. Here, we applied novel quantitative behavioral assays to assess restoration ofvisual function during homeostasis and regeneration in adult zebrafish. In a vision-dependentsocial preference test, vision is massively impaired early after lesion, but returns to pre-lesion levelswithin 7 days after lesion. In a quantitative optokinetic response assay with different degrees ofdifficulty, similar to vision tests in humans, we found that vision for easy conditions with highcontrast and low level of detail, as well as color vision, were restored around 7-10 days post lesion.Under more demanding low contrast and high detail conditions, vision was regained only laterfrom 14 days post lesion onwards. We conclude that vision, based on contrast sensitivity, spatialresolution and color perception, is regenerated in adult zebrafish in a gradual manner.

44 - The Role of Calcium, Akt and ERK Signaling in Cadmium-Induced Hair Cell Death

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Hair cells, the sensory cells responsible for hearing and balance, are extremely sensitive and can be kill be a number of compounds. These include certain therapeutic medications, like aminoglycoside antibiotics, and environmental toxins, such as heavy metals. Studies have found a correlation between higher levels of blood or urinary heavy metals, including cadmium, and hearing loss. It has also been shown that cadmium can kill hair cells in both mammalian and fish models, however, the mechanisms by which cadmium kills hair cells are largely unknown. We investigated if signaling pathways implicated in cadmium-induced cell death in other cell types, namely calcium, Akt and ERK, were also playing a role in cadmium-induced hair cell death. We found that while calmodulin inhibition did protect against cadmium-induced hair cell death, inhibition of CaMKII, the IP3 receptor, or mitochondrial calcium uptake failed to protect. The latter two have been shown to be able to protect against aminoglycoside-induced hair cell death. The calmodulin inhibition result may be due to calmodulin's role in the mechanotransduction process, something we have previously shown to be important for cadmium-induced hair cell death. We also observed an increase in both pAkt and pERK levels in hair cells following cadmium treatment. Inhibiting these signaling pathways showed a slight increase in the amount of hair cell death observed suggesting they are being activated by cells as a protective mechanism, which is different than some other cell types where their inhibition actually protects cells from cadmium-induced cell death. Overall our results show that calcium, Akt and ERK signaling pathways are not playing the same role in cadmium-induced hair cell death that they do in cadmium-induced cell death in other cell types and that cadmium and aminoglycosides appear to kill hair cells through distinct mechanisms.

45 - The role of cell division during Kupffer's vesicle (KV) lumen formation

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Previous studies from our lab identified that mitosis is required for lumen formation *in vivo* (Rathbun et al., 2020). Here we use the left–right organizer, Kupffer's vesicle (KV), in *Danio rerio* (zebrafish) to characterize lumen formation development. However, the specific mechanisms that drive lumen formation have not been fully elucidated. To note the positioning of post-mitotic cells in KV two approaches are employed. The first approach treats embryos with BrdU when KV cells are in a migratory stage pre-lumen formation. The second uses live cell imaging of H2B-mCherry to monitor mitotic divisions and subsequent daughter cell positioning in an engineered zebrafish line that specifically labels KV cell plasma membranes (Sox17:GFP-CAAX). My initial studies have identified that 50% of KV cells at the 6-somite stage incorporate BrdU, suggesting they are post mitotic. In addition, we find that this population is significantly constrained towards the notochord, which we call the top half of the KV. These findings suggest that not only is mitotic progression an essential component to KV morphogenesis, but that a specific patterning in mitotic divisions occur to assemble KV. My future directions are to examine how molecular components involved mitotic fidelity, spindle positioning, and subsequent daughter cell organization contribute to KV cell patterning and morphogenesis. One specific target is Pericentrin, a centrosome associated protein involved in spindle formation, positioning, and ciliogenesis. My studies using a Pericentrin mutant zebrafish model will identify mechanisms for the role of Pericentrin in directing KV cell patterning events.

46 - Blue and red light-mediated optogenetic activation of BMP, FGF, and Nodal signaling

Catherine E. Rogers[1], Patrick Müller[2], Katherine W. Rogers[1]

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Cells in the developing embryo experience distinct signaling dynamics, levels, and combinations that control differentiation into the diverse cell fates needed in healthy adults. To determine how signaling is decoded, experimental control over signaling inputs is required. Many contemporary approaches to manipulate signaling are irreversible and lack straightforward control over key signaling features. In contrast, molecular optogenetic approaches use an easy-to-control input—light—to manipulate biological processes. We are developing a suite of blue and red light-activated optogenetic tools to reversibly activate BMP, FGF, and Nodal signaling in living zebrafish embryos. We expect our suite of orthogonal optogenetic activators will provide experimental control over spatiotemporal signaling dynamics, levels, and combinations, and will be useful across fields including developmental biology, regeneration, and tissue engineering.
Funding: NIH Intramural Program

47 - In Vivo Isotope Tracing to Understand the Effect of Tumors on Organismal Metabolism

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The influence of localized alterations to normal physiology on organismal metabolism remains largely unexplored. However, changes such as the development of a tumor could have systemic metabolic implications. Adult zebrafish are a great model organism for such whole-body studies as they possess the same metabolic organs as mammals, and organ sizes are amenable to liquid chromatography/mass spectrometry (LC/MS) analyses of a single fish. Coupled with untargeted metabolomics, stable-isotope tracing studies are commonly used to probe mechanisms underlying metabolic perturbations. Here, by applying a workflow we developed for LC/MS-based metabolomics in adult zebrafish, we examined differences in organismal metabolism due to tumor burden. In melanoma-bearing *BRAF*^{V600E}; *p53*-null adult fish we found evidence for increased liver gluconeogenesis to compensate for tumor glucose demand. By using $^{13}C_6$-glucose, we traced carbon flux from M+6 glucose to M+3 alanine, and further found the source of this labeled alanine to be the tumor. Although tumor excretion of lactate has been well described, the so-called "Warburg effect", we found melanoma instead release alanine into circulation. This M+3 alanine is then converted in the liver to M+3 glucose via gluconeogenesis to maintain serum glucose levels in melanoma-bearing fish. Moreover, we found that we could attenuate this tumor-liver glucose-alanine cycle with the alanine aminotransferase inhibitor ß-chloroalanine, and this inhibition reduced tumor burden. This work presents a workflow for LC/MS-based metabolomic analyses and demonstrates the utility of adult zebrafish for organismal metabolic studies.

48 - Shedding (blue) light on BMP signaling during early embryogenesis

Catherine E. Rogers[1], Katherine W. Rogers[1]

[1]NICHD

During embryogenesis, zygotes reliably generate cells expressing distinct sets of genes leading to different fates and functions. Signaling dynamics, levels, and combinations are thought to activate these different gene expression programs. Many contemporary signaling manipulation methods are inflexible and don't provide the control needed to address how signaling is decoded. To address these limitations, we are developing optogenetic tools to precisely and reversibly activate signaling with blue or red light in zebrafish embryos, then observe how this impacts fate decisions. One of the tools we have developed, bOpto-BMP, activates BMP signaling with blue light. In the presence of blue light, zebrafish expressing bOpto-BMP phenocopy BMP overexpression and show elevated levels of the BMP signaling effector pSmad1. We also found that bOpto-BMP is not activated by red light. This will allow us to use bOpto-BMP orthogonally with red light activators of other pathways in one embryo. In addition, we are determining whether transient optogenetic BMP activation affects other signaling pathways. We expect our tools can be used to understand how cells decode signaling in different biological contexts. Funding: NIH Intramural Program

49 - Heterogeneous pdgfrb+ cells regulate coronary vessel development and revascularization during heart regeneration

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pdgfrb expression marks mural cells and is required for the mural cell association with the blood vessels. In a developing zebrafish heart, *pdgfrb* expression precedes the coronary vessel formation and begins expression in the epicardium around the atrioventricular canal where coronary vessels emerge. *pdgfrb*+ mural cells co-develop with the nascent coronary vessels and are essential for their development. In adult zebrafish hearts, *pdgfrb*+ cells form two separate clusters of cells, the epicardial derived cells (EPDC) and the mural cells, based on single-cell RNA sequencing analysis. The mural cells around zebrafish coronary arteries also express *cxcl12b* and smooth muscle cell markers. Interestingly, these mural cells remain associated with the coronary arteries even in the *pdgfrb* mutant hearts. *pdgfrb* expression dynamically changes in injured zebrafish hearts in which *pdgfrb*+ cells express genes important for heart regeneration. Our results demonstrate that heterogeneous *pdgfrb*+ cells are essential for coronary development and heart regeneration.

50 - Deciphering the species specificity code of Bouncer, an essential fertilization factor in fish

Krista Briedis Gert[1], Karin Panser[1], Benjamin Steinmetz[2], Alexander Schleiffer[1], Joachim Surm[3], Yehu Moran[3], Fyodor Kondrashov[4], Andrea Pauli[1]

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Despite the fundamental importance of fertilization for all sexually reproducing organisms, the molecular factors that govern this process have long escaped our knowledge. Recently, however, we identified the three-finger, egg membrane-expressed protein, Bouncer, which is essential for fertilization in zebrafish and species-specific between zebrafish and medaka (*Oryzias latipes*), two species that cannot cross-fertilize naturally. Remarkably, when zebrafish Bouncer is replaced with that of medaka on the zebrafish egg, these eggs can be fertilized by medaka sperm. Conversely, expressing zebrafish Bouncer on the medaka egg is sufficient to enable fertilization by zebrafish sperm. Thus, together with its currently unknown binding partner on sperm, Bouncer enables sperm-egg binding in a species-specific manner for zebrafish and medaka. What mediates species specificity in Bouncer and is therefore critical for binding, however, is unknown. To investigate Bouncer's functional domains, we used this species specificity as a tool by testing medaka/zebrafish Bouncer chimeras and predicted ancestral states for their ability to rescue fertilization. Fertilization assays with transgenic zebrafish *bncr*$^{-/-}$ females expressing medaka/zebrafish Bouncer chimeras revealed that a combination of two of Bouncer's "fingers" are needed for species-specific sperm-egg interaction. By testing predicted ancestral states of Bouncer, we found that the amino acid changes that underlie medaka sperm bias arose specifically in the *Oryzias* genus. Combining compatibility data from fish Bouncer homologs, medaka/zebrafish Bouncer chimeras, and ancestral states, we identified three candidate residues in addition to opposite N-glycosylation patterns in medaka and zebrafish Bouncer that together may provide species specificity and be critical for Bouncer's function. By defining the functional interaction sites within the Bouncer protein, this work provides insights into how Bouncer accomplishes specific sperm binding on the amino acid level and how species specificity between medaka and zebrafish arose.

51 - Zebrafish larvae utilised to monitor gastrointestinal motility in diabetes mellitus and after the administration of diabetic drugs - mainly targeting GLP-1 and GIP receptor agonist

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Background: Diabetes mellitus (DM) is a prominent chronic disease associated with extensive impact and mortality. Major organs systems, including the gastrointestinal (GI) tract, are being affected. Glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) are hormones that monitor glucose homeostasis and belong to class B G-protein coupled receptors (GPCRs). Zebrafish GPCRs share structural similarities with the human GLP-1 and human glucagon receptors. In addition to the transparency during early developmental stages, we hope to validate our DM model and to explore the role of GLP-1/GIP receptors in the zebrafish intestinal tract. Ultimately, assist in the discovery of novel therapeutics for GI dysmotility in DM.

Methodology: DM larvae models were established by (i) overfeeding, (ii) feeding with a high-fat diet (HFD), (iii) incubation in 30 mmol/L glucose concentration (SD-G) or (iv) a knockdown of the insulin promoter factor 1 (pdx1) gene. Body mass index (BMI), enzyme-linked immunosorbent assays (ELISA) for insulin levels and glucose assay were used to grade DM. As obesity is associated with diabetes, adipose deposits in the abdominal area were determined by imaging microscopy. DM drugs - exendin-4 and tirzepatide were microinjected into the GI. Peristalsis was quantified in anaesthetized larvae using light microscopy and MATLAB.

Results: Data indicates that significant differences in BMI, adipose deposit, insulin, and glucose levels are mainly observed in HFD and SD-G. There are significant differences between DM models and control-fed larvae in terms of mixing power, anterograde and retrograde actions. The exposure of exendin-4 and tirzepatide had differential effects on GI motility.

Conclusion: We established three DM models and an obesity model using zebrafish larvae. Advanced imaging and processing techniques revealed that there may be subtypes of dysmotility in DM and obesity. The addition of drugs that target GPCRs also suggest zebrafish larvae as a potential tool for drug analysis on the GI.

52 - Molecular screening of anti-angiogenic potential of bioactive compounds from marine actinomycetes using Zebrafish model

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Angiogenesis, sprouting of blood vessels from pre-existing vaculature, is a significant component in tumor progression. The present study aimed to screen the anti-angiogenic activity of crude extracts of bioactive compounds isolated from actinomycetes from echinoderms found in the coastal regions of Tamilnadu, India. As marine natural products have been proved to be a rich source of novel compounds in drug discovery especially in the area of small molecule based targeted anticancer chemotherapeutics identification, we chose it for our study. Since hypoxia is responsible for angiogenesis progression in many diseases, it is of our interest to mimic hypoxia in zebrafish embryos and upregulate the expression of main determinant factor like vascular endothelial growth factor (VEGF) and its receptor (VEGFR2), followed by downregulation of these angiogenesis- associated genes by the extracted bioactive compounds. In our study, the bioactive compounds were treated on zebrafish embryos at 24 hours post fertilization (hpf) and analyzed the morphology of the intersegmental vessels (ISVs) using RBC staining at 72 hpf followed by counting of ISVs and calculation of the inhibition ratio under normal conditions and during hypoxia. The potential of bioactive compounds to down-regulate the expression of angiogenesis- associated genes, at its mRNA level was analyzed using RT-PCR. The results indicated that the crude extracts of bioactive compounds inhibited ISVs formation in zebrafish embryos in a dose- dependent manner, with a significant anti- angiogenic activity observed at a concentration of 5µg, leading to an ISV inhibition ratio of 72.8±1.5%. Extracts significantly reduced the mRNA expression of HIF-1α, VEGFA and VEGFR2 at 5µg concentration. Accordingly, these bioactive compounds may be effective angiogenic inhibitors, which act via downregulation of HIF-1α signaling. Targeting the VEGF signaling pathway with VEGF inhibitor might be a promising approach in treatment of neovascular diseases and also tumor in near future.

53 - Genetic and neurological deficiencies in the visual system of mct8 mutant zebrafish

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Thyroid hormones (THs; T3 and T4) enter the cells using specific transporters and regulate development and metabolism. Mutation in the TH transporter monocarboxylate transporter 8 (MCT8, SLC16A2) is associated with brain hypothyroidism and neurological impairment. We established *mct8* mutant (*mct8-/-*) zebrafish as a model for MCT8-deficiency, which demonstrates endocrinological, neurological, and behavioral alterations. Here, we profiled the transcriptome of *mct8-/-* larvae. Among hundreds of differentially expressed genes, the expression of a cluster of vision-related genes was distinct. Specifically, the expression of the opsin 1 medium wave sensitive 2 (*opn1mw2*) decreased in two *mct8* mutants: *mct8-/-* and *mct8*$^{-25bp}$*-/-* larvae, and under pharmacological inhibition of TH production. Optokinetic reflex (OKR) assays showed a reduction in the number of conjugated eye movements, and live imaging of genetically encoded Ca^{2+} indicator revealed altered neuronal activity in the pretectum area of *mct8*$^{-25bp}$*-/-* larvae. These results imply that MCT8 and THs regulate the development of the visual system and suggest a mechanism to the deficiencies observed in the visual system of MCT8-deficiency patients.

54 - Zebrafish heart regeneration after coronary dysfunction-induced cardiac damage

Jisheng Sun[1], Elizabeth Peterson[1], Cheng Jiao[1], Xin Chen[1], Yun Zhao[1], Jinhu Wang[1]

[1]Emory University

Previous cardiac injury methods, including ventricular resection, cryoinjury, and genetic ablation of the myocardium or epicardium, demonstrated the zebrafish's robust capacity for heart regeneration. Examining innate regenerative mechanisms in zebrafish may lead to new methods to activate or enhance the limited endogenous regenerative programs in adult mammals to restore lost myocardium. However, the zebrafish model has no available coronary vessel injury method despite coronary dysfunction significantly contributing to heart disease like myocardial infarction. This deficiency is due to complications performing surgery on small zebrafish vessels and a lack of specific genetic tools. We identified the Notch ligand gene *deltaC* as a novel genetic marker for coronary endothelial cells and developed the *deltaC* fluorescent reporter line for coronary vessel visualization. Our *deltaC* reporter enabled specific detection of coronary vessel growth during both development and regeneration without interference from an endocardial cell background. In our adapted *ex vivo* system, we observed vigorous coronary growth on the surface of juvenile hearts and regrowth in wounded adult hearts. Further, vascular growth antagonists targeting the VEGF, EGF, and Notch signaling pathways demonstrated vessel growth inhibition in our *ex vivo* system, suggesting its potential to screen vascular growth regulatory molecules. Moreover, we developed a coronary genetic ablation system using the *deltaC* regulatory sequences in which severe coronary endothelial cell loss resulted in fish death. In contrast, fish with mild coronary endothelium ablation survived. Furthermore, depletion of coronary endothelial cells stimulated regenerative mechanisms, with cardiac tissue restoration within several weeks of injury. In summary, our work demonstrated the ability to use *deltaC* regulatory sequences for the high-resolution visualization of coronary endothelial cells, screening small molecules for their effects on coronary growth, and complete cardiac recovery after coronary-induced heart damage in adult zebrafish.

55 - Midkine-Ptprz1b signalling controls cell polarity during zebrafish hindbrain morphogenesis

Yao Le[1], Kavitha Rajasekhar[1], Jun Zhong[1], Paul Matsudaira[1], Thorsten Wohland[1, 2], Christoph Winkler[1]

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Midkine (MDK) and pleiotrophin (PTN) are related heparin-binding growth factors implicated in numerous biological processes, ranging from neurogenesis to metastasis. Some of these roles are attributed to their binding to receptor-type protein tyrosine phosphatase zeta 1 (PTPRZ1) but *in vivo* evidence supporting this ligand-receptor interaction is lacking. Mammals have one copy each of *MDK*, *PTN* and *PTPRZ1*, while the teleost-specific genome duplication resulted in two *mdk* (*mdka* and *mdkb*), one *ptn* and two *ptprz1* genes (*ptprz1a* and *ptprz1b*) in zebrafish. Complementary expression of these genes implies possible sub- or neofunctionalization of Mdk/Ptn-Ptprz1 signalling with different combinations of ligands and receptors at play during morphogenesis. Here, we performed proximity ligation assays as well as fluorescence cross-correlation spectroscopy in zebrafish embryos *in vivo* to show that Mdka, Mdkb and Ptn interact with Ptprz1a and Ptprz1b with different affinities. We also generated zebrafish mutants for *mdka*, *mdkb*, *ptn* and *ptprz1b* by CRISPR-Cas9. A loss of maternal *mdka*, *ptn* and *ptprz1b* led to a significantly delayed gastrulation with defective cell migration as shown by light sheet microscopy-based cell tracking. Importantly, maternal-zygotic (MZ) *mdka*, *mdkb*, *ptn* and *ptprz1b* mutants also showed hindbrain morphogenesis defects with impaired midline formation, supporting a functional interaction of these ligands and receptor. In particular, MZ *mdka* and MZ *ptprz1b* mutants manifested midline duplications that resulted from delayed neural keel convergence and misplaced midline-crossing cell divisions in the forming rhombomeres. These midline defects were completely rescued by overexpression of *Drosophila* Prickle, a key component of the Wnt/planar cell polarity (PCP) pathway. Our findings suggest that Mdka-Ptprz1b acts upstream of Wnt/PCP to control neural progenitor polarity and positioning of cell divisions that establish the midline in the developing zebrafish hindbrain. This project is supported by the Singapore Ministry of Education (MOE2016-T3-1-005).

56 - Hematopoietic potential of the endocardium in zebrafish embryos

Suman Gurung[1], Nicole Restrepo[1], Saulius Sumanas[1]

[1]University of South Florida

Myeloid progenitors which include macrophages and neutrophils constitute a part of the innate immune system and are thought to originate in the anterior lateral plate mesoderm (ALPM) of zebrafish embryos. Recent studies in murine embryos demonstrated that some myeloid cells, primarily macrophages, are also derived from the endocardium. However, the hemogenic potential of the zebrafish endocardium has not been reported to date. Here, we show that endocardium may give rise to myeloid cells in zebrafish embryos. Expression of an early myeloid marker *pu.1/spi1b* and pan-leukocytic marker *lcp1* was detected within the endocardial layer of the heart and partially overlapped with the endocardial marker *nfatc1* expression, suggesting the endocardial origin of these myeloid cells. Furthermore, using time-lapse imaging, we observed the emergence of migratory myeloid cells from the endocardium in live zebrafish embryos. Lineage tracing experiment using the Cre/loxP system confirmed the contribution of kdrl+ endocardial cells to the myeloid lineage. To identify molecular pathways involved in the formation of endocardial-derived myeloid cells, we analyzed the role of the transcription factors Etv2/Etsrp and Scl/Tal1 as well as Hedgehog signaling pathway (Hh). Intriguingly, inhibition of Etv2 or Scl function resulted in nearly complete loss of ALPM-derived myeloid cells, while endocardial-derived myeloid cells were largely unaffected, suggesting alternative pathways involved in their emergence. In contrast, inhibition of Hh signaling resulted in a greatly reduced number of endocardial-derived myeloid cells. We performed single-cell RNA-seq analysis to define the transcriptional profile of endocardial-derived myeloid cells. Our single-cell RNA-seq data followed by validation in zebrafish embryos suggest that endocardial-derived myeloid cells are primarily neutrophils. Our results identify a novel origin of myeloid progenitors in zebrafish, characterize their

57 - Extracellular calcium and calcium channel modulators alter aminoglycoside-induced hair cell loss in zebrafish

MING-HUAN CHAN[1], LIAO-CHEN CHEN[2], HWEI-HSIEN CHEN[2]

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Aminoglycosides are the essential antibiotics for treating gram-negative bacterial infections, however they act directly on inner ear, leading to hair cell death and hearing loss. This study aimed to investigate whether the calcium channel modulators and the extracellular calcium levels could alter aminoglycoside-induced ototoxic response in zebrafish (Danio rerio). The findings showed that a significant decreased number of neuromasts labeled with fluorescent dye FM1-43FX in the lateral lines of zebrafish larvae at 5 days post fertilization (dpf) after neomycin and gentamycin exposure for 30 to 90 min. The loss of hair cells in zebrafish after aminoglycoside treatment was protected by calcium channel blocker, verapamil. Moreover, verapamil attenuated aminoglycoside-induced toxic response in different external calcium levels. Interesting, increasing extracellular calcium levels protected hair cell loss from aminoglycoside exposure, while lower level of calcium facilitated hair cell death. In contrast, calcium channel activator Bay K8644 reversed the protective action of higher external calcium on aminoglycoside-elicited hair cell loss. The uptake of neomycin, labeled with Texas-red, into hair cells was prevented by verapamil and under high external calcium condition. Furthermore, the production of reactive oxygen species (ROS) in neuromasts exposed to neomycin was also reduced by verapamil and high external calcium. These data imply that prevention of hair cell damage against aminoglycoside toxicity by verapamil and high calcium might be associated with inhibition of excessive ROS production and aminoglycoside uptake. These findings suggest that calcium channel blockers and higher calcium level could be applied to protect aminoglycoside-induced listening impairment.

58 - Hematopoietic potential of the endocardium in zebrafish embryos

Nicole Restrepo[1], Suman Gurung[1], Saulius Sumanas[1]

[1]University of South Florida

Myeloid progenitors which include macrophages and neutrophils constitute a part of the innate immune system and are thought to originate in the anterior lateral plate mesoderm (ALPM) of zebrafish embryos. Recent studies in murine embryos demonstrated that some myeloid cells, primarily macrophages, are also derived from the endocardium. However, the hemogenic potential of the zebrafish endocardium has not been reported to date. Here, we show that endocardium may give rise to myeloid cells in zebrafish embryos. Expression of an early myeloid marker *pu.1/spi1b* and pan-leukocytic marker *lcp1* was detected within the endocardial layer of the heart and partially overlapped with the endocardial marker *nfatc1* expression, suggesting the endocardial origin of these myeloid cells. Furthermore, using time-lapse imaging, we observed the emergence of migratory myeloid cells from the endocardium in live zebrafish embryos. Lineage tracing experiment using the Cre/loxP system confirmed the contribution of kdrl+ endocardial cells to the myeloid lineage. To identify molecular pathways involved in the formation of endocardial-derived myeloid cells, we analyzed the role of the transcription factors Etv2/Etsrp and Scl/Tal1 as well as Hedgehog signaling pathway (Hh). Intriguingly, inhibition of Etv2 or Scl function resulted in nearly complete loss of ALPM-derived myeloid cells, while endocardial-derived myeloid cells were largely unaffected, suggesting alternative pathways involved in their emergence. In contrast, inhibition of Hh signaling resulted in a greatly reduced number of endocardial-derived myeloid cells. We performed single-cell RNA-seq analysis to define the transcriptional profile of endocardial-derived myeloid cells. Our single-cell data followed by validation in zebrafish embryos suggest that endocardial-derived myeloid cells are primarily neutrophils. Our results identify a novel origin of myeloid progenitors in zebrafish, characterize their transcriptional profile and identify molecular mechanisms involved in their emergence.

59 - Investigating the relationship between the immune response and ependymoglial activation during spinal cord regeneration in the adult and juvenile zebrafish model

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[1]University of Manitoba

Spinal cord injury (SCI) is a life changing condition affecting individuals within Canada and worldwide with no effective treatment to date. A limitation in humans, like other mammals, is that they cannot repair the damaged central nervous system after injury. By contrast, the zebrafish model has a remarkable ability to regenerate the spinal cord following complete transection, due to neural stem cell populations of ependymoglia. Previous work has shown that for ependymoglial-driven neural regeneration to occur, immune cells are a key requirement. However, in zebrafish the involvement of macrophages and the cytokine response during the process of spinal cord regeneration in post-larval stages remains poorly understood. In this study, I hypothesized that for functional recovery to occur, the pro-inflammatory response following SCI in zebrafish must be activated ahead of ependymoglial proliferation to initiate the regenerative process. To study this response, I developed a new juvenile model of SCI to then compare to the established adult model of SCI. By studying the spatiotemporal dynamics of immune cells post-SCI, I observed that overtime macrophages and microglia infiltrate into the injury site and contributed to cytokine release, correlating with a peak in proliferation of ependymoglia around the central canal. Interestingly, analysis of pro- and anti-inflammatory cytokines from RT-qPCR experiments demonstrated that pro-inflammatory cytokines are highly expressed shortly after injury, but are reduced to near control levels already by 3-days post-SCI. By contrast, anti-inflammatory cytokines appeared to play a minor role in the microenvironment post-SCI, remaining near control levels of expression. These findings propose that in order for successful spinal cord regeneration to occur in adult and juvenile zebrafish, a shorter pro-inflammatory response may be required to initiate ependymoglial proliferation in the spinal cord and restore functional repair.

60 - Rab11, but not Rab8, is essential for actin organization during KV lumen formation

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Using *Danio rerio's* (zebrafish) Left-Right organizer (LR) known as the Kupffer's vesicle (KV) as an *in vivo* vertebrate model to study lumen formation, we identified that Rab11-endosomes direct actin's apical organization independent of Rab8 during KV lumen formation. KV development involves the assembly of mesenchymal cells into a polarized rosette like structure, which then forms a cyst-like structure with a fluid filled cavity (lumen). During this process, Rab11- and Rab8-endosomes along with actin dynamically organize in the center of the rosette structure. As the rosette begins to transition into a cyst, Rab11 and actin get recruited to KV cell-cell junctions, whereas Rab8 gets organized at KV cell apical membranes. We found that optogenetically clustering Rab11-endosomes, but not Rab8-endosomes, caused an accumulation of an apically targeted cargo required for lumen formation, Cystic Fibrosis Transmembrane conductance Regulator (CFTR), and actin to accumulate on Rab11-clustered endosomes inhibiting their assembly at the forming apical membranes resulting in lumen formation failure. Together, these results support a temporal and spatial model that Rab11-endosomes first transport CFTR, and actin to apical membranes independent of Rab8 organization during the rosette stage of KV formation. During lumen formation and then expansion, actin redistributes to cell-cell junctions in a Rab11-dependent manner while Rab8 remains at the apical membrane. These findings suggest that Rab11 is a master regulator of KV lumen formation through apical targeting of CFTR and directing actin remodeling during lumen expansion.

61 - Integration of vascular progenitors into functional blood vessels represents a distinct mechanism of vascular growth

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During embryogenesis, the initial vascular network forms by the process of vasculogenesis, or the specification of vascular progenitors *de novo*. After the initial blood circulation has been established, the majority of later-forming vessels are thought to arise by angiogenesis from the already established vasculature. Here we show that new vascular progenitors in zebrafish embryos contribute to functional vasculature even after blood circulation has been established. Based on the expression analysis of vascular progenitor markers *etv2* and *tal1*, we characterized a novel site of late vasculogenesis (termed secondary vascular field, SVF), located bilaterally along the yolk extension. Using time-lapse imaging of *etv2* reporter lines, we show that SVF cells migrate and incorporate into functional blood vessels and contribute to the formation of the posterior cardinal vein and subintestinal vasculature, suggesting a novel mode of vascular growth. We further demonstrate that SVF cells participate in vascular recovery after chemical ablation of vascular endothelial cells. Inducible inhibition of *etv2* function prevented SVF cell differentiation and resulted in the defective formation of subintestinal vasculature. In addition, we performed single-cell RNA-seq analysis to identify the transcriptional profile of SVF cells, which demonstrated similarities and differences between the transcriptomes of SVF cells and early vascular progenitors. Our results characterize a distinct mechanism of how new vascular progenitors incorporate into established vasculature.

62 - Identification and validation of conserved enhancers in cardiac development

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The genetic control of heart development has been extensively studied, partly due to the prevalence of congenital heart disease (CHD). CHDs primarily arise from mutations affecting gene dosage or altering spatial/temporal expression patterns, making non-coding elements that control these aspects of gene expression particularly important. However, the annotation and functional dissection of non-coding elements is lacking when compared to the coding genome.

Recently, our lab identified 8866 human regulatory elements deeply conserved between zebrafish and humans. Dubbed accessible conserved non-coding elements (aCNEs), these open chromatin regions are enriched for enhancers and are conserved at the DNA sequence level between zebrafish and humans. Due to the deep conservation of these regions, we hypothesize that a subset of aCNEs are conserved critical regulators of cardiac development and implicated in cardiac disease.

To identify regions with a likely role in cardiac development, I used publicly available ChromHMM data from human fetal hearts to identify aCNEs with chromatin signatures representing enhancer states. I then used published ChIPseq datasets to identify regions bound by known cardiac transcription factors (TF), including NKX2.5, GATA4/5/6, and TBX5. These criteria identified 329 regions, capturing previously identified cardiac enhancers, and predicting 258 novel cardiac enhancers.

Functional examination of 17 of these novel regions showed that 88% were able to drive reporter gene expression in the developing zebrafish heart. Strikingly, two of these regions contain ultra-rare SNVs in patients with familial left-sided cardiac legions. Currently, we are working on linking upstream TFs and downstream target genes to aCNEs to gain molecular insight into how these regions function in cardiac development and how alterations may contribute to CHDs. Altogether, this work identified putative cardiac enhancers crucial in cardiac development and disease.

63 - The role of the apelin receptor in cardiac lineage specification in zebrafish

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The existence of an early cardiac progenitor (CP) population has long been supported by fate mapping experiments which identify presumptive CPs at specific embryonic positions as gastrulation begins. These presumptive CPs then migrate to the anterior lateral plate mesoderm and engage a conserved transcriptional network to direct specification, migration, and differentiation of the cardiac lineage. While later stages of cardiac development are well characterized, initial specification remains poorly understood, due to a lack of specific molecular markers for early CPs. To address the outstanding question of CP specification our group is studying the Apelin receptor (Aplnr), a highly conserved GPCR, first shown to regulate cardiac development in zebrafish where *aplnra/b* mutants do not express *nkx2.5*, the earliest specific CP marker, following gastrulation and do not develop cardiomyocytes. Fate mapping analysis has shown that migration of presumptive CPs during gastrulation requires Aplnr. Following loss of Aplnr function Nodal signalling fails to activate correctly and this failure disrupts mesendoderm specification. scRNA-seq has identified that with loss of Aplnr function a cardiac-like mesoderm population fails to develop during gastrulation. Our results indicate that Aplnr is required for the initial commitment of mesodermal cells to the cardiac lineage. Current work is focused on characterizing this specification failure to understand both the function of Aplnr and the mechanisms regulating early CP development.

64 - Efficient knock-in method enabling lineage tracing in zebrafish

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The CRISPR-Cas9 system aids the generation of knock-in zebrafish lines, but it has been hard to integrate large constructs and avoid disrupting the targeted genes. In this study, we devise a novel strategy via 3' knock-in of PCR-amplified dsDNA, i.e. in frame with the endogenous gene. Our PCR template comprised cassettes coding for fluorescence protein and Cre recombinase, which are separated from the endogenous gene and each other by two self-cleavable peptides. Primers with 5' AmC6 modifications were used to generate PCR amplicons harboring either short or long homologous arms, which serve to guide the donor. The 5' end-protected PCR products were co-injected with pre-assembled Cas9/gRNA ribonucleoprotein complexes for early integration. We targeted four genetic loci (*krt92*, *krt4*, *nkx6.1,* and *id2a*) at the 3' end and successfully generated ten knock-in zebrafish lines, which function as reporters for the endogenous gene expression. Additionally, we used the iCre or CreERT2 for lineage tracing to delineate differentiation paths in the pancreas and liver. We found that *nkx6.1* is initially present in multipotent pancreatic progenitor cells which contribute to all three major cell types in the pancreas (endocrine, ductal cells and acinar cells); whereas it gradually becomes restricted to intrapancreatic ductal cells. Likewise, *id2a* expressing cells are multipotent progenitors giving rise to all cells in the pancreas, liver, and intestine before 24 hours postfertilization (hpf); whereas it shows specific expression in pancreatic and hepatic ductal cells starting from 24 and 36 hpf, respectively. Furthermore, temporally controlled long-term lineage tracing confirmed that endocrine cells in the secondary islets can originate from *nkx6.1*$^{+}$ intrapancreatic ductal cells. In sum, we present an efficient knock-in technique with widespread use for both cellular labeling and lineage tracing.

65 - High-throughput drug screen for investigating cranial neural crest cell dynamics in the developing zebrafish

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Cranial neural crest cells (cNCCs) are a highly migratory multipotent stem cell population that gives rise to many essential cellular derivates, such as craniofacial tissues. Perturbations of this early cell population, through intrinsic or environmental factors, can result in drastic cellular/genetic changes. These changes can result in aberrant development of many tissues and organs, namely those in the cranium, such as craniofacial tissue, cartilage, and bone, the eyes, and the brain. Numerous malignancies, such as neuroblastoma, and craniofacial malformations such as cleft palate, and skin and ocular disorders such as albinism and Axenfeld-Rieger syndrome arise from perturbations in cNCC development. The identification of drugs that affect NCC developmental dynamics allows for an investigation into the subcellular elements that cause these abnormalities in the cNCC population and subsequent disease. To identify such drugs, we used the zebrafish model, *Danio rerio*, along with a clinical drug library from the Texas A&M Institute of Biosciences and Technology and a robotic high-throughput microfluidics-based system called the VAST Bioimager and Large Particle Sampler. With these tools, we created a workflow for a high-throughput drug screen that led to the identification of two drugs that have given us insight into the dynamics and fate of cNCCs when in, or induced to be in, a disease state.
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66 - SH2 domain protein E (SHE) and ABL signaling regulate vascular lumen size.

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Blood vessels in different vascular beds vary in lumen diameter, which is essential for their function and fluid flow along the vascular network. 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 vascular lumen size in zebrafish embryos and mammalian cell culture. Zebrafish *she* mutants displayed enlarged dorsal aorta (DA) and defects in blood flow. Vascular endothelial specific overexpression of *she* resulted in a reduced diameter of the DA lumen. 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. Enlargement of the DA lumen in *she* mutants correlated with an increased endothelial expression of *claudin 5a (cldn5a*), which encodes a protein enriched in tight junctions. Inhibition of *cldn5a* expression partially rescued the enlarged DA in *she* mutants, suggesting that She regulates DA lumen size, in part, by promoting *cldn5a* expression. SHE knockdown in human endothelial umbilical vein cells resulted in a similar increase in the diameter of vascular tubes, arguing that SHE function is evolutionarily conserved. These results establish novel roles for She and Abl kinase signaling in regulating lumen size during vascular tubulogenesis.

67 - Single-cell analysis reveals regional and subtype-specific gene regulatory architecture of skeletal joints

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Skeletal joints are complex structures optimized for local function and composed of diverse cell types. The zebrafish cranium is an excellent model to study how diverse joints are specified, as it hosts an immense variety, from synovial joints to fibrocartilaginous bridges. One feature all these joints share is lifelong cartilage, which is distinct from the replacement cartilage that templates endochondral bones during development. The mechanisms for ensuring lifelong cartilage, as well as patterning cell types within functionally distinct joints, remain unclear. To begin to address this, we created a *ucmab:GFP* reporter that specifically labels cartilage-containing joints from early development through adulthood in zebrafish. We then used this reporter to purify joint cells at multiple stages for integrated single-nuclei gene expression and chromatin accessibility sequencing (multiome). By analyzing gene expression, we identified distinct types of cells associated with diverse joints. We then used cluster-specific open chromatin regions (i.e. putative enhancers) to generate a series of transgenic lines labeling joint cell populations. While some enhancers are active widely across cranial joints, others are active in only one or a few types of joints, suggesting the presence of both general and joint-specific enhancers. We also identified enhancers that drove expression in different layers of the joint (i.e. superficial versus deeper chondrocytes). Motif enrichment suggests a potential role for the Jun/Fos AP-1 family in distinguishing lifelong from replacement cartilage, which we are currently testing functionally. We are also using a series of mutants to test how regionally expressed transcription factors interact with more general joint-promoting factors to establish joint-specific architecture and cell type composition at the level of specific enhancers. Through these studies we hope to identify the gene regulatory mechanisms underlying specification of the functionally diverse types of joints in the body.

68 - Attenuation of BMP signaling reduces enteric progenitor numbers in the developing gut

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The vertebrate enteric nervous system (ENS) consists of a series of interconnected ganglia within the muscle walls of the gut and is largely responsible for coordinating peristalsis, water balance, and regulation of hormonal secretions. During development, neural crest cells (NCC) that contribute to ENS migrate into the primitive foregut and migrate caudally along its length, during which time they are referred to as enteric NCCs (ENCCs). While ENCCs migrate as a group along the gut, they receive various extrinsic signals from the surrounding tissue and neighboring NCCs that promotes their proliferation, migration, differentiation, and multipotency. Due to the complex and combinatorial nature of the signaling mechanisms involved in the establishment of the ENS, it has been difficult to tease apart the major driving forces of many of the defects involved in aberrant ganglion formation in the gut. Through single-cell RNA sequencing analysis of zebrafish NCCs and NCC-derived tissues, we identified differential expression patterns of several BMP pathway member encoding transcripts in NCC and ENCC populations during development. Through immunohistochemical and hybridization chain reaction (HCR) the analysis we demonstrate active BMP signaling and expression of target genes in NCC and ENCC populations in vivo, across space and time. We also discovered that broad chemical attenuation BMP signaling using the small molecule inhibitor K02288 during specific phases of ENCC development reduces the number of enteric cells in the most distal gut, in the zebrafish model. Overall, these results suggest that BMP plays a key role prior to and during the very early colonization phases of ENS development. The elucidation of BMPs as important membersof the enteric gene regulatory network will help to delineate the effects of BMP signaling, which serves as a driver for proper ENS colonization and formation. This project was funded by NICHD F31-HD104474 and CPRIT RR170062.

69 - Rewarding effects and reinstatement of methamphetamine-induced conditioned place preference in zebrafish

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The rewarding effects of drugs of abuse have been demonstrated in conditioned place preference (CPP) procedure in zebrafish. However, the addictive potential (ie, the vulnerability to relapse, measured by the ability to induce reinstatement of an extinguished response) of abused drugs, remains poorly investigated in zebrafish. In the present study, the effects of methamphetamine (MA) on the acquisition, extinction, and reinstatement of CPP were evaluated using a biased apparatus in zebrafish. MA via oral gavage dose-dependently increased the time spent in the drug-paired compartment during the post-conditioning test. Confined and non-confined extinction training significantly reduced the time spent in the MA-paired compartment. Following extinction, this place preference could be reinstated by exposure to a low dose of MA or stress. In addition, a CPP retest conducted after 14 days demonstrated the extinction persisted. Subsequent MA priming significantly reinstated CPP, indicating the unrelenting propensity to elicit MA-associated memory. This work demonstrates an incremental value of using CPP in zebrafish as an animal model in addiction research.

70 - Linking molecular abnormalities to behavioral deficits using a zebrafish model for tauopathies

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Tauopathies are neurodegenerative diseases characterized pathologically by accumulation of abnormal Tau in the brain. However, it remains unclear how these molecular and cellular dysfunctions lead to behavioral deficits, especially during the early stages of pathogenesis. To dissect disease mechanisms across multiple biological scales, we generated a zebrafish model of progressive supranuclear palsy (PSP), a primary tauopathy causing unexpected falls in patients early in disease progression, by expressing human 0N/4R-Tau in the evolutionarily conserved vestibulospinal (VS) nucleus. Human Tau-expressing zebrafish exhibit impaired balance control during free-swimming while maintaining normal locomotor ability compared to their siblings. Functional imaging of the VS nucleus shows impaired directionality index in Tau-expressing neurons in response to tilt stimulus. This altered neuronal activity correlates with Tau phosphorylation in VS neurons. Interestingly, we also observed ectopic accumulation of acidic organelles in the cell bodies of Tau-positive neurons, suggesting abnormal lysosomal function. Taken together, our zebrafish PSP model allows us to understand molecular and cellular mechanisms of balance deficits in tauopathies and can be a powerful system for preclinical drug screening and evaluation of potential therapeutic targets.

71 - Platelet factor Pku390 regulates myocardial and endothelial cell proliferation during zebrafish heart regeneration

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Zebrafish heart regeneration is a complex process consisting of multiple cell interactions and regulations. Previous studies have revealed essential roles of myocardial, endocardial, and epicardial signaling in heart regeneration, but it remains much unknown how leukocytes including platelets are involved in this regenerative process. Here, we report platelet pku390-PDGF signaling regulates zebrafish heart regeneration. We found that pku390 was specifically expressed in platelets and erythrocytes in adult hearts, and haploinsufficiency of pku390 disrupted myocardial/endothelial cell proliferation and heart regeneration with elevated cardiac fibrosis and collagen deposition after ventricular resection. By applying single-cell transcriptomic analysis of pku390+/- and control sibling hearts at different regenerative stages, we captured whole landscape of more than 60,000 single heart cells including cardiomyocytes, endothelial cells, epicardium, fibroblasts, macrophages, platelets, red blood cells, T cells, and B cells. Bioinformatic analysis of the single-cell transcriptomic data revealed close ligand-receptor connections between platelets and other heart cells, and in particular, the interaction of several platelet-specific expressing ligands including PDGFs and myocardial/endothelial receptors. Quantitative RT-PCR data confirmed that the platelet-specific expression of these ligands were induced during different stages of injured hearts, and that siRNA-mediated knockingdown of each of these ligands decreased myocardial and endothelial cell proliferation of injured hearts. Together, this work discovers the platelet-specific Pku390-PDGFs signaling on regulating zebrafish heart regeneration, which may also be exploited for promoting mammalian organ regeneration.

72 - A Collagen10a1 mutation disrupts cell polarity and causes skeletal defects in a medaka (Oryzias latipes) model for Schmid Metaphyseal Chondrodysplasia

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Small teleost species such as zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) have been increasingly used as models for human skeletal diseases, facilitating the investigation of disease mechanisms as well as the screening of drugs. Schmid Metaphyseal Chondrodysplasia (SMCD) is an autosomal dominant skeletal disorder characterized by growth plate abnormalities, hip deformities and dwarfism. Studies using SMCD patient samples and mouse models have shown that in SMCD, mutations in the trimerization domain of Collagen type 10a1 (Col10a1) lead to improper trimer assembly and an elevated level of endoplasmic reticulum (ER) stress. However, downstream pathways triggered by ER stress remain unclear. To elucidate the SMCD pathomechanism, we therefore established a medaka model for SMCD that is accessible to live imaging at high cellular resolution. Using CRISPR/Cas9, a *col10a1*^{D633a} medaka mutant that harbors a SMCD-relevant mutation was generated. We show that heterozygous *col10a1*^{D633a} mutants recapitulated SMCD phenotypes, such as elevated ER stress, skeletal deformities and dwarfism. In addition, carbamazepine, the only drug proposed for the treatment of SMCD and currently being tested in a clinical trial, rescued skeletal defects in *col10a1*^{D633a} mutants. Further examination of mutants using live confocal microscopy, correlative light-electron microscopy, as well as microfocus- and synchrotron-based X-ray tomographic microscopy revealed disorganization of skeletal tissues in mutants, suggesting aberrant matrix secretion and an impairment of cell polarity in *col10a1* cells. In line with this, transcriptome profiling of mutant *col10a1* cells revealed dysregulation of genes involved in the regulation of cell polarity, such as *refilin A* (*rflna*). Together, our data imply a critical role for cell polarity in SMCD pathogenesis and highlight the *col10a1*^{D633a} medaka mutant as an attractive novel model for SMCD drug screens.

73 - Tamalin function is required for neuronal survival and myelination in the zebrafish CNS

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Tamalin is a postsynaptic scaffold protein that regulates cytoskeletal events including neuronal growth and actin reorganization through Arp6 G-protein. Recent in vitro studies have shown that Tamalin plays an important role in synaptic plasticity by controlling endocytosis of group 1 metabolic glutamate receptors. Abnormal regulation of glutamate receptors in the central nervous system is known to be associated with glutamate-mediated neurodegenerative disorder. However, the in vivo function of tamalin in neuronal survival and neurological disease development is still unknown. In this study, we observed that the tamalin gene is expressed exclusively in neurons of the zebrafish central nervous system. Next, by analyzing knockout mutations in the tamalin gene in zebrafish and mice, we found that loss of Tamalin function induces neurodegeneration along with myelin degeneration in the central nervous system. Interestingly, hypomyelination was also observed independent of axonal defects in the CNS of tamalin knockout zebrafish and mouse, suggesting that Tamalin function is also associated with myelination. Furthermore, we revealed that the expression of group 1 metabolic glutamate receptors was increased in tamalin knockout zebrafish, suggesting that neurodegeneration in the absence of tamalin function was induced by excitotoxicity mediated by abnormal regulation of glutamate receptors. Taken together, our findings demonstrate that Tamalin function plays an important role in neuronal survival and axonal myelination in the central nervous system.

74 - Tissue-scale processes control the timing of vertebrate muscle cell fusion

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Skeletal muscle fibres consist of multinucleated cells that are precisely organised for efficient force transmission and motor function. The formation of skeletal muscle fibres requires the fusion of multiple muscle precursor cells, while the tissue is being formed through an interplay of signalling pathways and dynamic cell rearrangements. Current knowledge about this process is mainly derived from cultured cells and invertebrates, while the dynamics of muscle fibre morphogenesis in vertebrates *in vivo* remains largely unexplored. To address this, we live imaged myotome formation in zebrafish embryos at high spatiotemporal resolution and quantified cell behaviour. We generated complete *in toto* maps, following every muscle fibre during the first 16 hours of myotome formation. Analysing over 300 cells from four embryos revealed significant heterogeneity in cell morphology, orientation, and position during fusion. While these fusion events appeared stochastic at the cellular scale, a clear tissue-scale pattern emerged along the mediolateral axis of the animal. This corresponded to the spatiotemporal expression pattern of a fusion-specific gene, *Myomaker*. Perturbing FGF and Shh signalling disrupted the patterns in cell fusion and *Myomaker* expression. Interestingly, inhibiting the migration of neighbouring slow muscle cells altered the timing of fusion events without changing *Myomaker* expression, indicating that the physical movements of cells are key to tissue patterning. Our findings highlight how vertebrate muscle fusion differs from invertebrates in its dependence on tissue-level morphogenetic processes that shape the myotome.

75 - Noise-induced hearing loss in zebrafish model: Characterization of tonotopy and sex-based differences

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Hearing loss caused by persistent exposure to loud noise is one of the most common diseases in modern society. Many studies have demonstrated the characteristics of noise-induced hearing loss in human and non-human vertebrate models, including frequency-specific noise-induced hearing loss and sex-biased differences. Despite the increasing popularity of zebrafish as a model for NIHL, a better understanding of this model is needed to determine sex differences in NIHL. To study the features of zebrafish as they relate to an NIHL model, we tested various phenotypes after frequency-specific noise stimulation. The degree of damage to hair cells and hearing loss were investigated after exposing zebrafish to 200 Hz and 1 kHz continuous waves and broadband white noise with a bandwidth from 50 Hz to 1 kHz. After exposure to all frequencies, the larvae showed lateral line hair cell damage, which is superficially located. In adult zebrafish, the threshold of auditory-evoked potential signals is elevated. Moreover, the number of hair cells remarkably decreased in the rostral region of the saccule, after exposure to 1 kHz and white noise, whereas zebrafish exposed to 200 Hz noise showed a decrease in hair cells in the caudal region. Moreover, male zebrafish were found to be more vulnerable to noise than female zebrafish, as is the case in humans and other mammals. Cortisol levels also increased in the noise-exposed male group, as compared to the noise-exposed female and control male groups. However, there was no difference in cortisol levels when the noise-exposed female group was compared to the control female group. Our study demonstrates not only that noise-induced hearing loss is frequency-dependent but also that the degree of hearing loss is affected by sex in zebrafish, emphasizing the need to consider sex in NIHL studies.

76 - Using single-cell genomics approaches to uncover a conserved Gata4/5/6 gene regulatory network in cardiopharyngeal development

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GATA4/5/6 transcription factors play essential, conserved roles in heart development. To understand how GATA4/5/6 mediate the mesoderm-to-cardiac fate transition, we labelled, isolated, and performed single-cell gene expression analysis on cells that express *gata5* at pre-cardiac time points spanning zebrafish gastrulation to somitogenesis. We found that most mesendoderm-derived lineages had dynamic *gata5/6* expression. In particular, *gata5* expression was maintained in the cardiac lineage, yet gradually silenced in the pharyngeal mesoderm, suggesting that the modulation of *gata5*expression may be crucial for cardiopharyngeal cell-fate divergence. In the absence of Gata5/6, the population structure of mesendoderm-derived cells is substantially altered. In addition to the expected absence of the cardiac mesoderm, we confirmed a concomitant expansion of cranial-pharyngeal mesoderm. Moreover, Gata5/6 loss led to extensive changes of chromatin accessibility near cardiac and pharyngeal genes (e.g. *tbx1*, *ebf*) prior to the establishment of either fate, consistent with the known pioneering role of the GATA factors in establishing permissive chromatin states for cellular competence. Functional analyses in zebrafish and the tunicate *Ciona*, which possesses a single GATA4/5/6 homolog, revealed that GATA4/5/6 likely acts upstream of *tbx1*and *ebf2* to exert essential and cell-autonomous roles in promoting cardiac and inhibiting pharyngeal mesoderm identity. Through single-cell open chromatin profiling at corresponding stages, we identified *gata5 cis*-regulatory elements (CREs), which could be responsible for maintaining its cardiac-specific expression. Notably, one CRE with cardiac-enriched accessibility compared to pharyngeal mesoderm displays high sequence conservation with human and mouse and harbors a TBX1 motif. As enhanced *tbx1* expression is a conserved feature for the pharyngeal mesoderm, and *Tbx1/10* antagonizes *Gata4/5/6* in Ciona, we propose that balancing the cardiac and pharyngeal mesoderm fates is controlled by a deeply conserved GATA4/5/6-TBX1 antagonistic regulatory circuit.

77 - Improving transgenic Zebrabow lines for advanced colorimetric barcoding

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Transgenic multicolor labeling strategies, such as Zebrabow, are well-established and powerful tools allowing the investigation of cellular behavior at a population level and tracking of individual cells over time. The Zebrabow system is based on Cre-loxP recombination and creates a stochastic choice of expression among fluorescent proteins, resulting in indelible labeling with multiple distinct colors. The predominantly used Zebrabow system has several limitations including suboptimal fluorescence intensity, failure to fill all cell compartments, and unproportioned expression of non-recombined fluorophores in the transgenes. Here, we generate new Zebrabow plasmids and create stable transgenic lines to overcome these limitations. The new transgenic Zebrabow lines have increased color varieties and minimal sequence homology of the fluorophores compared to the classic Zebrabow system. Additionally, these advanced Zebrabow transgenic lines harbor three pairs of incompatible loxP sites, allowing the insertion of a 'stop' cassette into the first position of the genetic cassette. This modification leads to the expression of the three fluorophores only in Cre-positive cells, giving more spectral diversity correcting the color imbalance caused by default fluorophore expression in Cre-negative cells. This 'stop' cassette consists of a non-fluorescent marker in the default position to facilitate screening purposes, such as uncovering cassette insertion numbers or performing immunolabeling. The transient activation of Cre recombinase expression results in time-specific color labeling, and all daughter cells will inherit the same color as their progenitor, allowing lineage tracing. We show that the newly generated Zebrabow transgenic lines have a high recombination efficiency and Cre-positive specific expression in embryonic stages. In the future, we are planning to elucidate recombination, color stability, and stochastic analysis in adult stages and diverse tissues. The Zebrabow system, presented here, could provide a new resource for next-generation color-based lineage analyses in zebrafish.

78 - Pleotropic Embryonic Defects Due to Loss of Fucosylation

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GDP-mannose 4-6-dehydratase (*GMDS*) plays key role in *de novo* synthesis of cellular GDP-fucose. In mammals, GDP-fucose is required for essential biological functions such as posttranslational modifications and as such, deficient fucose metabolism due to *GMDS* loss of function has been implicated in multiple diseases. Genome-wide association studies (GWAS) have revealed that single nucleotide polymorphisms (SNPs) in the *GMDS* gene have significant associations with stroke and primary open-angle glaucoma (POAG) risk, indicating that this gene could play important roles in the development and maintenance of vision and cardiovascular health. In this study, we developed a novel *gmds* loss of function zebrafish model using CRISPR Cas9 mutagenesis to investigate the phenotypic consequences of the *gmds* gene mutation in vascular development and glaucoma onset. Homozygous zebrafish *gmds* mutant embryos exhibit cerebral hemorrhages at 2 days post fertilization (dpf), with defects in vascular patterning and maturation that may be dependent on Notch and Cxcl12 /Cxcr4 signaling. These data clearly indicate a novel role for *GMDS* in vascular development and maintenance in vertebrates.

Homozygous *gmds* mutant embryos die by 8 dpf, and no defects are observed in the eye or optic nerve at this time. Thus, heterozygous *gmds* mutants were utilized to study the effect of *GMDS* loss of function in POAG development. We examined the retinal layers' structure and optic nerve health of heterozygous *gmds* mutants at 6 months, 12 months, and 19 months respectively, using optical coherence tomography (OCT) imaging and ocular histology. Significant loss of retinal ganglion cell layer was observed in 6 months and 19 month old *gmds* heterozygous mutants. Studies to elucidate molecular pathways regulated by *GMDS* for ocular maintenance and glaucoma onset are ongoing, and will help to better understand the role this gene plays in human glaucoma.

79 - Ndrgs: Molecular integrators of hypoxia and circadian rhythm signaling

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Hypoxia-induced injury is a contributing cause to many human diseases, including stroke, pulmonary vascular disease, and chronic kidney disease. Research using hypoxia-tolerant organisms such as zebrafish can shed light on mechanisms of hypoxia adaptation and reveal potential therapeutic targets for the prevention or treatment of hypoxic-ischemic injury. Currently, the Brewster lab is investigating N-Myc Downstream Regulated Gene 1a (Ndrg1a), a member of the α/β hydrolase superfamily that plays a vital role in hypoxia adaptation by promoting hypometabolism. Recent work in our lab has found that in the kidney, Ndrg1a works to downregulate the sodium-potassium ATPase pump, normally a large consumer of ATP. We hypothesize that it also prevents ionic imbalance and cellular edema by coordinating the removal of this pump with that of other ion and water channels. Additionally, Ndrg1b, a paralog of Ndrg1a, has previously been demonstrated to alter its expression patterns in a circadian manner. Given that Ndrg1a is responsive to hypoxia, Ndrg1b is regulated by circadian rhythms, and that these pathways are involved in crosstalk at the molecular level, we hypothesize that both Ndrg1a and Ndrg1b may act as integrators of environmental signals (circadian rhythms and oxygen levels). Oxygen consumption in diurnal organisms cycles in a circadian manner, thus we posit that these proteins may play similar roles with regard to both signals. Preliminary data suggest that Ndrg1a expression may be regulated in a circadian manner and Ndrg1b may be hypoxia-responsive. These findings raise the question of whether Ndrg1a/b play a broader role in metabolic regulation under conditions in which energy supply and demand are limited. Future work will explore the mechanism by which these proteins function and is expected to address a significant gap in our understanding of metabolic regulation in zebrafish.

80 - Neuroepigenetic regulation of a tunable behavioral circuit

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Our ability to form and retain memories relies on neural circuits being flexible and adaptive. At the same time, neurons must maintain essential synaptic connections throughout the entire lifespan of an organism, striking a fine balance between flexibility and stability. Disruption of this balance can lead to neurodevelopmental and neuropsychiatric disorders. I utilize larval zebrafish to investigate the genetic and epigenetic mechanisms driving the development and activity of behavioral circuits, with a focus on the acoustic startle response. This is a conserved behavior, driven by a well-characterized neural circuit and modulated by experience, that is frequently disrupted in a variety of disorders, including schizophrenia. I am currently investigating how chromatin regulators drive startle circuit development and modulate behavioral plasticity. I first took a pharmacological approach and screened a library of 148 inhibitors to epigenetic targets. From this screen, I identified multiple compounds that disrupted startle habituation, including HDAC and SET7/9 inhibitors. Based on these results and additional genes associated with human neurological disorders, I am generating mutants in epigenetic regulators to investigate how these proteins regulate circuit development and/or function. In addition, I am developing tools to investigate gene expression and chromatin changes in startle circuit neurons both during normal development and with epigenetic dysregulation that disrupts behavior. My results so far confirm that epigenetic regulation affects startle behavior in larval zebrafish, and with my future studies, I aim to generate a comprehensive view of how epigenetic regulation during development contributes to establishment and function of a conserved behavioral circuit.

81 - Differential expression of cyp26b1 in the tooth germ controls tooth morphogenesis in fish

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Fish display an impressive diversity in tooth shape and size. While many factors have been identified in the process of tooth morphogenesis, however, little is known about how cell signaling participates in establishing organ shape during odontogenesis. Our previous work has identified the signaling molecule retinoic acid (RA) as one of the main actors of tooth induction in fish. By exposing embryos to exogenous RA and to an RA inhibitor we have been able to quantify changes in tooth shape. Exogenous RA exposure in zebrafish make the developing tooth narrower and more elongated resembling the tooth shape observed in the wild like in the white cloud minnow. RA exposure in goldfish, with shorter and wider tooth makes it also narrower and more elongated making it more like a zebrafish tooth. Expression of tooth epithelium and mesenchyme markers *dlx2a*; *dlx3b*; *pitx2a*; *runx2b* and *lhx6* is expanded in RA treated zebrafish embryos. Our data reveal that the levels of RA in the tooth germ is controlled by the timing and level of expression of the RA degrading enzyme *cyp26b1* in a subset of cells of the tooth. *cyp26b1* expression is delayed in the white cloud minnow compared to zebrafish and the odontogenic gene expression *dlx2a* in the minnow tooth resembles the expression of *dlx2a* in the zebrafish tooth when Cyp26 activity is blocked. We identified a specific enhancer region of *cyp26b1* controlling the expression of this gene in the zebrafish tooth germ. We propose that modifying the onset of *cyp26b1* expression in the tooth germ will change the level of RA available, ultimately altering the shape of the tooth. This project has revealed that fine tuning of RA level by *cyp26b1* expression in the developing tooth is a crucial player in the vast tooth diversity observed in fish species in the wild.

82 - Investigating the combined use of cannabinoids to treat epilepsy in a zebrafish model

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Though decades of anecdotal and cultural evidence support the treatment of epilepsy with cannabis, the medical community is hesitant to accept its use due to inadequate information on the mechanism of action and long-term effects. The phytocannabinoid cannabidiol (CBD) is approved to treat patients with severe epilepsy syndromes, but there are many individuals with less severe epilepsies whose quality of life is negatively affected by side-effects from current antiepileptics. This research aims to determine which cannabinoids have antiepileptic activity and to evaluate which treatments are the most effective, using a chemical model of epilepsy. Using a high-throughput format, zebrafish larvae were treated with phytocannabinoids and then exposed to the convulsant pentylenetetrazole (PTZ). Seizures were quantified simultaneously in 96 larvae using a high-resolution camera to determine potential antiepileptic effects, while a novel HPLC analysis of larvae quantified cannabinoid uptake. Treatment with cannabinol (CBN) and Δ^8-tetrahydrocannabinol (Δ^8-THC) decreases seizure intensity and had similar uptake as compared to CBD. Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabichromene (CBC), and cannabigerol (CBG) showed antiepileptic effects at high dosage, however tissue uptake of CBC and CBG was decreased as compared to CBD. Paired treatments suggest the activity of CBD can be enhanced in the presence of other antiepileptic phytocannabinoids. Interestingly, treatment with the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA) did not provide seizure relief. Moving forward, we will use RT-qPCR to study the effects of phytocannabinoid treatment on expression of seizure markers and endocannabinoid associated enzymes, providing insight into the mechanism of action. Our data supports the hypothesis that phytocannabinoids are promising antiepileptics and could be used in tandem for more effective treatment. The mechanism of action of these compounds will give the medical community more information for use of cannabinoids as treatments.

83 - The Calcium-Sensing Receptor (CaSR) regulates zebrafish sensorimotor decision-making via a genetically defined cluster of hindbrain neurons

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Decision-making is a function of nervous systems critical for both human well-being and animal survival. Sensorimotor decision making, defined as the rapid selection of a single motor response from a defined set of potential responses to a sensory stimulus, is a comparatively simple form of decision-making that is highly tractable for circuit and genetic analyses. We previously established larval zebrafish as a model for sensorimotor decision-making and identified the G protein coupled *Calcium-sensing Receptor (CaSR)* to be critical for this process. Here we report that *CaSR* function is dispensable for sensory transduction and motor output of the decision-making process, instead dynamically regulating the bias between two mutually exclusive, acoustically evoked behaviors. We show that transgenic expression of CaSR in neurons, but not glial cells, restores typical decision-making bias in *CaSR* mutants, demonstrating a neuronal role for CaSR in decision-making. By employing a whole-brain computational strategy called Multivariate Analysis of Variegated Expression in Neurons (MAVEN), we identify a single, genetically defined cluster of a few hundred neurons as a candidate site for CaSR function. Moreover, we demonstrate that CaSR expression targeting this cluster, which has never before implicated in behavior, shifts decision-making in wild-type larvae and restores deficits in *CaSR* mutants, providing compelling evidence that this cluster is indeed a key site for CaSR function. Combined, our data provide a rare example of a G protein coupled receptor that biases vertebrate sensorimotor decision-making via a defined group of hindbrain neurons.

84 - Spinal inhibitory circuits: new roles in locomotion

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Movement and locomotion are coordinated from head to tail along the long axis of the spinal cord. The spinal cord is home to many neurons that project long ascending or descending axons along this axis, but synaptic targets of these long projections remain unknown. Most assays of synaptic connectivity are carried out in spinal cord slices, where this long-range connectivity is entirely lost. However, long-range connections are vital to both motor coordination and spinal cord repair. Using *in vivo* recordings, we show that the spinal V1 ($En1^+$) population projects long axons over ~6 muscles segments. Surprisingly, the postsynaptic contacts of these V1 neurons change over the length of the axon projection. We find that V1 neurons inhibit motor neurons and other premotor targets at short range, but at long range selectively inhibit sensory targets instead. With modeling, we demonstrate that this local inhibition onto motor neurons provides strong temporal control, preventing inappropriate activity outside of the desired movement. Furthermore, we extend this approach to the V2b (Gata3+) population to show that descending inhibition is more broadly spread through the rostrocaudal axis. Together these data reveal that the rostrocaudal structure of the spinal cord circuit is more complex than previously appreciated. We conclude that mapping the spinal cord from head to tail will provide a better understanding of locomotor control.

85 - In vivo profiling of site-specific human cancer cell states in zebrafish

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We present a quantitative high-resolution imaging assay of cancer cell morphology in zebrafish xenografts to probe functional adaptation of single cells. We focus on Ewing Sarcoma, a pediatric cancer driven by a single oncogenic fusion protein EWSR1-FLI1, and with little to no additional somatic mutations, making it a prototypical form of cancer whose adaptation to microenvironments is likely driven by acute, non-genomic mechanisms. Computer vision analysis of 3D cell shapes reveals systematic shifts in the distribution of morphological states as a function of cell type and seeding site, as well as tissue-specific cellular organizations that recapitulate those observed in human tumors. Loss of EWSR1-FLI1 causes a shift to more protrusive cell states, with increased morphodynamics and decreased tissue-specific specialization. Combining these data, we propose a model where changes in EWSR1-FLI1 expression levels enable differential modes of Ewing Sarcoma cancer cell adaptation.

86 - Dynamic buffering of extracellular chemokine to enable robust adaptation during directed tissue migration

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How developing tissues adapt to achieve phenotypic stability in response to highly variable genetic and environmental inputs is poorly understood.

Here, we take an experimental approach that combines quantitative imaging with inducible perturbation experiments to address this fundamental question using the zebrafish lateral line primordium, a migrating tissue guided by Cxcr4-mediated chemokine signalling. Upon exposure to abrupt ‘chemokine floods’, migrating tissues arrest transiently before recovering their baseline migratory behaviour under the continued presence of elevated chemokine. Investigation into the mechanism that underlies this ‘perfect adaptation’ revealed a chemokine-triggered phosphorylation of the scavenger receptor Cxcr7b that reroutes it from constitutive ubiquitination-regulated degradation to cell surface recycling, thus coupling scavenging capacity to extracellular chemokine levels. We finally show that Cxcr7b phosphorylation is not required for directed tissue migration in the presence of physiological chemokine, but essential when chemokine levels are increased.

This work uncovers a new feedback strategy where the adaptation burden is shifted from a canonical GPCR to an autonomously acting scavenger receptor that directly and dynamically buffers the extracellular guidance cue. We predict that this ‘outsourcing’ strategy for adaptation may be general to other cell and tissue mobilisation contexts such as immune response, stem cell homing, and pathological invasion.

87 - Flow-dependent and flow-independent functions of unconventional type 1 Myosins in zebrafish Left-Right Asymmetry

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While similar mechanisms control animal antero-posterior and dorso-ventral polarity, different species display striking variations in the establishment of Left-Right (LR) asymmetry. In spite of this apparent diversity, we previously identified the unconventional Myosin Myo1D as a conserved regulator of LR asymmetry. While *Drosophila* Myo1D acts locally and in the absence of cilia to control organ chirality, zebrafish Myo1D controls motile cilia orientation and the establishment of a symmetry-breaking fluid flow in Kupffer's Vesicle, the animal's central LR Organizer (LRO).

In addition to Myo1D, the zebrafish genome encodes the closely related protein Myo1G. Although *myo1g* mutations impair laterality and enhance the defects of *myo1d* mutants, *myo1g* and *myo1d* exert distinct functions. *myo1d* mutations impact all lateralized organs, in accordance with a role in the generation of the central LRO flow. In contrast *myo1g* mutations impair heart and brain laterality but have no effect on the viscera, raising the question whether *myo1g* might exert a flow-independent function? Accordingly, *myo1g* inactivation further modifies the laterality phenotypes of mutants that already completely lack a LRO flow. We will present evidence that in contrast to the flow-dependent control of LR asymmetry exerted by Myo1D, Myo1G is important for the Nodal-mediated transfer of laterality information.

88 - Single-Cell Analysis of the Zebrafish Inner Ear

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A major cause of human deafness is the permanent loss of the mechanosensory hair cells of the inner ear. On the contrary in the zebrafish lateral line system, hair cells can regenerate from neighboring supporting cells throughout life. Here we sought to characterize the zebrafish inner ear as an alternative model for studying hair cell regeneration. To do so, we performed single-cell RNA and ATAC sequencing of the zebrafish inner ear at multiple stages to catalog the diversity of hair, support, and progenitor cells and their homology to mammalian counterparts. We identify bipotent progenitors for hair and support cells that appear to be unique for the zebrafish inner ear versus the lateral line. We identify several types of support cells, including distinct types in the macula versus cristae. In the macula, we identify two types of hair cells that share gene expression with either mammalian type I or type II cells. In situ hybridization reveals that these hair cell subtypes occupy distinct spatial domains within the two major macular organs, the utricle and saccule, consistent with the reported distinct electrophysiological properties of hair cells within these domains. These findings suggest that primitive type I and II hair cells likely arose in the last common ancestor of fish and mammals, with subsequent modifications along the mammalian lineage. Similarities of inner ear cell type composition between fish and mammals support zebrafish as a relevant model for understanding inner ear-specific hair cell regeneration.

89 - The effects of social experience on synergistic neuromodulation of motor circuits

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We investigated the effect of social dominance on the activation of the escape and swim motor circuits. Using a non-invasive technique, we recorded the activation of the escape and swim circuits in dominant and subordinate fish and showed social-status dependent differences. Subordinates increased escape response activation and decreased swimming activation. Whole brain western blot analysis showed a significant decrease of both the dopamine transporter (DAT) and dopamine receptor type 1 (DRD1b) in subordinates compared to their dominant counterpart. To investigate how status-dependent expression changes in the dopamine signaling pathway may be involved in altered motor circuit activation, we blocked DRD1b activity with the antagonist, SCH23390, and with a CRISPR/Cas9 knockout line. Inactive DRD1b shifted dominant behavior from favoring swim to escape, resembling a subordinate phenotype, supporting the role of DRD1b in status-dependent motor circuit activation. We further showed that this shift in dopaminergic balance of circuit activity is mediated indirectly through activation of glycinergic interneurons using the antagonist, strychnine. To quantify differences in DRD1b expression on hindbrain glycinergic nuclei that provide inhibitory input onto the escape circuit, we generated a transgenic *Tg*(Glyt2a:GFP) line and stained for DRD1b. Our results reveal how changes in relative excitability of multiple neuromodulatory inputs provide a mechanism for the nervous system to adapt to changes in social conditions, allowing animals to select a socially appropriate behavioral response.

90 - Automated Ultra-High-Throughput Behavioral Motion Tracking of Larval Zebrafish

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Zebrafish (Danio rerio) are commonly used as a model organism for behavioral studies aiming to elucidate patterns of movement that correlate to specific stimuli or developmental states. These studies observe a large number of larvae with microscopes, typically at low magnifications (1x - 4x), to relate experimental conditions to the behavior of 3 to 7 dpf larvae. Traditionally this type of experiment is either laboriously executed using a conventional microscope one fish at a time, limiting throughput of experiments, or with a low resolution system, limiting the behavioral measurements that can be captured. Here, we propose and demonstrate how one can use a novel micro-camera-array-microscope (MCAM™) system to record and stimulate zebrafish, through optical flashes or mechanical vibration, in standard well plate configurations including, 96-, 48-, and 24-well at both high spatio-temporal resolutions, and high throughput. The high resolution images and videos acquired from the MCAM enable one to not only track the overall movement of the larvae (position, velocity, and acceleration), but to identify the different body parts of the zebrafish to measure key kinematic metrics such as tail bend angle, and beat frequency. Videos of zebrafish larvae in these configurations can be captured, simultaneously, at up to 120 frames per second. The videos are then processed automatically using convolutional keypoint detection and yield high precision coordinates of eight key points localized in cartesian space for each frame. Results are reported in spreadsheet format (CSV files) for synthesis and visualization. We've also extended the imaging protocol of MCAM based assays to image embryos in well plates with mesh inserts to facilitate wash and transfer protocols during an experiment. Utilizing the MCAM and associated analysis software, one can increase the throughput of behavioral assays of zebrafish larvae requiring high speed and high resolution observation while improving the statistical significance of experiments.

91 - foxg1a is required for hair cell development and regeneration in the zebrafish lateral line

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***foxg1a* is required for hair cell development and regeneration in the zebrafish lateral line**

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University of Missouri Kansas City

Mechanosensory hair cells, located in the inner ear, mediate the sensations of hearing and balance. Mammalian inner ear hair cells lack the ability to regenerate following damage, resulting in permanent sensory deficits. In contrast, aquatic vertebrates like zebrafish *(Danio rerio)* have a specialized class of mechanosensory hair cells found in the lateral line system, which allows them to sense changes in water current, and are robustly able to regenerate following damage. The canonical Wnt signaling pathway is critical for development and regeneration of the lateral line. To identify Wnt target genes, we used RNA-sequencing to compare the transcriptomes of wild-type and mutants with a lesion in the Wnt pathway transcription factor *lef1.* We identified the forkhead box transcription factor, *foxg1a*, as a Wnt target in the lateral line. In mice, Foxg1 downregulates Wnt activity by repression of *wnt8b* (Hardcastle et al. 2000), and functions to promote normal development of mammalian hair cells as well as other tissue. Foxg1 mutations in humans result in severe neurological and developmental disabilities. The zebrafish *foxg1a*^{a266} mutant was generated using CRISPR-Cas9 genome editing (Thyme et al. 2019). Examination of *foxg1a*^{a266} mutants found that while the initial formation of the lateral line appears normal, mutants develop significantly fewer hair cells compared to heterozygous controls. In addition, *foxg1a*^{a266} zebrafish show reduced hair cell regeneration following damage with neomycin. This work will allow us to better describe the molecular and cellular basis for mechanosensory hair cell development and regeneration.

92 - The effect of adolescent idiopathic scoliosis (AIS)-associated gene FNDC1 on zebrafish vertebrae morphology

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Adolescent idiopathic scoliosis (AIS) is the most common spinal deformity presenting in adolescents, with some cases becoming severe enough to warrant major surgical intervention. AIS a complex trait; therefore onset is likely the result of multiple gene variants interacting directly or indirectly. However, genes associated with curve progression are largely unknown. To examine candidate variants responsible for severe AIS curve progression, we performed a gene burden analysis on whole exome data from patients suffering from severe AIS and two independent control data sets. We observed an enrichment of ultra-rare variants in core matrisome genes in severe AIS patients- the most significant enrichment of ultra-rare variants associated with severe AIS was in FNDC1 (fibronectin type III domain containing 1). To characterize the morphological effects of perturbance of this gene on skeletal development and morphology, we examined FNDC1 mutant zebrafish containing a nonsense mutation. While we did not observe a gross phenotype in the structure of the skeleton, upon skeletal examination via microCT, we found higher bone mineral density in mutant vertebrae, as well as larger vertebral cross-sectional area. The vertebral centra also exhibited a higher pMOI score, indicating that the shape of the vertebrae is altered in FNDC1 mutants. This suggests that mutations in FNDC1 may act as a risk factor for severe AIS by affecting the structure of vertebral bone tissue. These clinical and zebrafish data support a model in which FNDC1 gene defects have functional effects on vertebral development which may be a contributing factor to AIS.

93 - An intricate immune balance necessary for natural spinal cord regeneration

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At present, spinal cord injury (SCI) in mammals causes irreversible sensory and motor function loss. Adult zebrafish naturally regenerate a fully severed spinal cord (SC), and thus provide a system for the discovery of factors necessary to promote regeneration. Following injury, the immune system is necessary to clear damaged tissue and provide a permissive environment for tissue repair; however, it is unclear what aspects of the immune response are beneficial or detrimental to neural regeneration. Zebrafish possess an immune system evolutionarily conserved with mammals, allowing us to use the adult zebrafish to tease apart the immune balance required to support successful SC regeneration. We have discovered the immune response to SCI in adult zebrafish is distinct from that of mammals in cell composition, timing, and clearance. Using pharmacological and genetic approaches to deplete immune cells, we found leukocyte activation is necessary for SC regeneration, specifically in the acute phase of injury. Combining single-cell transcriptional datasets of the regenerating SC with CRISPR/Cas9 neurobehavioral screening, we have identified *transcription and immune response modulator* (*tcim*) as a previously unknown microglia/macrophage-specific gene that regulates the clearance of post-injury inflammation. In *tcim* mutants, the SC does not regenerate, microglia and macrophages adopt a more pro-inflammatory state, and an excess of blood-derived leukocytes amass and persist within the lesion site. Together, our data indicate an intricate choreography of post-injury inflammation comprised of leukocyte identity, activation state, and gene expression that is necessary for natural SC regeneration.

94 - Defining the Critical Period for Initial Swim Bladder Inflation in Larval Zebrafish (Danio rerio)

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Deficits to swim bladder inflation in teleost fish can cause major developmental issues including inflammatory responses, malformed bodies, deficits in food conversion, decreased life span, and most commonly, mass larval mortality. In zebrafish, we observe 100% mortality among larvae that fail to initiate swim bladder inflation. Because initial inflation coincides with the beginning of feeding, it is assumed that uninflated larvae are unable to acquire food and die from starvation. However, preliminary evidence suggests that uninflated larvae starve because of a failure to consume food, even when it is readily available. Understanding why uninflated larvae die even though food is easy to acquire requires a better understanding of initial inflation, including the temporal limits of initial inflation in zebrafish. Larvae of most teleosts initiate inflation by gulping air and forcing it into the swim bladder through the pneumatic duct that connects the bladder and gut. In physoclistous fish, the pneumatic duct degenerates during larval development, delineating the critical period during which initial inflation must occur but zebrafish are physostomes who normally retain their pneumatic duct through adulthood. Therefore, it is unknown whether zebrafish also have a critical period for initial inflation. This project will define the critical period for swim bladder inflation in zebrafish larvae with the goal of understanding the unexplored connection between hypoinflation and starvation. The physiological and metabolic mechanisms for this under-feeding phenomenon will be examined as part of future research.

95 - An inflation sensation: Initial swim bladder inflation in larval zebrafish is mediated by the mechanosensory lateral line

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Larval zebrafish achieve neutral buoyancy between 3-4 days post-fertilization by gulping air from the water's surface to inflate their swim bladders. We define this behavior of swimming to the air-water interface as "surfacing." Little is known about the sensory basis for this under-appreciated behavior of larval fish. A strong candidate is the mechanosensory lateral line, which is a hair cell-based sensory system that detects hydrodynamic information from sources like water currents, predators, prey, and surface waves. However, the influence of the lateral line on the larval behaviors that mediate swim bladder inflation remain unexamined.

To explore the connection between the lateral line and surfacing behaviors, we utilize a genetic mutant (*lhfpl5b*$^{-/-}$) that specifically silences the lateral line from birth. We observe that 46% of lateral line mutants over-inflate their swim bladder during initial inflation and become positively buoyant. Thus, we hypothesize that larval zebrafish use their lateral line to sense the air-water interface and regulate swim bladder inflation. In support of the hypothesis that lateral line defects in mutants are responsible for swim bladder over-inflation, we show (i) that ototoxic ablation of the wild type lateral line reproduces the mutant phenotype and (ii) that transgenic rescue of the mutant restores normal inflation. To test the hypothesis that over-inflation is caused by abnormal surfacing behaviors in lateral line mutants, we report that (i) the over-inflation phenotype requires access to the air-water interface and that (ii) lateral line mutants visit the surface more frequently and spend more time at the surface than wild type siblings. In summary, we have discovered a novel sensory basis for achieving neutral buoyancy where larval zebrafish use their lateral line to sense the air-water interface and regulate swim bladder inflation.

96 - Zebrafish MeCP2 mutation produce behavioral alterations reminiscent of Rett Syndrome

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Rett syndrome is an autism spectrum disorder and one of the most common causes of mental retardation in women. Sporadic mutations on the transcription factor MeCP2 has been found as one of the most frequent causes for Rett syndrome in humans but how MeCP2 mutations cause of Rett syndrome is still largely unknown (Kyle et al. 2018). In recent years multiples models of Rett syndrome had been developed in mice and zebrafish (Pietri et al. 2013). Mice models show affected social behavior, but so far there are no good descriptions of how MeCP2 mutations affect the social behavior adult zebrafish. To elucidate the role of MeCP2 in behaviors, I examined behavioral phenotypes in zebrafish MeCP2 mutants. Interestingly, MeCP2 mutant adult zebrafish showed increased anxiety and fear response, as well as reduced thigmotaxis, which are reminiscent of the Rett syndrome phenotype, specifically high anxiety. On the other hand, they showed normal social preference. Next, we examined whether zebrafish MeCP2 mutants harbor defects in brain development. Our imaging analysis revealed that zebrafish MeCP2 mutants showed increased size in the DM region of the telencephalon, a region homologous to the amygdala in mammals and responsible for fear behaviors, compared with wild-type siblings. These data suggest that MeCP2 plays a crucial role in neural circuit formation responsible for fear response in telencephalon.

97 - The origin and lineage of cells that give rise to the zebrafish fin

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While studies have unraveled the overall processes of embryonic development, the process of post-embryonic development, where small animal prototypes acquire species-specific size and morphology, is not well understood. Recent cell-tracking studies in zebrafish and medaka fish have shown that cells of adult fin, mesenchymal cells, osteoblasts, and osteoblast progenitor cells (OPCs), are derived from the somites of embryonic stage. However, it is still an open question when and how the mesenchymal cells differentiate into respective cell types.

In this study, we used the Cre-*loxP* system in zebrafish to label and track the lineage of *sox9a+* somite cells. The labeled *sox9a+* cells in the somites contributed to the fin fold mesenchymal cells of larvae and to the mesenchymal cells, osteoblasts and OPCs of adult fin, indicating that these were derived from the *sox9a+* somite cells. However, the single cell-tracking analysis showed that the larval fin fold cells mostly disappear during larval stage without contributing to the adult fin. Instead, cells originated from the basal region of the fin fold proliferated to become the adult cells including the mesenchymal cells, osteoblasts and OPCs. Thus, it was revealed that a small population of somite-derived cells are destined to contribute to the adult appendages. Additionally, the cell tracking analysis has also shown that a somite cell contributes to either the osteoblast-lineage or the mesenchymal cells of adult fin, and that they do not change the fate during growth and regeneration. These observations suggest that the fate decision, either of osteoblast-producing lineage or mesenchymal lineage, has already occurred in the somite stage, and that the memory of cell types is maintained throughout the life. Thus, *sox9a+* somite cells contain three different committed fates, larval fin fold mesenchyme, adult osteoblast-producing lineage, and adult fin mesenchyme.

98 - Functional validation of a new XLID gene and its use in drug discovery

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X-linked intellectual disability (XLID) syndromes are associated with X chromosome predominantly affecting males. Previous studies have revealed that a microdeletion encompassing the *CNU162* gene in human XLID patients is associated with motor dysfunction, intellectual disability and autism. We cloned the zebrafish *cnu162* gene and found its spatio-temporal expression in a subset of interneurons and myotomes during early stages of development. Induction of loss of function was done by the CRISPR-Cas9 mediated knockout (KO) technology and two alleles of +34 bp or -11 bp mutation were established. Through early morphological and behavioral analyses, we observed that *cnu162* homozygous KO mutants have defects in locomotion pattern after tactile response when compared to wild type siblings between 48 to 70 hpf. The KO mutant phenotype is rescued by overexpression of wild type Human *CNU162* mRNA. Then, we screened small molecules using *cnu162* KO zebrafish and, to our surprise, identified a chemical (Chemical A) which can partially rescue the mutant phenotype, aberrant locomotion. We synthesized various Chemical A-based derivatives, performed phenotype-based screening, and finally found another bioactive compound (Chemical B). We are currently trying to understand the underlying molecular mechanisms involved in the signaling pathway and to use this disease model system in developing therapeutic approaches for the *CNU162*-related XLID syndromes.

99 - Towards a molecular understanding of zygotic genome activation in zebrafish

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During embryo development, the zygotic genome transitions from a transcriptionally silent to a transcriptionally competent and active state (also known as zygotic genome activation, ZGA). Thus, in the context of transcriptional regulation, the embryo is a good system to study transcription. In zebrafish, the transcription factors (TFs) Pou5f3/OCT4, Sox19b/SOX2, and Nanog are responsible for the activation of >75% of zygotic genes (1, 2). Genome-wide analyses of the chromatin landscape have revealed correlations between gene expression and chromatin accessibility during ZGA. One suggested mechanism is that, like its mammalian counterparts, the zebrafish ZGA regulators could act as pioneer factors and bind to nucleosomal DNA, make chromatin accessible, and prime genes for activation. Our goal is to obtain a more mechanistic understanding of the molecular events that set the genomic stage for transcriptional engagement.

Here, we present a biochemical, imaging, and genomics framework to study how transcriptional machineries come together to regulate ZGA. To identify interacting partners of the ZGA regulators, we used biotinylated oligonucleotides that mimic TF binding sites as pulldown baits. TF-capture experiments were performed in zebrafish embryonic extract. We will present our preliminary results and discuss mechanistic insights into the coordinated actions of TFs and their interacting partners in regulating gene expression during early embryo development.

1. Lee et al. Nature. 2013.2. Leichsenring et al. Science. 2013.

100 - 5-Bromoprotocatechualdehyde derived from Polysiphonia japonica inhibits parkin degradation and preserves pancreatic β-cells against palmitate toxicity

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Diabetes is a major health issue increasing worldwide. Currently, nearby half a billion people have diabetes, which will increase by 25% in 2030 and 51% in 2045. Hyperlipidemia is a key pathological feature of diabetes; it exerts dysfunction and death on β-cells through production of cytotoxic metabolites and activation of harmful signaling pathways. Hence, maintaining β-cells health and preventing β-cell degeneration will be essential approaches in prevention and/or treatment of diabetes. In our previous study, it has been confirmed that the crude extract of red seaweed, *Polysiphonia japonica* has prominent effect on insulin secreting β-cells against palmitate-induced lipotoxicity, and thus, the substance was isolated to confirm its standard component, 5-bromoprotocatechualdehyde (BPCA). BPCA can induce β-cell proliferation without any cytotoxicity and protect the cells against palmitate-induced lipotoxicity by preventing cell damage, ROS overproduction, as well BPCA attenuates the dysfunction of glucose-induced insulin secretion against palmitate treatment. BPCA also reduced mitochondrial damage by maintain parkin protein expression. Moreover, BPCA was observed to have a protective effect on palmitate-induced β-cells dysfunction *in vivo* zebrafish model. These findings suggested that BPCA could be a potential therapeutic agent to protect insulin secreting β-cell for the prevention of diabetes.

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101 - Controlled furrow constriction in cytokinesis ensures lumen size maintenance in tubular epithelia

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Epithelial tubules consist of a curved monolayer of epithelial cells surrounding a central lumen. They are the structural unit of many organs including the kidney and lumen shape and size maintenance is essential for their function. Cell division controls a vast majority of proliferative epithelial tissue morphogenesis. Cytokinesis, the last stage of cell division, is essential to trigger and control de novo lumen formation but its contribution to the maintenance of lumen shape remains unknown. Combining spherical cysts grown from MDCK cells and the zebrafish pronephros, we characterize here how cytokinesis dynamically and spatially organizes in a 3D epithelial environment and show that controlled furrow constriction in cytokinesis is required for lumen shape and size maintenance. More specifically, using 3D culture of MDCK cells, we show that perturbations of furrow constriction in cytokinesis, without preventing cytokinetic abscission, lead to defects in lumen shape. Then, taking advantage of 3D time-lapse imaging of the zebrafish pronephros, a simplified yet highly conserved system to study kidney tubular epithelial organization in vivo, we characterize how cytokinesis dynamically and spatially organizes in a 3D tubular environment. We show that furrow constriction perturbations lead to tubule enlargement due to increased lumen size. We further show that lumen enlargements occur in proliferative regions of the tubule and can be rescued using cell cycle inhibitors that prevent cell division. This demonstrates that defects occurring in division are responsible for lumen size defects. Altogether, our findings show that proper control of furrow constriction during cell division ensures lumen shape and size maintenance and thus proper morphogenesis of kidney epithelial tubule.

102 - Time-course RNA-Seq revealed internal abnormality of WRN mutants zebrafish

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The mechanism of aging is a big question in science. To elucidate the molecular basis underlying aging, we aimed to produce a Werner syndrome model in zebrafish. Werner syndrome is a genetic progeria that show premature aging symptoms at the onset of 20 years old with the 60% of the patients being Japanese. While the adult-onset symptoms of the pathology are premature dyslipidemia and diabetes, young-onset syndromic signs have not been explored. The responsible gene of the Werner syndrome is *WRN* encoding for a RecQ type DNA helicase. We successfully generated *wrn* mutant (mut) zebrafish using the CRISPER/Cas9 genome editing. We found no difference in the appearance between wild type (WT) and mut until 21 day post fertilization (dpf). Eventually, however, 90% of the mut underwent premature death between 7 and 21 dpf. Furthermore, the rest 10% mut, which survived more than 21 dpf, died at between 56 and 84 dpf with their body size smaller than WT and damaged skin. We employed time-course transcriptome analysis (RNA-Seq) to elucidate what is going on in mut. We analyzed gene expression of WT and mut zebrafishes at 4 ~ 35 dpf, in total 192 individuals, covering the dying stage for 90% mut. Based on the gene expression profiles, we could classify mut into two groups: premature dying group with down-regulation of pancreas-specific genes, and late dying group that down-regulates of pancreas-specific genes along with the up-regulation of DNA damage markers. Furthermore, our measurement of glucose levels from whole larval body at 21 dpf revealed that mut showed lower glucose level than WT. These results suggest that premature dying mut underwent early death by the impairment of pancreas and subsequent malnutrition. This malnutrition would also take place in human carrying a *WRN* mutation before displaying the late-onset premature aging symptoms.

103 - Single cell transcriptome analysis reveals heterogeneity and a dynamic regenerative response of quiescent radial glia in zebrafish adult brain

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In zebrafish telencephalon, radial glial (RG) show a remarkable ability to regenerate damaged neural tissue by reentering cell proliferation and produce neural precursors to rebuild the lost neural circuit. However, it is not fully understood how RG respond to brain damage and initiate the regenerative response. Here we applied single-cell transcriptomics to RG in zebrafish adult telencephalon and identified five RG subtypes, which are classified into four quiescent RG (qRG) and one proliferating RG (pRG). Four qRG differentially express a distinct subset of qRG markers, suggesting heterogeneity of qRG in zebrafish adult brain. Interestingly, one RG subtype shows high expression of ribosomal proteins, and its fraction is increased in response to brain damage. Consistently, the mTOR pathway is activated in RG near the injury site. It was reported that inflammatory response of brain-resident immune cells, microglia, is required for inducing regenerative response of RG in zebrafish. Genetical elimination of microglia not only suppressed damage-induced regenerative response of RG but also decreased fraction of the ribosomal expression-enriched RG. Lastly, our pseudo-time analysis revealed a lineage to generate the ribosomal expression-enriched RG. Our findings reveal heterogeneity of qRG in zebrafish adult brain and their dynamic regenerative response to brain damage.

104 - mTORC1 hyperactivity causes sensory integration impairment in the zebrafish model of Tuberous Sclerosis Complex

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Tuberous Sclerosis Complex (TSC) is a rare genetic disease that manifests with early symptoms, including cortical malformations, childhood epilepsy, and TSC-associated neuropsychiatric disorders. Neuropsychiatric symptoms comprise anxiety and autism spectrum disorder. In autism spectrum disorder, patients suffer from reduced sensory integration and aberrant response to various stimuli. We found that lack of Tsc2 in zebrafish resulted in hyperactivation of the mTorC1 pathway in habenulae specifically in the left dorsal region. Although we previously proved that Tsc2-deficient fish suffered from anxiety (Kedra et al, PNAS 2020), they exhibited a lack of light preference in the light-dark box test. We observed the aberrant function of light-responsive neurons in the left dorsal habenula of Tsc2-deficient fish. The afferent connectivity of habenulae was intact in fish lacking Tsc2, but the habenula commissure was thinner compared to sibling controls. Both, the lack of light preference and aberrant function of light-responsive neurons in the left habenula could be rescued by the mTorC1 inhibitor, rapamycin, suggesting that mTorC1 hyperactivity is responsible for impaired integration of light response in the zebrafish model of TSC.

105 - The role of A-to-I RNA editing by Adar in zebrafish development

Katarzyna Niescierowicz[1], Cristina Navarette-Hernandez[1], Cecilia Winata[1], Leszek Pryszcz[1], Eugeniusz Tralle[1], Agata Sulej[1], Karim Abu Nahia[1], Matthias Bochtler[1]

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Adenosine deaminases (ADARs) catalyse the deamination of adenosine to inosine, also known as A-to-I editing, in RNA. Although A-to-I editing occurs widely across animals, new biological roles are still being discovered. Here, we study the role of A-to-I editing by Adar, the zebrafish orthologue of mammalian ADAR1. Knockdown and overexpression experiments revealed that maternal *adar* is essential for zebrafish development, particularly during the earliest steps of antero-posterior and dorso-ventral patterning, and that this function is dependent on an intact deaminase domain. Genome-wide editing discovery revealed pervasive editing in maternal and the earliest zygotic transcripts, the majority of which occurred in the 3'-UTR. Interestingly, transcripts implicated in gastrulation as well as dorso-ventral and antero-posterior patterning were found to contain multiple editing sites. Adar knockdown or overexpression affected gene expression without so much altering global editing patterns by 12 hpf. Analysis of *adar*-/- zygotic mutants further revealed the role of Adar in regulating innate immune response – a function which is conserved with that of mammalian ADAR. Our study therefore established distinct maternal and zygotic function of RNA editing by Adar in embryonic patterning and regulation of innate immune response, respectively.

106 - An improved Erk-specific reporter reveals oscillatory Erk dynamics linked to the cell cycle during early development

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In the early zebrafish blastoderm, long-range FGF signalling at the embryonic margin patterns the mesendoderm. ‘Snap-shot’ views of development in fixed tissues reveal that although FGF signalling forms a gradient at the margin, using phosphorylated Erk (pErk) as a read-out, the levels of activity are highly variable between neighbouring cells. Interestingly, differential Erk signalling dynamics over time have been shown to influence cell fate decision making and cellular behaviour. We therefore sought to investigate the temporal dynamics of FGF/Erk signalling during early development to ask whether this is the source of heterogeneity in signalling levels. To do this, we have improved the specificity of an Erk-Kinase Translocation Reporter (KTR) to enable real-time visualisation of Erk activity in developing tissues *in vivo*. We find that Erk signalling is extremely dynamic and suggest that mitotic erasure of Erk activity introduces oscillations in Erk signalling. We also observe that the rate of Erk signal restoration post-mitosis is a source of heterogeneity in the developing zebrafish mesendoderm. Finally, we show that signalling downstream of pErk does not reflect the shape of the pErk gradient, however, the mechanisms that shape the pErk gradient regulate the sensitivity of cells to changes in signalling levels over time. Going forward, our modified KTR will be an important resource for the *in vivo* study of Erk signalling dynamics during development and our future work will ask how Erk signalling dynamics and heterogeneity influence tissue patterning.

107 - Jam2b-positive Lateral Plate Mesoderm Gives Rise To The Secondary Vascular Field

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Lateral plate mesoderm (LPM) gives rise to early vascular endothelial and hematopoietic lineages during zebrafish embryonic development. However, it is unclear if LPM-derived cells can differentiate into vascular endothelial cells after the initial vascular network has been established. We recently identified the secondary vascular field (SVF) located bilaterally along the yolk extension, which gives rise to late-forming *etv2*+ vascular progenitors. Here we identified Junctional Adhesion Molecule Jam2b as a molecular marker for the SVF-forming region. Based on in situ hybridization analysis, *jam2b* is expressed in the lateral portion of the LPM and does not label vascular progenitors during early vasculogenesis. However, its expression overlaps with the late vascular progenitor or SVF markers such as *etv2*. We used CRISPR-mediated homology-independent DNA repair approach to knock-in a transcriptional activator Gal4 into the endogenous *jam2b* locus. *Jam2b* reporter line recapitulated endogenous *jam2b* expression in the lateral portion of the LPM. Time-lapse imaging showed that LPM-derived *jam2b*+ cells incorporate into the posterior cardinal vein after 24 hpf when blood circulation has been initiated. Single-cell RNA-seq analysis of combined *jam2b* and *etv2* reporter lines demonstrated substantial heterogeneity of *jam2b*+ mesoderm and defined a transcriptional signature of SVF cells. Our results identify a unique marker for LPM that gives rise to late-forming vascular progenitors and demonstrate their contribution to the functional embryonic vasculature.

108 - Monitoring Erk signalling dynamics with a modified Erk-kinase translocation reporter (KTR) reveals signalling dynamics linked to the cell cycle during early development

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In the early zebrafish blastoderm, long-range FGF signalling at the embryonic margin promotes mesoderm induction. ‘Snap-shot’ views of development in fixed tissues reveal that although FGF signalling forms a gradient at the margin, using phosphorylated Erk (pErk) as a read-out, the levels of activity are highly variable between neighbouring cells and is completely lost during mitosis. These observations suggest that FGF/Erk signalling is dynamic and heterogeneous at the single-cell level. Interestingly, differential Erk signalling dynamics have been shown to influence cell fate decision making and cellular behaviour. We therefore sought to investigate the temporal dynamics of FGF/Erk signalling during mesendoderm patterning. To do this we tested a previously described Erk-kinase translocation reporter (KTR) that reports Erk activity in 48hpf embryos. We found extensive non-specific reporter activity in the early blastoderm and show that this is due to off-target CDK1 activity. We suggest that this non-specificity is made more obvious here by the rapid cell cycles of the early blastoderm. To improve the specificity of the Erk-KTR, we modified a putative cyclin-binding domain found within the Elk1-derived Erk docking domain. While our modifications do not perturb the Erk signalling response, the reporter no longer displays non-specific activity in the early blastoderm. While the CDK1-responsivity of the original reporter was made obvious here by a quirk of development, this is likely a general issue with the reporter in growing tissues in many organisms. Therefore, this modified reporter will be an important resource for the study of Erk signalling dynamics during development. We also present preliminary data revealing the extremely dynamic nature of Erk signalling during the period of mesendoderm induction. Going forward, we will use this reporter to investigate the role of signalling dynamics and heterogeneity in mesendoderm patterning.

109 - Nodal signaling establishes a competency window for stochastic cell fate switching

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Specification of the germ layers by Nodal signaling has been held as paradigmatic example of the Wolpert's French flag model, were cells exposed to graded levels of a morphogen acquire different fates at different locations in the embryo. However, such a deterministic model cannot explain why, in the zebrafish embryo, endodermal cells are specified in a "salt and pepper" pattern, where their immediate neighbors, equidistant from the morphogen source, acquire the mesodermal fate. Combining pharmacology, quantitative imaging and single cell transcriptomics we show that Nodal signaling establishes a bipotential progenitor state where cells initially fated to become mesoderm can switch to an endodermal fate. Intriguingly, rather than being strictly dependent on signalling levels, switching into endoderm is a stochastic event, relying on whether a cell will turn on the expression of the endoderm master regulator *sox32*. The likelihood of the switch being positively regulated by Nodal signalling and negatively regulated by Fgf signalling. This inherently imprecise mechanism nevertheless leads to robust endoderm formation as embryonic robustness at later stages obviates the requirement for a precise number of progenitors to be initially produced. Therefore, in contrast to previous deterministic models of morphogen action, Nodal establishes a temporal window when cells are competent to undergo a stochastic cell fate switch, rather than determining fate itself.

110 - INVESTIGATING MUTATIONS IN THAP12 AS A NOVEL GENETIC ETIOLOGY OF LENNOX GASTAUT SYNDROME

Katarzyna Ochenkowska[1], Meijiang Liao[1], Uday Kundap[1], Eric Samarut[1]

[1]CRCHUM

Patients with IS (infantile spasms) will later develop other types of seizures such as Lennox-Gastaut syndrome (LGS), characterized by seizures and cognitive dysfunction. Recently, two new variants in the THAP12 gene have been identified in two siblings with idiopathic IS and LGS. Since no other disease-causing mutations in THAP12 have ever been referenced, the role of THAP12 mutations in causing IS/LGS needs to be demonstrated. We hypothesize that THAP12 mutations cause IS and LGS and we aim at proving it in vivo using patients' cells and zebrafish models.

This project aims at demonstrating that the variants in THAP12 identified in patients are causing LGS by showing that these mutations cause a gene loss-of-function (LoF). We will check the subcellular localization of THAP12 in patients' fibroblasts versus unaffected by immunofluorescence and confocal imaging. We will also check changes of the level of expression of THAP12 transcript and the protein. We will test the effect of THAP12 LoF using zebrafish and confirm that it leads to neurodevelopmental defects. We generated a THAP12-KO model in zebrafish and by crossing it with fluorescent reporter lines, we will check the development of neuronal cells. To check if THAP12 LoF induces an abnormal brain activity, we will record the electrical activity from the larval brains in THAP12-KO larvae compared to WT.

There are two orthologs of the human THAP12 gene in the zebrafish genome (thap12a, thap12b). The sequence similarity between zebrafish and human THAP12 proteins is high and their functional domains are conserved, which makes studying THAP12 LoF in zebrafish clinically relevant. We showed that THAP12 mutations in zebrafish larvae lead to abnormal brain development that is consistent with the symptoms of LGS patients. We also found that the brain size of THAP12 CRISPRs larvae is reduced with an abnormal organization of neuronal cells.

111 - EGFR-mediated endocytosis is required for Wnt9a/Fzd9b signaling during hematopoietic stem cell development

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Wnt genes encode a highly conserved family of secreted growth factors that drive diverse signaling cascades required for a large variety of processes during embryonic development and tissue homeostasis. There are at least 19 Wnt ligands and 10 different Frizzled (Fzd) receptors, depending on the vertebrate species, suggesting that different downstream signals are selected in part by receptor-ligand pairings. Yet, the field has been bogged down by the assumption that most Wnt proteins can drive signals through many (if not all) Fzd receptors. Because of this, the *in vivo* identification of cognate Wnt-Fzd pairings, and the mechanisms required for eliciting downstream signaling remain very poorly understood. Using zebrafish developmental biology as a jumping off platform, we have established that the ligand Wnt9a is uniquely required to initiate hematopoietic stem and progenitor cell (HSPC) proliferation, though the receptor Fzd9b. Surprisingly, the epidermal growth factor receptor (EGFR) is required for mediating Wnt9a/Fzd9b signaling specificity; however, we do not yet understand the molecular mechanisms for this requirement in Wnt9a signaling. Using fluorescently-tagged, signaling competent Wnt9a and Fzd9b molecules, we have identified a requirement for endocytosis of the receptor complex in initiating the Wnt9a signal. Using an unbiased proximity-labeling proteomics approach, we have also identified a molecular mechanism for initiating this signal. Given the diversity of reports for the requirements and dispensability of endocytosis in Wnt signaling, we believe that this represents a mechanism of signaling specificity. This work was supported by NIH K99, R00 and R35 grants.

112 - Ectopic MYCN expression promotes a neural crest-like signature in developing sympathoadrenal progenitors

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Neural Crest Cells (NCC) are a transitory multipotent stem cell population that arises from the neural tube during vertebrate embryogenesis. NCCs migrate to distinct sites and give rise to a variety of cell types within the developing embryo, including neurons and glia of the sympathetic nervous system. During normal embryonic development, a subpopulation of NCC migrates ventrally towards the dorsal aorta and acquires a sympathoadrenal fate, subsequently maturing into adrenal chromaffin cells or sympathetic neurons. It has been suggested that failure in sympathoadrenal progenitor (SAP) differentiation leads to neuroblastoma (NB). Interestingly, the molecular mechanisms that lead to NB onset remain largely unknown. In NCCs, MYCN is transiently expressed to promote migration and its downregulation precedes neuronal differentiation. However, in NB, overexpression of *MYCN* has been linked to high risk and aggressive disease progression. By using a previously published zebrafish line overexpressing MYCN in the SAP lineage, we have found that MYCN-overexpressing cells aberrantly maintain the expression of NCC markers, while also expressing SAP differentiation markers, during early larval stages. These results suggest that ectopic MYCN expression leads to an aberrant "NCC-like" undifferentiated population that retains the expression of NCC genes past their normal window. This failure in proper SAP fate acquisition could be one of the earliest mechanisms leading to MYCN-driven NB onset. Overall, we aim to increase the understanding behind MYCN effects on correct cell fate acquisition that could help shed light on the cellular mechanisms driving high-risk NB onset and identify potential targets for drug discovery that could be used to improve patient outcome.

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113 - Differential regulation of Wnt signaling by negative feedback regulators Axin2 and Nkd1

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Wnt signaling is a crucial developmental pathway involved in the regulation of multiple events during early development as well as stem cell maintenance in adults. The pathway signals through the stabilization of β-catenin in the cytoplasm allowing for β-catenin to relocate to the nucleus to activate Wnt target genes. Aberrant Wnt signaling is linked to many diseases including cancer and neurological disorders demonstrating the need to better understand its regulation. Axin2 and Nkd1 are negative feedback regulators of Wnt signaling and the current model suggests that Axin2 reconstitutes the b-catenin destruction complex, whereas Nkd1 functions to inhibit the nuclear localization of β-catenin. Based on this model, we hypothesized that Axin2 and Nkd1 cooperate to attenuate Wnt signaling. To test this, we generated Axin2-/-, Nkd1-/-, and Axin2-/-;Nkd1-/- zebrafish lines. Curiously, all three of the mutant lines have no overt embryonic phenotype and are viable, although the Axin2 -/- adults have swollen gills and spinal curvature, which was rescued in the double mutant. Observing Wnt target gene expression during blastula stages revealed significant differences between the single mutants: Where *sp5* expression had no response in Axin2-/-, it was significantly increased in Nkd1-/- compared to wild-type or in response to Wnt8 overexpression. *sp5* expression in the double mutant was indistinguishable from the Nkd1-/- single mutant, suggesting that Nkd1 functions downstream of Axin2. These results further suggest Nkd1 and Axin2 function in different capacities to regulate Wnt signaling, which we are currently evaluating through RNA-seq. Understanding the differences between Axin2 and Nkd1 will provide critical insight into the balance Wnt signaling needs to achieve and maintain cellular homeostasis.

114 - Somatic KRAS mutations in the endothelium drive sporadic brain arteriovenous malformations

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Arteriovenous malformations (AVMs) are abnormal direct connections between an artery and a vein without an intervening capillary network. These malformations are prone to rupture and are the leading cause of hemorrhagic stroke in children and young adults. Until recently, the cause of these lesions was unknown. We discovered that somatic activating mutations in KRAS occur in the endothelium of the majority of patients with sporadic brain AVMs. To better understand how KRAS mutations result in AVMs, we expressed active KRAS in the endothelium of zebrafish. This revealed the sufficiency of active KRAS to drive AVMs. At the cellular level, active KRAS led to ectopic sprouting as well as an increase in cell size, leading to an expansion of the diameter of the lumen. Active KRAS also resulted in direct connections between arteries and veins. Inhibition of the MAPK/MEK pathway downstream of KRAS reversed abnormal arteriovenous shunts and ectopic sprouting. We are currently using cell culture, mouse and zebrafish models to further understand the molecular mechanisms responsible for AVM formation and maintenance and are seeking to identify pharmacological treatments to reverse AVMs.

115 - Zebrafish calpain3b mutants recapitulate limb-girdle muscular dystrophy

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Limb-Girdle Muscular Dystrophy Type 2A (LGMD2A) is characterized by progressive hip and shoulder muscle weakness. Mutations in *CAPN3* that codes for calpain 3, represent the most common cause of LGMD2A. In zebrafish, *capn3b* was previously identified as a mediator of Def-dependent degradation of p53, but not described as a muscle-specific gene. We have established *capn3b* as a *bona fide* muscle-expressed gene. To model LGMD in zebrafish, we generated loss-of-function mutants in *capn3b*, as well as a mutant in dystrophin (*dmd*) (positive control Duchenne muscular dystrophy model) using CRISPR/Cas9. Two mutants in *capn3b* were generated, one using a multi-exon deletion and the second using an RNA-less promoter deletion approach, resulting in relatively normal adult-viable animals over >4 generations. Both these mutants exhibited loss of *capn3b* expression. Mutants in *dmd* were homozygous-lethal and exhibited overt muscular dystrophy phenotypes by 3 days post-fertilization (dpf). Treatment with 0.8% methylcellulose (MC), thus making movement harder, of both *capn3b* mutants for 2-4 days starting at 2 dpf resulted in pronounced structural muscle abnormalities in 20-30% of embryos detectable by birefringence as well as increased mortality, none of which were seen in the MC-treated casper embryos or in the control embryos. The Evans Blue Dye staining assay for loss of sarcolemma integrity produced expected negative results in casper and mutant control-treated embryos and expected positive results in *dmd* homozygotes, whereas MC-treated *capn3b* mutants did not exhibit loss of membrane integrity. These new mutant fish provide a tractable model not only for studying the mechanisms underlying muscle repair and remodeling, but also as a preclinical tool for whole animal therapeutics and behavioral screenings. This is important when assessing the specificity, efficacy, and toxicity of small molecules with the potential to identify novel treatments that can safely alter the natural history of progressive muscle weakness in LGMD patients.

116 - Automated 3D Imaging of Zebrafish Larvae Using the VAST BioImager

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3D tomographic visualizations have become a powerful approach in medical and scientific imaging. Generating tomographic projection datasets needed for 3D visualizations is often done by manually manipulating samples. We have developed software and methods to generate 3D reconstructions of live and fixed zebrafish larvae in an automated manner using the VAST BioImager in combination with an upright microscope. The VAST BioImager (Vertebrate Automated Screening Tool) is a modular, expandable platform for the high throughput imaging and sorting of zebrafish larvae 2-7 dpf. The system reliably and reproducibly detects, orients, and rotates the larvae to a user-defined field of view, eliminating the step of manual manipulation. Because zebrafish larvae can be rotated to user-defined positions within a glass capillary, we can collect a full rotation's worth of larvae images, enabling tomography.

Here, we show volumetric reconstructions of craniofacial features, hearts, and tumors and neuromast in zebrafish larvae. Reconstructions were typically acquired and processed in 5-15 minutes. Using the 3D tomographic software, we were able to extract morphologically relevant data such as jaw angles and heart volumes. In conjunction with the VAST system's high throughput positioning and orientation of zebrafish larvae, large numbers of 3D reconstructions of organs to whole fish are easily collected, enabling insights into morphology, structure, and phenotype.

117 - Building the atrial muscular wall involves cell elongation and reorganization of tissue polarity

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During vertebrate development, the cardiomyocytes, or muscle cells of the heart, undergo complex cellular rearrangements important for the formation of the mature organ. Previous studies have mostly focused on the formation of the trabecular network in the ventricle; however, morphogenetic processes that drive atrial wall complexity, which is crucial to propagate the action potential for cardiac contraction, have largely been overlooked. Our study uses zebrafish larvae to elucidate cardiomyocyte behaviors during atrial development, as they allow for high-resolution live imaging and are easily amenable to genetic modifications.

Using live 3D confocal imaging of zebrafish hearts, combined with mosaic labelling and temporal tracking of individual atrial cardiomyocytes, we found that atrial wall morphogenesis is driven by complex cell behaviors. Specifically, we observed that atrial cardiomyocytes in zebrafish larvae form membrane protrusions and adopt an elongated shape in a non-stochastic orientation that establishes atrial tissue-level polarity. These shape changes lead to restricted multilayering between neighboring cardiomyocytes and the formation of new cell contacts, resulting in populations of elongated cardiomyocytes that span the atrium in an orientation parallel to the direction of blood flow. These cell behaviors lead to the appearance of muscle ridges on the inner surface of the atrium. Notably, these atrial cardiomyocyte behaviors appear to be regulated by the Wnt signaling pathway, but independent from factors important in ventricular morphogenesis such as Erbb2 and Notch signaling. Altogether, these data suggest that atrial morphogenesis is driven by oriented cell elongation as well as by distinct molecular factors, all of which are under investigation.

118 - Ga13 controls the convergence of pharyngeal endoderm by regulating the expression of E-Cadherin

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During segmentation, pharyngeal endoderm cells undergo convergence and extension (C&E), a process that is critical for endoderm pouch formation and craniofacial development. Our previous published data showed that the sphingosine-1-phosphate G protein-coupled receptor S1pr2 signals through a Ga_{13}/RhoA-dependent pathway to regulate endoderm convergence. However, the underlying cellular mechanisms remain unknown. Using transgenic lines that specifically label endodermal cells, our confocal imaging reveals that during the 6-12 somite stage, endodermal cells gradually form stable cell-cell contacts and migrate collectively toward the midline. Furthermore, the cells in the lateral-most region of the endodermal sheet migrate ventrally and undergo apical constriction, with groups of cells forming multicellular rosettes whose centers are enriched for the apical markers, including a-PKC and ZO-1. These morphogenetic changes reduce cell's apical surface, contributing to endoderm C&E. In Ga_{13}-deficient embryos, the endodermal cells fail to form stable contacts, and apical constriction and endoderm convergence are impaired. Notably, the expression of E-Cadherin (Cdh1) in Ga_{13}-deficient embryos is reduced, and *cdh1*-deficient endoderm cells show similar defects in endoderm convergence. Furthermore, we found that Cdh1 and Ga_{13} display synergistic effects on endoderm C&E, and overexpressing Cdh1 rescues the defects of endoderm convergence in Ga_{13}-deficient embryos. Thus, our study indicates that Ga_{13} regulates pharyngeal endoderm convergence by regulating Cdh1expression, uncovering a new mechanism that controls pharyngeal endoderm morphogenesis.

119 - Investigating the role of collagen 11a2 in vertebral development and congenital scoliosis

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Collagen 11a2 is a minor cartilaginous collagen, which maintains spacing and fibrillar integrity of the major cartilaginous collagen. *COL11A2* has been previously associated with human skeletal dysplasias and, in a whole exome sequencing study of 26 patients with isolated vertebral malformations (VMs), our collaborators identified 2 patients with *COL11A2* missense variants. Knockdown of *col11a2* in zebrafish embryos has previously been shown to disrupt the structure of the notochord, which is the precursor tissue to the spine. I have shown that *col11a2* is strongly expressed in the craniofacial cartilage and notochord of zebrafish embryos, supporting a potential role for Col11a2 in vertebral development.

To investigate the role for Col11a2 in zebrafish spine development, I employed CRISPR/Cas9 gene targeting approaches to generate *col11a2* nonsense and transcriptless alleles. Homozygosity of either allele results in vertebral fusion defects, with fish homozygous for the *col11a2* deletion exhibiting more severe phenotypes. Vertebral fusions become apparent in *col11a2* zebrafish mutants at 5mm standard length, and primarily affect the caudal vertebrae of the spine. Intervertebral discs are initially present in these mutants, but regress as developing vertebrae fuse. Notably, fish heterozygous for the *col11a2* deletion allele also develop vertebral fusion defects, demonstrating haploinsufficiency of the *col11a2* locus. This raises the intriguing possibility that heterozygosity of damaging *COL11A2* variants may cause VMs in humans.

To test the functional consequence of identified VM-associated *COL11A2* variants, I assayed the ability of orthologous alleles to suppress vertebral fusion phenotypes using a transgenic assay. Expression of wildtype *col11a2* in notochord sheath cells suppressed VMs in *col11a2* mutant zebrafish. However, expression of patient-associated variants failed to suppress vertebral fusions, supporting a pathogenic role for identified mutations. Together, our results indicate an essential role for Col11a2 in zebrafish vertebral development, and support a causal role for damaging *COL11A2* mutations in human VMs and congenital scoliosis.

120 - Adgrg6 variant analyses: from chemical screening in zebrafish to virtual screens and in vitro assays

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The adhesion GPCR Adgrg6 (Gpr126) is required for vertebrate Schwann cellmyelination and zebrafish inner ear morphogenesis. In humans, recessive mutationsin *ADGRG6* are causative for the lethal contracture syndrome arthrogryposis multiplexcongenita (AMC), characterised by a lack of Myelin Basic Protein expression in theintramuscular nerves. Other *ADGRG6* variants are associated with a range of diseasestates.

We have used the zebrafish embryo as a model system to identify compounds thatcan rescue phenotypic defects in *adgrg6* mutants, which could therefore providepromising starting points for the development of therapeutic compounds. Wepreviously identified molecules that could rescue a hypomorphic phenotype, using anallele-specific experimental design to differentiate hit compound activity(Diamantopoulou et al., eLife 2019). This approach identified lead agonist compoundsthat might interact directly with Adgrg6.

To validate lead compounds from the zebrafish screens, we are developing *in vitro*assays to test compound activity. Initial work has focussed on testing proteinlocalisation and activity of different zebrafish and human receptor variants *in vitro*.Different Adgrg6 mutant constructs revealed variable protein expression and basalcAMP activity relative to the wild type. For example, a single amino acid change in theGPS region of the receptor appeared to increase basal cAMP activity of the zebrafishreceptor but decrease activity in the human receptor.

In parallel, we are taking three approaches to identify inhibitors of the Adgrg6 signallingpathway. Firstly, we have isolated additional hit compounds from the original screensthat exacerbated the hypomorphic mutant phenotype. Next, we will perform a screen onwild-type embryos to identify compounds that can induce phenotypes resemblingthose of *adgrg6* mutants. Finally, we are combining these *in vivo* approaches with *insilico* ligand-based virtual screening techniques. Compounds of interest will beselected for further testing in both our *in vitro* and *in vivo* platforms

121 - Characterization of Deeply Conserved Enhancers Implicated in Congenital Heart Disease

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Many genes have been implicated in congenital heart disease (CHD), however the roles in heart development and disease of regulatory elements that control their expression are largely not understood. To search for such elements, our lab identified conserved non-coding regions of the zebrafish genome that show chromatin accessibility in mesendoderm progenitors that depends on the activity of Gata5, a master regulator of genes involved in heart development. These regions show sequence conservation in the human genome and have been termed accessible conserved non-coding elements (aCNEs).

While the effects of coding sequence mutations in genes involved in heart development are often relatively easy to predict, annotation of non-coding elements is difficult and relies on the results of *in vivo* studies. Here, we aimed to validate and characterize the expression patterns driven by of four Gata5-sensitive aCNEs using a transgenic GFP reporter assay in zebrafish. We show that three out of four of these aCNEs act as cardiac enhancers, driving GFP expression in zebrafish hearts at 48 hours post fertilization (hpf). These enhancers are nearest to either of two genes encoding paralogous forms of Zfpm2, a cofactor of Gata5 that represses Gata5 activity and is required for normal heart development. This suggests that these enhancers may regulate *zfpm2* expression. In two of these enhancers, single nucleotide variants have been identified in human CHD patients by the SickKids Cardiac Genome Clinic, suggesting that alterations of these enhancers may contribute to CHD. Identifying these regions as heart enhancers conserved between zebrafish and humans could to greatly increase our understanding of CHD and normal heart development in humans in the future.

122 - Molecular and functional heterogeneity in the developing vestibulo-ocular reflex circuit

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All vertebrates need to stabilize gaze during movement. As animals develop, gaze stabilization behavior improves. However, it is unclear how the developing brain comes to stabilize gaze. Functional imaging in this circuit has uncovered a unique readout for response dynamics between neurons of a given population. I hypothesize that these response dynamics differentially drive behavior maturation.

In the larval zebrafish, the gaze stabilization circuit is a tractable three-neuron arc. We first generated lines of transgenic zebrafish to express the calcium indicator GCaMP6s in two of these nuclei: the central vestibular nucleus and extraocular motor neurons of the trochlear nuclei. I measured neural activity with a multiphoton microscope while delivering whole-body tilts in the pitch and roll axes. To assay neuronal changes that occur during behavioral improvement, I recorded from the same cells at 3, 5, and 7 days post fertilization. I observed systematic changes in neural activity, including differential responses to identical tilts, as well as increasing sensitivity to particular directions of tilt, implicating a strengthening period of tuning.

While neural responses were found to be heterogeneous, preliminary data reveals a negative correlation between a neuron’s response strength at an early time point and the magnitude of that neuron’s response strengthening over time. I have adopted a multipronged approach including correlative imaging and perturbative assays to directly address how an individual neuron’s response differentially influences the maturation of behavior. Finally, we are working to characterize the molecular correlates of these changes with transcriptomic approaches. This work will speak to the mechanisms responsible for the development of gaze stabilization.

123 - Synaptic dysfunction in a C9orf72 loss of function model rescued by pharmacological intervention

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motoneurons causing muscular atrophy, paralysis and ultimately death. Presently, no curative treatment exists. Understanding the physiopathological mechanisms will help develop new efficient treatments. In 2011, an expansion of a repetition of a hexanucleotide (GGGGCC) in the first intronic region of the *C9orf72* gene has been discovered as the first genetic cause of ALS. To investigate the role of *C9orf72* loss of function in ALS, we used synthetic micro-RNAs to specifically target the zebrafish C9orf72 gene (C9-miRNA) and have developed a stable zebrafish C9-miRNA line with reduced expression of *C9orf72*. Upon loss of function of *C9orf72*, we observed that zebrafish C9-miRNA mutants display severe motor deficits beginning 6 days postfertilization (6 dpf) and a majority die prematurely at 15 dpf. Analysis of the neuromuscular junctions using specific presynaptic and postsynaptic markers SV2 and alpha-bungarotoxin respectively, revealed a significant decrease in the number of synaptic contacts in the C9-miRNA line at 6 dpf correlating with a decreased synaptic vesicles turnover. Electrophysiology recordings using patch clamp technique on muscle fibres showed a decrease of amplitude and frequency of the spontaneous miniature end plate currents, which suggests a decreased number of presynaptic endings. Also, TDP-43 has been shown to aggregates at 6 dpf in our C9-miRNA. Among the few fishes that survived until adulthood, we observed a significant motoneuron and muscle atrophy. Mass spectrometry showed a decreased expression of the neuroprotective protein, calpastatin in our mutant. Thus, we have been pharmacologically compensating the loss of calpastatin and observed some rescue of the number of synaptic contacts and vesicle turnover indicating that this pathway may have a role in the synaptic dysfunction. Altogether, our zebrafish C9-miRNA replicates aspects of ALS and showed that C9orf72 has a role in the synaptic transmission at the NMJ.

124 - Identification of a conserved tbx20 enhancer with mutations in a CHD patient

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Congenital heart diseases (CHDs) include a range of structural and functional heart defects and affect nearly 1% of all live births. Most familial CHD cases have no identifiable genetic origin. Variation in the non-coding genome can contribute to CHDs, as mutations in regulatory elements such as enhancers lead to improper spatiotemporal expression of key cardiac or developmental genes. Previously, our lab has identified 8366 non-coding regions conserved between humans, mice, and zebrafish, termed accessible conserved non-coding elements (aCNEs). As these aCNEs are both conserved and active early in development, we hypothesize these to be enriched for functional elements. Through collaboration with the SickKids Cardiac Genome Clinic, 615 ultra-rare single-nucleotide variants (SNVs) in aCNEs have been identified in a cohort of 94 CHD patients. From these hits, a CHD patient with hypoplastic left heart syndrome has been identified with two SNVs in aCNE20, which is predicted to regulate *tbx20* expression based on proximity and chromatin looping data. As Tbx20 is a transcription factor with an essential role in early cardiac development, we hypothesize that SNVs in aCNE20 result in misregulation of *tbx20* and subsequent cardiac defects. To test this hypothesis, a transgenic zebrafish reporter line was generated, demonstrating that wild-type (WT) aCNE20 drives pancardiac gene expression. Using the same reporter assay, I will examine the effect of the CHD patient SNVs on aCNE20's ability to drive cardiac gene expression in comparison to WT aCNE20. I will also generate aCNE20 deletion mutants and look for abnormalities in cardiac morphology or function, as well as changes in expression of *tbx20*. Overall, this project aims to investigate the contribution of non-coding elements to cardiac development and CHDs. More broadly, it will provide a framework for the functional validation of non-coding variation.

125 - Upregulation of TgBAC(ankrd1a:EGFP) in zebrafish skeletal muscle during regeneration and in response to mechanical stretch

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Functionally pleiotropic cardiac ankyrin repeat protein ANKRD1 (CARP) is predominantly expressed in the heart, where it participates in transcriptional regulation, sarcomere assembly and mechano-sensing as a component of I-band complex organized on titin N2A region. *ANKRD1* expression is significantly upregulated in diseased skeletal muscle of patients with muscular dystrophy, congenital myopathy and motor neuron diseases. This gene is also involved in muscle stress response pathways initiated after acute resistance exercise, eccentric contractions, stretching or injury. Here we investigated activation of zebrafish homolog, *ankrd1a* gene, in stressed skeletal muscle, using transgenic line *TgBAC(ankrd1a:EGFP)*. We detected transgene upregulation in cells in close proximity of the needle stab wound during regeneration. Subjecting larval skeletal muscle to stretching also increased transgene expression in muscle cells, corroborating stretch-responsiveness of *ankrd1a* observed for its mammalian homologue. Our results implicate *ankrd1a* in zebrafish skeletal muscle tissue repair and remodeling as a sensor of stressed muscle.

126 - Discovery of Novel Pain Therapies Using CRISPR-Cas9 Targeted Mutagenesis and Phenotype-based Drug Screening in Larval Zebrafish

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Introduction: Opioid drugs provide effective pain relief but come with considerable side-effects including addiction and respiratory depression, which can be lethal with overdose. During the COVID19 pandemic, the opioid epidemic reached almost 100,000 deaths in the United States, highlighting the urgency to identify safe-to-prescribe pain killers. Using our novel biotechnology platforms in larval zebrafish, we propose to identify new molecular targets and drugs with potent analgesic properties, reduced respiratory liability, and without causing addiction.**Methods:** Combining targeted mutagenesis and phenotype-based drug screening approaches in larval zebrafish *in vivo*, we can quickly identify new molecular targets and screen new drug compounds to either target respiratory depression or analgesia. We first screened customized drug libraries targeting voltage-gated calcium channels and the adenylyl cyclase pathway, two molecular pathways involved in pain. We are also creating mutants using CRISPR-Cas9 in embryo F0 founders to identify new molecular targets. Using our new models, we quantified respiratory depression by fentanyl in 7-day post-fertilization (dpf) larvae, as well as the nociceptive response to formalin.**Results:** To perform targeted mutagenesis, we injected 4 single guide RNAs targeting the *orpm1* gene, which encodes the μ-opioid receptor. We then measured respiratory depression by fentanyl in 7dpf crispant larvae or controls. In *oprm1*$^{-/-}$ crispants, respiratory depression by fentanyl was substantially reduced compared to controls, therefore confirming the validity of our targeted mutagenesis approach. We are currently targeting the genes coding for calcium channels and phosphodiesterases to determine whether they can be molecular targets for respiratory depression and/or analgesia.**Discussion:** The successful identification of new drug combinations to prevent respiratory depression while preserving analgesia suggests that our drug discovery platform in larval zebrafish will substantially accelerate the identification of new opioid drugs with reduced morbidity and mortality. Our platform is also being leveraged to discover non-opioid pain killers with reduced side-effects.

127 - Cisplatin disrupts mitochondrial bioenergetics in the zebrafish lateral-line organ

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Cisplatin, a commonly used chemotherapy, causes hearing loss in more than 50% of patients. This hearing loss is due to the loss of hair cells—the sensory receptors of the auditory system. No targeted therapies currently exist to treat cisplatin-induced hearing loss partly because the underlying mechanisms of cisplatin-induced hair cell death are not completely defined. Zebrafish may offer key insights to cisplatin ototoxicity because they contain superficial hair cells in their lateral line organs that are remarkably similar to those within the cochlea, but are optically accessible, permitting observation of cisplatin injury in live intact hair cells. Since mitochondria have been implicated in cisplatin cytotoxicity, we hypothesize that dysfunctional mitochondrial activity is a critical cellular event that precedes cisplatin-induced hair cell death. We therefore used a combination of genetically encoded biosensors and fluorescent indicators to define changes in mitochondrial bioenergetics in response to cisplatin in larval zebrafish. First, we examined lateral line hair cell mitochondrial activity, and observed significantly increased mitochondrial membrane potential following cisplatin exposure. Since mitochondria are integral for intracellular calcium homeostasis, we then exposed zebrafish expressing a mitochondrial-localized calcium indicator within hair cells to cisplatin. These experiments revealed that mitochondrial calcium levels are elevated immediately following completion of cisplatin treatment and that calcium levels spike immediately prior to death. Next, using a genetically encoded indicator of mitochondrial age and redox history, we observed older hair cells with greater cumulative activity were more susceptible to cisplatin than younger hair cells with less redox history. Examination of cisplatin on hair cell oxidative stress using a fluorescent ROS indicator revealed that these changes in mitochondrial function were associated with increased oxidative stress. Lastly, cisplatin exposure induced caspase-3-mediated apoptosis. Altogether, these findings support that cisplatin disrupts hair cell mitochondrial bioenergetics and may play a key role in cisplatin ototoxicity.

128 - Who lurks at the crossing: Slit2 and Slit3 act together in optic chiasm axon sorting

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Retinal ganglion cells (RGCs) extend axons from the neural retina to their innervation site at the dorsal midbrain (the optic tectum in non-mammals), with the two optic nerves joining at the ventral midline to form the optic chiasm. Here, RGC axons are sorted depending on binocularity, and in the case of animals with no binocular vision like the zebrafish, all cross to innervate the contralateral optic tectum. In different species, the Slit-Robo signaling pathway has been reported to be paramount in regulating this process. In the zebrafish, it has been shown that RGCs express the Slit receptor Robo2, whose mutant *astray* shows a severe axon guidance phenotype around the chiasm. On the other hand, two *slit* mRNAs are expressed around the chiasm area: *slit2* and *slit3*. Using the CRISPR/Cas9 system, combined with transgenic or anterograde tracer labeling of RGCs and live imaging, we analyzed the role these secreted factors play in the organization of optic axons, from the optic nerves to the optic tracts and tectum. *slit2*-deficient embryos presented defects in axon organization around the midline, as well as minor guidance errors and a reduction in growth cone velocity at the optic chiasm. *slit3-deficient* embryos, on the other hand, showed no noticeable defects in RGC axon guidance or organization. Interestingly, blocking the expression of both *slit2* and *slit3* simultaneously led to the appearance of severe retinal misprojections at the midline, including projections to the contralateral retina and the ipsilateral optic tract. Moreover, some of these misprojected axons appeared to reach the ipsilateral optic tectum, preferentially innervating its ventral-most region. Our current observations, added to previous research, point to a joint role of Slit2 and Slit3, possibly acting through Robo2, in the regulation of retinal axon guidance and sorting at the ventral midline.

129 - Knowing where to re-grow: Identification of extrinsic cues promoting target-selective axon regeneration

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Regenerating axons in the peripheral nervous system must extend over long distances to reconnect with their original synaptic targets for functional recovery. However, 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. To visualize regenerating axons as they navigate stepwise choice points, we established the larval zebrafish pectoral fin, equivalent to tetrapod forelimbs, as a vertebrate model system in which to study this process. Recently, we reported muscle-specific differences in the mechanism of neuromuscular synapse development between the pectoral fin and the axial muscle of the trunk (Walker et al. Development 2021), highlighting the value of this system for new discovery. Pectoral fin motor nerves sort at a plexus to choose the abductor or adductor muscle and then topographically innervate specific muscle domains. Using a laser, we transect these nerves and monitor axon regeneration in real time. By labeling single axons, we observe robust, specific, and functional regeneration of axons back to their original domains, indicating the existence of unknown local cues within the fin to guide selective reinnervation. To identify extrinsic injury-dependent cues, we employed RNAseq in denervated fins at timepoints that precede important axon guidance decisions, predicting that changes in gene expression may reflect regional cues important for axon growth and guidance. From upregulated candidate genes, we find that the cation channel *trpv6*, which regulates calcium homeostasis, is required for axon growth in regeneration. *Trpv6* is expressed in cells, likely sodium-potassium-ATPase-rich (NaR) ionocytes, that localize near the fin motor nerves and at the base of the fin, suggesting its effects on axon growth may be long-range. We report progress in understanding how *trpv6*, calcium homeostasis, and NaR ionocytes impact axonal regrowth. Funding: (LW)K01NS119496 (MG)EY024861, (MG)NS097914.

130 - A signaling switch in zebrafish underlies the induction and maintenance of the spinal cord specification and patterning regulatory gene cdx4

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Early central nervous system (CNS) specification, regionalization and patterning rely on a limited number of signaling factors used iteratively during development. How these signals –FGF, BMP, Wnt, Nodal, Retinoic Acid– direct different developmental programs in the CNS over time remains unknown. We investigated the influence of these signals on the regulation of the zebrafish *cdx4* gene that codes for a transcription factor involved in the specification of the spinal cord and the induction, establishment and maintenance of expression of *hox* patterning genes. Using various loss and gain of function approaches we identified two phases of *cdx4* transcription: an early initiation and a late maintenance phase. During the early phase, *cdx4* expression domain along the gastrula margin depends on BMP ventrally and Nodal dorsally, with Wnts needed to sustain high transcription levels. In contrast to reports in *Xenopus*, we do not find evidence of FGF regulating *cdx4* during this early phase. This phase of expression coincides with Cdx4 function in the specification of spinal cord fates and the initiation of *hox* gene transcription. During the late phase, *cdx4* transcription in the spinal cord is under the control of an FGF-dependent autoregulatory loop that is required for the maintenance of *hox* gene transcription. At this stage, our previous work shows that Retinoic Acid limits the anterior extent of *cdx4* transcription to the spinal cord territory. Taken together, these results support a biphasic model of *cdx4* regulation that is dependent on different signaling factors; the first phase specifying and the second patterning the spinal cord. **(This work was supported by the NSF grant IOS-1755386 and UR School of Arts and Sciences.)**

131 - The combination of E-box and AP-1 motifs function as the regeneration-response enhancer

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Whereas mammals cannot regenerate many of their organs when they are severely injured, fish and urodeles have higher tissue regeneration capacity such that fins or limbs can fully regenerate after amputation. During regeneration, amputation triggers transcriptional activation of a set of specific genes via regeneration-response enhancers (RREs), and their function is thought to be crucial for regeneration. However, the exact entity of the RREs is still an open question.

Here, we used an EGFP reporter transgenic assay in zebrafish and identified three RRE elements from the *fn1b* upstream region, two of them function only when it was combined with *fn1b* 0.7kb promoter, the other one acts as a standalone RRE. From the comparison of RREs, we found that two transcription factor binding motifs, E-box and AP-1, are commonly contained. Indeed, the tandem E-box repeats could drive the RRE response when it was placed with the 0.7kb promoter or the tandem AP-1 repeats, confirming that their cooperative function is the entity of RRE.

The RRE response is suppressed by inhibition of Jun N-terminal kinase, an upstream mediator of AP-1, or by expression of the dominant-negative Lef1, which downregulates the binding of Tcf/Lef to the E-box, indicating that the signals through both motifs are necessary for the RRE response.

Furthermore, we showed that RREs are only activated by injuries that induce blastema formation, and that they are also activated by tissue amputation of other tissues or species, such as the pectoral fins, scales, and heart of the zebrafish and the *Xenopus* limb bud. Our data suggested that the elements containing E-box and AP-1 universally function as RREs beyond tissues or species.

132 - The zebrafish cerebellar neural circuitry is involved in social behavior

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Deficits in social behavior are found in neurodevelopmental disorders including autism spectrum disorders (ASDs). Since abnormalities in cerebellar morphology and function are observed in ASD patients, it has been considered that the cerebellar neural circuitry is important for social behavior. However, it remains unknown how the cerebellum is involved in social behavior. To address this issue, we investigate the role of cerebellar neural circuitry in social behaviors using the zebrafish cerebellum as a model.

Adult zebrafish are social animals and exhibit preference for conspecifics, aggregate, shoal, and school in both nature and the laboratory. Adult zebrafish pairs are known to show the synchronized orienting swimming, called orienting behavior, which reflects social attention. It is a swimming behavior between two individuals in which, when they are spatially separated and can view each other through a partition, they prefer to approach and behave stereotyped orienting pattern. To investigate the involvement of the cerebellum in the orienting behavior, we used adult wild-type zebrafish, transgenic zebrafish that express botulinum toxin, which inhibits release of neurotransmitters, in either granule cells (GCs) or Purkinje cells (PCs), and *reelin* mutants that show abnormal positioning of PCs. In spatially separated and mutually visible conditions, pairs of wild-type fish stayed near the partition separating the two fish and exhibited a specific range of body angles (orienting angles) compared to the condition without visual stimuli. The fish expressing botulinum toxin in GCs or PCs, and the *reelin* mutants showed significantly reduced percentages of positioning near the partition and showing the orienting angles in the presence of visual stimuli, compared to their control siblings. We also found increased expression of *c-fos* and *egr1*, markers for neural activity, in the cerebellum after the orienting behavior. These results suggest that the zebrafish cerebellum plays important role in the social orienting behavior.

133 - Lifelong Cartilage Regeneration in Zebrafish

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Cartilage has limited capacity for repair, as demonstrated by the increasing prevalence of osteoarthritis in the aging population. Here, we have developed a genetic ablation model to assess the ability of zebrafish to regenerate cartilage throughout their lifetime. In *col2a1a*:mCherry-NTR fish, addition of metronidazole as early as 7 days and as late as one year after fertilization results in robust ablation of craniofacial cartilage. Within 3 days following ablation, we observe substantial new cartilage forming around the dead cartilage, which we can monito r by re-expression of a *col2a1a*:GFP transgene. Formation of new cartilage correlates with upregulation of the Bmp responsive *BRE*:GFP transgene. Interestingly, new cartilage is formed from the perichondrium adjacent to growth plates but not from the periosteum, suggesting a loss of competency of cells to respond to cartilage loss as they transition into periosteal zones. The cartilage outgrowths observed are reminiscent of those seen in the disease Hereditary Multiple Exostoses (HME), caused by mutations in *EXT1* or *EXT2* genes involved in heparan sulfate proteoglycan synthesis. As the drug palovarotene has shown promise in reducing exostoses, we tested whether it could also prevent cartilage outgrowths in response to cartilage ablation. Indeed, treatment of zebrafish with palovarotene immediately following cartilage ablation completely abolished the cartilage regenerative response. Here we show that zebrafish are capable of mounting a cartilage regenerative response through adulthood. Beyond offering insights into boosting cartilage regeneration, this cartilage ablation and regeneration model may also provide a high-throughput way to investigate new drugs to treat HME and other forms of heterotopic ossification.

134 - Regulatory Evolution of Isl1 is Linked to the Origin of Paired Appendages in Vertebrates

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The molecular basis of paired appendage origin is a central question in vertebrate evolutionary history. Studies in mice identified *Isl1* as an initiator of hindlimb development, but the requirement of *Isl1* in fishes is elusive because *Pitx1* was shown to be necessary and sufficient for pelvic fin development. Here, we provide evidence that the initiation of pelvic appendages by *Isl1/2* is a conserved feature of jawed vertebrates. We found that although the developing pelvic fin of zebrafish does not express *isl1a/b*, it expressed *isl2a/b*. Through a sequence analysis, we identified an *Isl1/2* enhancer that originated in jawed vertebrates, coincident with the origin of paired appendages. The conserved enhancer can drive reporter expression in the cardiopharyngeal field and lateral plate mesoderm region presumptive of pelvic appendages. Following the divergence of *Isl1* and *Isl2* in jawed vertebrates, zebrafish lost the *isl1* enhancer while retaining the *isl2* enhancer. Strikingly, conditional activation of *isl1* is sufficient to induce premature pelvic appendage outgrowth in a small population of zebrafish embryos, suggesting the existence of a latent development potential initiated by *isl1*. These findings indicate that pelvic appendage development in the common ancestor of zebrafish and mice relies on *Isl1/2*, and supports a model where jawed vertebrates evolved *Isl1/2* enhancers that express in the lateral plate mesoderm and drove the evolution of paired pelvic appendages.

135 - Short telomeres modulate anxiety-related behavior in zebrafish

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Telomeres function as molecular clocks that count the number of primary cells division. Telomere shortening plays a central role in the aging process in humans since mutations in genes that control for telomere maintenance are associated with premature aging. Aging affects multiple cognitive and social behaviors, and telomere shortening has also been reported in patients with anxiety-related disorders. First generation of telomerase-deficient zebrafish (*tert*$^{-/-}$) has become a reference model of premature aging, telomeropathies, and associated neuropsychological disorders.

In the present study, we tested *tert*$^{-/-}$zebrafish (one year old) using the Novel Tank Diving (NTD) test, which measures the anxiety response to novelty. Typically, when zebrafish adults are introduced to a new environment, they display bottom dwelling behavior at the beginning as a sign of anxiety, and once their anxiety/stress is relieved, they start vertical exploration of the water column.

Our results showed that *tert*$^{-/-}$ mutants remained more on top of the tank, not showing the same anxiety levels compared to their wild type counterparts. Both wild type and *tert*$^{-/-}$ mutants showed similar swimming activity, indicating that behavioral differences were not related to overall motor activity.

This neurobehavioral phenotype was recovered in a double *tert*$^{-/-}$*/tp53*$^{-/-}$ mutant, suggesting molecular control mechanism mediated by tp53 signaling pathways.

To our knowledge, this is the first study that a behavioral impairment was rescued through a double genetic modification. Further work is in progress to unravel the underlying molecular mechanism.

136 - Identification of regulatory elements active in skeletal tissues associated with craniosynostosis risk genes

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Craniosynostosis (CS), one of the most common craniofacial birth defects, is when one or more cranial sutures are prematurely replaced by bone, which reduces flexibility of the skull and restricts brain expansion. A genome-wide association study (GWAS) for CS identified two risk loci, one downstream of *BMP2*, and one within the *BBS9* gene, adjacent to *BMPER*, encoding an extracellular modulator of BMP signaling. We hypothesized that distal regulatory elements for *BMPER* located within *BBS9* accounted for the CS risk and aimed to identify *BMPER* enhancers active in skeletal tissues. From a 1.3 Mbp region encompassing *BMPER* and a portion of *BBS9,* including the GWAS risk locus, we selected conserved noncoding sequences as candidates. Using zebrafish transgenesis, we identified two enhancers. The -117*BMPER* is broadly active in early osteoblasts, whereas -707*BMPER* regulates expression in cranial cartilage and harbors a sequence variant linked to CS risk. We similarly examined the risk locus near *BMP2* and identified two enhancers; +402*BMP2* is active in bone, while +421*BMP2* is active in cartilage and also contains a risk-related variant. To further identify the potential transcription factor (TF) interactions with each enhancer, we screened them against a library of >1100 annotated human TFs via an enhanced yeast one-hybrid (eY1H) assay. We are currently performing a targeted screening of the two enhancers containing CS risk-associated variants, to look for disease-related differences in TF bindings. In summary, we have found conserved enhancers for genes in the BMP pathway, and through the eY1H screen, identified signaling pathways that may regulate their activity. Both genes play conserved roles in skeletal development, and our analysis offers insights into their regulation across species. Both genes are also implicated in genetic risk for CS, and our identification of enhancers from the risk loci is a critical step in understanding the mechanisms underlying CS pathogenesis.

137 - Single-cell atlas of the regenerating spinal cord in adult zebrafish

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Unlike mammals, zebrafish have the innate ability to regenerate their spinal cord (SC) within 6-8 weeks post-injury (wpi). To determine the transcriptomic signature of regenerating SCs, we performed single nuclear RNA sequencing at 0, 1, 3 and 6 weeks post-injury. Our analysis revealed dynamic changes in cell populations across time points and uncovered the emergence of specific cell clusters after injury. Using various bioinformatic tools, we identified differentially expressed signaling pathways as well as pseudo cell trajectories during the course of regeneration. By analyzing neurotransmitter gene expression, both *in silico* and *in vivo*, we characterized the excitatory and inhibitory landscape during successful regeneration. Further analysis of neuronal subclusters identified one injury-induced population that is exclusively present at 1 wpi and that we refer to as iNeurons. We hypothesize that genes expressed in iNeurons include factors that promote neuron survival, axon growth, and SC regeneration. We used HCR *in situ hybridization* to confirm the expression of iNeurons top markers and high-efficiency CRISPR/Cas9 to elucidate their regenerative roles and the contribution of iNeurons to SC repair. Additionally, we are performing cross-species transcriptomic comparisons between zebrafish and mice to identify differences and similarities between regenerative and non-regenerative systems. Our study provides a comprehensive resource of cell populations that direct successful SC regeneration, cross-species comparisons of different SC injury response and novel therapeutic targets for neural repair.

138 - Srsf6b-induced splicing defects contribute to selective motor neuron vulnerability in a zebrafish model for Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease caused by the deficiency in the Survival of Motoneuron (SMN) protein. It is characterized by a loss of lower α-motor neurons and progressive muscle atrophy. Although SMN protein deficiency is systemic, MNs become selectively vulnerable during SMA disease conditions. To elucidate the molecular mechanisms behind this selective vulnerability, we used bulk transcriptomics in a zebrafish SMA model and identified the splice factor Srsf6b as a novel target of SMN. It was found to be enriched in MNs and aberrantly spliced under SMN-deficient conditions. Phenotypic analysis showed that knockout of *srsf6b* in zebrafish causes early motor axon pathfinding and synaptogenesis defects. Transcriptomics analysis identified that Srsf6b is crucial for splice regulation of specific genes important for MN development and synaptogenesis at neuromuscular junctions (NMJs). An *srsf6b* deficiency also exacerbated defects in a *smnA6T* zebrafish model that emulates intermediate types of SMA. This suggests that *srsf6b*, when wrongly spliced in Smn deficient MNs, amplifies splicing defects in MN-specific genes at an early disease stage. This leads to neuromuscular defects, potentially causing selective vulnerability of MNs during SMA disease conditions.

139 - Optogenetic control of intracellular second messengers in neurons and heart muscle cells

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Optogenetics is a breakthrough technology for non-invasive manipulation of neural activity. The channelrhodopsins (*Cr*ChRs) from the green algae *Chlamydomonas reinhardtii* have been used to control neuronal activities. Intracellular signaling involving G-protein coupled receptors (GPCRs), Ca^{2+}, cAMP and cGMP are involved in synaptic plasticity and higher brain function, but optical manipulation of these signals has not been well established.

In this study, we attempted to regulate intracellular signaling using microbial rhodopsins and animal rhodopsins to control the intracellular signals in zebrafish. The microbial rhodopsins were Na^+-selective channel rhodopsin (*Gt*CCR4) from the cryptophyte *Guillardia theta*, cation channel rhodopsin (*Kn*ChR) from filamentous terrestrial algae *Kebsormidium nitens*, and guanylyl cyclase rhodopsin (*Be*GC1) from aquatic fungus *Blastocladiella emersonii*. The animal GPCR rhodopsins were bistable rhodopsins that do not release the chromophore after light absorption, including Gq-GPCR rhodopsin from jumping spider (SpiRh1) and Gi/o-GPCR rhodopsin from mosquito and lamprey (Mos Opn3, Lamp PP). We expressed these tools in the hindbrain reticulospinal V2a neurons, which are involved in locomotion, or cardiomyocytes, by Gal4-UAS system. Light stimulation of V2a neurons with *Gt*CCR4 or *Kn*ChR immediately induced tail movements comparable or more efficiently than *Cr*ChR2, whereas stimulation with *Be*GC1 induced swimming with a short delay, presumably through upregulation of intracellular cGMP or cAMP. Similarly, stimulation with the Gq-GPCR SpiRh1 induced swimming with a short delay. Studies with the Ca^{2+} indicator GCaMP6s revealed that the SpiRh1-mediated activation led an increase in Ca^{2+} in the V2a neurons, indicating that SpiRh1 activated the Gq-GPCR→PLCβ→IP3→Ca^{2+} pathway. Light-stimulation with Gi/o-GPCRs Mos Opn3 or Lamp PP in the cardiomyocytes immediately stopped heart beats, which lasted a few minutes. The Gi/o-GPCR activity was found to be mediated by the regulation of inward-rectifier K^+ channels. Our results indicate that these optogenetic tools are useful to manipulate the second messenger-mediated signaling in zebrafish *in vivo*.

140 - CRISPR/Cas9-mediated constitutive loss of VCP (Valosin containing protein) impairs proteostasis and leads to defective striated muscle structure and function in vivo

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CRISPR/Cas9-mediated constitutive loss of VCP (Valosin containing protein) impairs proteostasis and leads to defective striated muscle structure and function *in vivo*

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Valosin Containing Protein (VCP) acts as a key regulator of cellular protein homeostasis by coordinating protein turnover and quality control. In humans, mutations in VCP lead to (cardio-)myopathy and neurodegenerative diseases such as inclusion body myopathy with Paget disease of the bone and frontotemporal dementia (IBMPFD) or amyotrophic lateral sclerosis (ALS). To date, due to embryonic lethality, no constitutive VCP knockout animal model exists.

Here, we generated a constitutive CRISPR/Cas9-induced *vcp* knockout zebrafish line. Similar to the phenotype of *vcp* morphant knockdown zebrafish embryos, we found that systemic *vcp* deficiency leads to significantly impaired cardiac and skeletal muscle function. By ultrastructural analysis of skeletal muscle fibers and cardiomyocytes, we observed severely disrupted myofibrils, accumulation of lysosomal structures and inclusion bodies, as well as mitochondrial degeneration. *vcp* knockout was associated with a significant accumulation of ubiquitinated proteins, suggesting impaired proteasomal function. These results reflect dysregulation of the pleiotropic functions of VCP in protein and organellar quality control pathways.

In conclusion, our findings demonstrate the successful generation of a stable constitutive *vcp* knockout zebrafish line that will enable the characterization of the detailed mechanistic underpinnings of *vcp* loss-of-function, particularly the impact of disturbed protein homeostasis on organ development and function in vivo.

141 - A newly identified cilium controls meiotic chromosomal pairing mechanics and germ cell morphogenesis in zebrafish and mouse

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Meiosis is a cellular program essential for the production of haploid gametes. The hallmark of meiosis is chromosomal pairing and synapsis via synaptonemal complexes, but chromosomal pairing also depends on cytoplasmic counterparts that tether and rotate telomeres on the nuclear envelope. Telomeres slide on perinuclear microtubules, shuffling chromosomes and mechanically driving their homology searches. Pull of telomeres towards the centrosome drives formation of the “zygotene chromosomal bouquet”. These telomere dynamics are essential for pairing and fertility, and the bouquet, discovered in 1900, is universally conserved. Nevertheless, how cytoplasmic counterparts of bouquet formation are mechanically regulated has remained enigmatic. Here, we report the “zygotene cilium” - a previously unrecognized cilium, in oocytes. We show in zebrafish that this cilium specifically connects to the bouquet centrosome, constituting a cable system of the cytoplasmic bouquet machinery. Furthermore, zygotene cilia extend throughout the germline cyst, a conserved germ cell organization. Using multiple ciliary mutants and laser-induced excision, we demonstrate that the zygotene cilium is essential for chromosomal bouquet and synaptonemal complex formation, germ cell morphogenesis, ovarian development and fertility. Mechanistically, we provide evidence that the cilium functions at least partly via anchoring the bouquet centrosome in order to counterbalance telomere rotation and pulling. We also show that the zygotene cilium is conserved in both male and female meiosis in zebrafish, as well as in mammals. Our work uncovers the novel concept of a cilium as a critical player in meiosis and sheds new light on reproduction phenotypes in ciliopathies. We propose a cellular paradigm that cilia can control chromosomal dynamics.

A preprint describing this work is available on *bioRxive*:
https://www.biorxiv.org/content/10.1101/2021.02.08.430249v2

142 - The SWI/SNF complex subunit Smarce1 is a key regulator of cardiomyocyte proliferation during development

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Molecular pathways that regulate organ growth during embryonic development are often reactivated in adulthood to orchestrate organ repair and regeneration after injury. The molecular mechanisms that regulate heart growth during development and regeneration after injury are only poorly defined yet, but of major clinical importance for therapeutic heart repair. In search for novel master regulators of cardiac growth, we isolated in an ENU mutagenesis screen the zebrafish mutant *heart of stone* (*hos*), which displays massively increased cardiomyocyte proliferation during development. By positional cloning, we found that loss of the SWI/SNF chromatin remodeling complex member Smarce1 is causing uncontrolled cardiomyocyte proliferation in *hos*, whereas Smarce1 overexpression represses cardiomyocyte proliferation in the developing zebrafish heart, demonstrating fine-tuned Smarce1 expression crucial for orchestrated cardiomyocyte proliferation in embryonic zebrafish hearts. Smarce1 thereby orchestrates cardiomyocyte proliferation by regulating cyclins, cyclin-dependent kinases and their inhibitors, in zebrafish via pro-proliferative Jak/Stat3 signaling. Thus, tight regulation of the SWI/SNF chromatin remodeling complex subunit Smarce1 is essential to control cardiomyocyte proliferation/cell cycle activity during development in the vertebrate heart.

143 - Dissecting the molecular networks of cardiomyocyte proliferation to guide heart regeneration

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Myocardial infarction is a life-threatening disease of the human heart and leads to reduction of functional cardiac muscle. Although, current therapeutic approaches can strongly reduce the mortality in myocardial infarction patients, they are still limited due to unknown molecular-based strategies to induce myocardial regeneration. Unlike human hearts, zebrafish hearts is naturally undergoing heart regeneration after injury mediated by proliferation of resident cardiomyocytes. Regeneration of the adult zebrafish heart is due to the reactivation of embryonic signaling pathways guiding cardiomyocyte proliferation during heart development.

To dissect molecular networks underlying endogenous cardiomyocyte proliferation during zebrafish embryogenesis we compared the zebrafish lines '*heart of stone*' (*hos*) and '*liebeskummer'* (*lik*). They have a similar phenotype of cardiac hyperplasia due to increased cardiomyocyte proliferation.

Using the state-of-the-art technology 'RNA-Seq', differentially expressed genes (DEGs) of both fish lines have been identified by comparing the expression levels of the mutants with their wildtype siblings. To investigate differential expression of heart specific genes, the DEGs of both fish lines were filtered for genes expressed in the heart and compared.

Tumor necrosis factor receptor superfamily, member 1a (tnfrsf1a) and *S100 calcium binding protein A10b* (*s100a10b*) are upregulated in both fish lines and their role in cardiomyocyte proliferation during early embryogenesis have been investigated. Knockdown of these genes via Morpholino injection led to a severe phenotype including changes in the body axis as well as a reduced number of cardiomyocytes. These results suggest that our approach of comparing RNA-seq generated DEGs of mutant fish lines is suitable to identify genes with a regulatory function during cardiomyocyte proliferation, which will be further analyzed.

144 - TLR/NFκB-mediated indirect negative feedback regulation of Wnt/β-catenin signaling controls precise size of zebrafish dorsal organizer.

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Since an NFκB homolog Dorsal was reported as an initiator of *Drosophila* axis formation, enormous efforts have been made to understand the molecular mechanisms underlying the initial process of axis formation during the past decades. In *Drosophila* axis initiation, specific activation of Toll receptor at the ventral region stimulates the activation of Dorsal, which promotes ventral specification. Dorsal also activates the expression of a Wnt family of extracellular protein which functions as a direct antagonist of Toll receptor. This Wnt-mediated negative feedback regulation of Toll/NFκB signaling controls correct size of embryonic ventral region. On the other hand, in vertebrate including fish and amphibians, the initial cue of axis formation is Wnt/β-catenin signaling. Wnt/β-catenin is specifically activated at the dorsal region and promotes the formation of dorsal organizer which controls dorsal specification. However, it still remains unclear whether Toll-like receptor (TLR)/NFκB signaling is involved in axis initiation in vertebrate. Here, by combination of *in vivo* reporter analysis, CRISPR/Cas9-mediated knockout, and morpholino knockdown, we show that TLR/NFκB signaling is activated at the dorsal region in early zebrafish embryos and participates in axis initiation. Mechanistically, Wnt/β-catenin signaling stimulates the activation of an NFκB homolog Rel specifically at dorsal region through Toll-like receptor 4 (Tlr4). Activated Rel then stimulates the transcription of a Wnt antagonist *frizzled related protein* (*frzb*), thereby restricting the Wnt/β-catenin-active area and dorsal organizer region. Thus, Tlr4/NFκB-mediated indirect negative feedback regulation of Wnt/β-catenin signaling controls precise size of zebrafish dorsal organizer. In addition, the crosstalk between TLR/NFκB and Wnt in axis initiation is evolutionarily conserved but, interestingly, the roles of them as an initial cue and a feedback mediator are inverted between *Drosophila* and zebrafish.

145 - The HPT axis induces AgRP1 neuron proliferation in Oatp1c1-deficiency

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Thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3), regulate growth, metabolism, and neurodevelopment. THs secretion is controlled by the hypothalamic thyrotropin-releasing hormone (TRH) and the hypothalamic-pituitary-thyroid (HPT) axis. To facilitate TH activity in the cell nucleus, the organic anion-transporting polypeptide 1C1 (OATP1C1/Slco1c1) and the monocarboxylate transporter 8 (MCT8/SLC16A2) transport primarily T4 and T3, respectively. Mutation in *OATP1C1* is associated with brain hypometabolism, gradual neurodegeneration, and impaired cognitive and motor functioning in adolescent patients. In order to understand the role of Oatp1c1 and the mechanisms of the disease, we profiled the transcriptome of *oatp1c1* mutant (*oatp1c1-/-*) and *mct8-/-xoatp1c1-/-* adult zebrafish brains. Among dozens of differentially expressed genes, agouti-related neuropeptide 1 (*agrp1*) expression increased in *oatp1c1-/-* adult brains. Imaging analysis showed enhanced proliferation of AgRP1 neurons in *oatp1c1-/-* larvae and adults, and increased food consumption in *oatp1c1-/-* larvae. Similarly, the number of AgRP1 neurons increased in thyroid gland-ablated zebrafish. Pharmacological treatments showed that the T3 analog 3,3',5-tri-iodothyroacetic acid (TRIAC), but not T4, normalized the number of AgRP1 neurons in *oatp1c1-/-* zebrafish. Since *trh* expression increased in *oatp1c1-/-* brain, we inducibly overexpressed *trh* in wild-type larvae and found that Trh promotes the proliferation of AgRP1 neurons. These results revealed functional upregulation of the Trh-AgRP1 circuitry and feeding under brain hypothyroidism, suggesting the AgRP1 system as a therapeutic target in OATP1C1-deficiency.

146 - Olfactory rod cells: transcriptomic and functional characterisation of a rare cell type in the vertebrate olfactory system

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The basic senses have been essential for the survival and evolution of most animal species. It is therefore unsurprising to find many specialised cell types that contribute to sensation — from different classes of olfactory sensory neuron and chemosensory taste receptor, to mechanosensory hair cells in the auditory and vestibular systems. Olfactory rod cells are a rare and newly reported cell type in the zebrafish olfactory epithelium (Cheung et al., Front. Physiol. 2021) which differ from the known classes of olfactory sensory neuron. These cells each bear a large actin-rich rod-shaped apical projection extending about 10 µm above the epithelial surface and have a rounded cell body positioned apically in the epithelium. Olfactory rods appear in the olfactory epithelium at around 36 hours post-fertilisation and are posterolaterally clustered in the olfactory pit. Olfactory rod cells are labelled by actin reporters and express calcium indicators driven by neuronal promoters. Due to their location in a sensory system and the presence of an apical projection protruding into the external environment, it is hypothesised that these mystery cells are a type of multimodal sensory cell. To better understand the neurophysiology of the olfactory system, we now aim to further define the cell type by means of transcriptomic and functional analyses. Here, we report our current methods for performing RNA sequencing, calcium imaging, and whole-cell patch-clamp electrophysiology for such characterisation.

147 - Quantitative videomicroscopy reveals the impact of transcriptional asymmetry on hair cell rotations

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Multicellular rotations are common but poorly understood processes that occur *in vitro* and during organogenesis. Theoretical and *in vitro* studies have conceptualized cell-pair rotations as self-organizing systems of identical particles that stochastically break symmetry to start a monotonous and continuous angular movement within an inert substrate. However, it is unclear if this notion can be extrapolated to a natural context, where rotations stop in a very reproducible manner and heterogeneous cells interact with an active epithelium.

Here we pursue this problem in neuromasts of zebrafish, where sibling hair cells acquire different identities via Notch1a-mediated asymmetric repression of the transcription factor Emx2. Next, hair-cell pairs rotate ~180° around their geometric center. We show that this rotation is three-phasic, starts spontaneously, and progresses via coincident strong homotypic coupling and coherent heterotypic junction remodeling. Rotating cells exert independent but equivalent active forces and interact asymmetrically with the surrounding epithelium. Contrary to expectation, the Notch/Emx2 status of the cells does not correlate with this asymmetry. Moreover, eliminating Notch1a affects the precision and the accuracy of the angular movement, while loss of Emx2 has no significant impact.

These findings are compatible with a mechanistic model under which stochastic emergence of local instabilities enables departure from a metastable state to initiate rotations, whereas deterministic cell-pair asymmetry reduces rotational noise.

148 - Role of Ccn2a in zebrafish intervertebral disc maintenance and regeneration

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Intervertebral disc (IVD) degeneration is the primary cause of low back pain in humans, levying a significant load on the clinical system. The current solutions focus on surgical intervention instead of treating degenerated tissue to improve the pathological conditions. The knowledge about IVD maintenance and the etiology of the pathogenesis of IVD degeneration is poorly studied. The cellular communication network factor-2 (CCN2), a matricellular protein, has been shown to play a role in fibrotic and regenerative processes in different organs. This study shows the role of Ccn2a, zebrafish orthologue of mammalian CCN2, in zebrafish IVD maintenance. We find that *ccn2a* is expressed in the notochord as well as in adult IVDs. *ccn2a*$^{-/-}$ show decreased cell proliferation and increased cell death leading to IVD thinning and degeneration. Moreover, ectopic expression of Ccn2a induces cell proliferation and cell survival in aged wild-type zebrafish IVDs. With the help of loss- and gain-of-function, pharmacological, and biochemical studies, we found that mechanistically, Ccn2a maintains cellular homeostasis of IVDs and can promote IVD regeneration by inducing FGFR1-SHH signaling. These findings enrich the understanding of the cellular and molecular mechanisms in IVD homeostasis by Ccn2a with its potential therapeutic implications to promote regeneration in degenerated human discs

149 - Yolk cell cytoskeletal dynamics and interactions during zebrafish epiboly

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The main drivers of blastoderm and yolk syncytial layer (YSL) epiboly are actomyosin based motors in the yolk cell. An array of microtubules is also found in the yolk cell and disruption of either actin or microtubule cytoskeletal networks leads to epiboly delay. It is currently unknown whether the organization or function of each network depends on the other. We are exploring the possibility that microtubules and actin interact in the yolk cell to facilitate epiboly. Based on work in other systems, structural and regulatory interactions are two potential mechanisms of cross regulation. Our focus is the YSL, where the actomyosin ring forms via retrograde flow to drive epiboly. We used live imaging to examine the spatiotemporal relationship between actin and microtubules in the YSL. Using EMTB-3GFP, which does not affect microtubule dynamics (Eckerle et al., 2017), we identified a horizontal array of microtubules that accumulates during epiboly and is positioned just vegetal to the actin ring. Formation of the microtubule array coincides with the formation of the actin ring and appears to be linked to retrograde flow. The function of the horizontal microtubule array is unclear, but it may act as a fence to concentrate and/or spatially restrict the actomyosin ring. We are currently examining the effects of disrupting one cytoskeletal structure on the other. We find that yolk restricted expression of constitutively active myosin phosphatase results in disorganized marginal microtubules. We are also examining potential candidates that might be involved in actin-microtubule interactions. Camsap2a is a microtubule minus-end binding protein that act as a site for microtubule stabilization and polymerization. Camsaps can also mediate interactions between microtubules and actin. Zebrafish *camsap2a* is expressed in the YSL during epiboly and CRISPR/Cas9 mutant lines, currently being characterized, exhibit reduced actomyosin ring formation and epiboly delays.

150 - A New Mechanism of Dorsal Axial Organizer Repression by Integrator Complex Subunit 6

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The vertebrate body axis is specified by an embryonic signaling center called the dorsal organizer, which can duplicate the body axis when grafted into a host embryo. A zebrafish loss-of-function mutant isolated in our laboratory, called *ints6*^{p18ahub}, provides new insights into how the organizer is repressed to specify the proper balance of dorsoventral tissues. In this maternal-effect mutant, the progeny of homozygous *ints6*^{p18ahub} mutant females (M-*ints6*^{p18ahub} embryos) display an expanded organizer and multiple body axes, a unique zebrafish loss-of-function mutant phenotype. The mutated gene, *integrator complex subunit 6* (*ints6*), encodes a member of the Integrator complex, an RNA processing complex that has not previously been implicated in embryonic patterning. Integrator's first discovered function was in the maturation of spliceosomal small nuclear RNAs, but the complex has several additional mechanisms of regulating gene expression. In this project, I aim to uncover the mechanism by which Ints6 represses the organizer. I have found that the *ints6*^{p18ahub} allele is hypomorphic and temperature-sensitive. Employing temperature shift experiments, I have determined that the critical window of Ints6 function is between high and sphere stages (3.3-4 hpf) to repress the organizer. I also found that a separate maternal-effect nonsense allele of *ints6* causes an earlier embryonic arrest phenotype at the mid-blastula stage. These findings suggest that Ints6 has at least two functions: first in developmental progression past the mid-blastula stage, and second in organizer repression. Furthermore, my analysis of RNA-seq in M-*ints6*^{p18ahub} embryos supports the hypothesis that Ints6 represses the expression of many genes in the embryo, via a recently discovered transcriptional attenuation function of the Integrator complex. I am currently regionally expressing Ints6 to determine where in the embryo Ints6 functions to repress the organizer. These studies will reveal a new mechanism of dorsal organizer repression and will provide new insights into Integrator complex function.

151 - Regulation of dynein function in the axon terminal by NudC is essential for both microtubule dynamics and initiation of retrograde cargo transport

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Microtubule dynamics are essential for axon outgrowth and maintenance of mature axons. While we have learned much about the role of microtubule dynamics and their regulation during axon outgrowth, how microtubule stability is regulated in mature axons is less clear. This is particularly true in the case of the dynamic microtubule plus ends in the axon terminal. In axon terminals, microtubule plus ends play a number of roles, including functioning as a docking site for the retrograde motor protein complex Cytoplasmic dynein. Dynein interaction with microtubule plus ends is required for efficient cargo loading and initiation of retrograde transport. In addition, in vitro dynein can also increase microtubule stability. The role of dynein in axon terminal microtubule regulation was largely unexplored. Using a forward genetic screen, we identified NudC as a regulator of dynein activity in axon terminals. NudC is a chaperone protein that can stabilize Lis1, an essential dynein activator for cargo transport. In *nudc* mutants there is a complete loss of Lis1 protein in the axon terminal. Analysis of axon terminal ultrastructure in *nudc* mutants revealed several phenotypes including: 1) high levels of dynein motor proteins; 2) accumulation of vesicular cargo; and 3) nets of stabilized microtubules. Aberrant microtubule stability and cargo accumulation was suppressed by exogenous Lis1 expression. Lis1 facilitates cargo loading but is not known to regulate microtubule dynamics. Because dynein can enhance microtubule stability in vitro and dynein levels are increased in *nudc* mutant axon terminals, we postulated that the increased motor presence could underlie the microtubule stabilization. Treatment with the dynein inhibitor ciliobrevin suppressed microtubule netting in *nudc* mutants, confirming increased dynein activity causes this phenotype. Together our data implicate NudC in the compartment-specific regulation of dynein localization and function through promoting Lis1 stability in axon terminals.

152 - Beyond a common cloaca: partitioning of distal gastrointestinal and urinary tracts in adult zebrafish

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Introduction: Historically, adult zebrafish have been thought to excrete urine directly into a cloaca which is a single common channel for excretion that includes urinary, gastrointestinal and genital tract terminations. Humans have separate tracts for these systems. Studies in some fish species have identified separate pores for urinary tracts. The distal course of urine has not been examined directly in adult zebrafish.

Methods: Adult casper zebrafish (roy$^{-/-}$, nacre$^{-/-}$, one to two years old) were anesthetized with tricaine solution. Pericardial injections of dextran-conjugated Alexa 488 dye were performed by hand. Dextran-conjugated Alexa 568 was used for counter-injections into the cloaca. While still anesthetized, fluorescent videos were acquired of the caudal abdomen between the pelvic and anal fins. Confocal images were obtained with an Andros spinning disk confocal microscope.

Results: Fish survived injections for up to 30 minutes before sacrifice. The Alexa 488 tracer circulated to the mesonephros, filled the collecting duct and distal urinary system, and was excreted after ten minutes. Fluorescent urine first filled a bladder-like structure in the caudal abdominal region and was then excreted externally from an orifice ventral to the bladder in a pulsatile manner. Cloacal counter-injections with fluorescent dextran filled the gastrointestinal tract only. Fluorescent urine maintained a path distinct from the gastrointestinal tract. Closer inspection of the exit site revealed an external urethral structure from which all urine was expelled. Confocal images confirmed the urethral location as posterior to the cloaca. In females, this structure is visible with light microscopy.

Conclusions: Adult zebrafish have a urethral structure that expels urine through an external urethral meatus. Urine appears to be stored and secreted from bladder- and urethral-like structures that are distinct from the gastrointestinal tract. This anatomical insight opens the possibility to employ zebrafish for studying congenital abnormalities of the distal urinary tract.

153 - The selective VCP inhibitor CB-5083 impairs striated muscle function in zebrafish and disrupts cardiomyocyte protein homeostasis

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Mutations in valosin-containing protein (VCP/p97) are associated with multisystem proteinopathy with (cardio-)myopathy and neurodegenerative diseases. Pathogenesis in VCP disease stems from dysregulation of multiple protein quality control (PQC) pathways. Striated cardiac and skeletal muscle are particularly susceptible to disruptions in proteostasis due to the complex maintenance requirements of the sarcomere. Selective VCP inhibitors represent a promising therapeutic avenue for VCP disease based on studies in patient myoblasts and iPS-derived motor neurons. However, the effects of pharmacological VCP inhibition on cardiac function have not been sufficiently investigated.

VCP loss-of-function produces significant (cardio-)myopathic phenotypes in zebrafish embryos. We hypothesized that the VCP inhibitor CB-5083 would reproduce phenotypes observed in *VCP* knockdown embryos. Embryos treated with CB-5083 displayed myopathic and neuromuscular phenotypes, characterized by reduced muscle organization, impaired motility, abnormal motor neuron development, and complete loss of cardiac contractility by 72 hpf, recapitulating knockdown phenotypes. Next, we investigated biochemical markers of PQC pathways. Treated embryos showed increased levels of ubiquitinated protein and Hspa5/BiP, consistent with ERAD impairment. Ultrastructural analysis revealed sporadic myofilament degeneration in skeletal muscle fibers and enlarged, dysmorphic mitochondria in both skeletal and cardiac muscle of treated embryos, consistent with roles of VCP in the regulation of mitophagy. To further support a direct effect of VCP inhibition on cardiomyocyte proteostasis, we treated neonatal rat cardiomyocytes (NRVMs) with CB-5083 and again evaluated PQC markers. Inhibitor-treated NRVMs showed a dose-dependent increase in ubiquitinated protein and elevated Hspa5/BiP and phospho-Rps6, a downstream target of mTOR Complex 1, suggesting altered regulation of cardiomyocyte protein synthesis via the mTOR pathway.

These findings demonstrate potential cardiotoxicity of pharmacological VCP inhibition mediated by disruption of cardiomyocyte proteostasis. Additionally, we support growing evidence that VCP function is necessary for maintaining myocardial homeostasis.

154 - Alone in a crowd: Effect of a non-functional lateral line on expression of the social hormone, parathyroid hormone 2.

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Parathyroid hormone 2 (Pth2) is a vertebrate-specific neuropeptide whose expression in the thalamus of fish and rodents is dynamically regulated by social environment - high expression in the presence of conspecifics but low expression in isolation. In zebrafish, social interactions fail to stimulate *pth2* expression in isolates whose mechanosensory lateral line has been chemically ablated, suggesting that the social modulation of *pth2* levels is acutely dependent on lateral line input. However, it is unknown how a chronic, genetic loss of lateral line function influences the ability of zebrafish to interpret their social environment.

We hypothesize that zebrafish born without a functional lateral line will be unable to appropriately sense their social context, leading to chronically low levels of *pth2*. To test this prediction, we use zebrafish mutants that lack either lateral line function only or both inner ear and lateral line function. Socially-raised lateral line mutants express low levels of *pth2* relative to their social wild type siblings, but there is no further reduction in mutants that lack both lateral line and inner ear function. Interestingly, social isolation of hair cell mutants causes a further reduction in *pth2* expression, indistinguishable from isolated wild type siblings. These results suggest that social modulation of *pth2* expression requires the lateral line along with additional unidentified sensory cues that do not involve the inner ear. Lastly, we created GFP and RFP transgenes that are driven by the *pth2* promoter and find that both isolated and lateral line mutant larvae exhibit a decrease in *pth2*-expressing cells compared to social wild type siblings. Altogether, these data suggest that lateral line mutants experience a chronic sense of isolation, even when raised in a social environment.

155 - Regulation of cell plasticity by mitochondrial metabolism-induced expression and mitochondrial localization of Sgk1

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Many types of differentiated cells are endowed with the ability to reenter the cell cycle and proliferate. This cell plasticity is critical for tissue homeostasis and regeneration. The underlying mechanisms, however, are poorly understood. Here we report a metabolism-regulated intra-mitochondrial signaling mechanism regulating epithelial cell plasticity. Using a zebrafish model, in which a population of differentiated epithelial cells (ionocytes) become reactivated and proliferate in response to a physiological stress, we discovered that this cell plasticity is associated with increased mitochondria membrane potential, TCA cycle/OXPHOS gene expression, and ATP production. The elevated mitochondrial activity and mitochondrial metabolism are both required and sufficient in driving these differentiated cells to proliferate. The elevated mitochondrial TCA cycle/OXPHOS is associated with increased mitochondrial ROS generation, which in turn induces the expression and mitochondrial localization of glucocorticoid-regulated kinase 1 (Sgk1), a conserved AGC family serine/threonine protein kinase and an effector of the PI3 kinase. Genetic perturbation and pharmacological inhibition experiments showed that Sgk1 kinase activity is required for cell reactivation and proliferation. Mechanistically, Sgk1 links the mitochondrial metabolism change to ATP synthesis by modulating the F_1F_o-ATP synthase phosphorylation state. Importantly, this mitochondria metabolism-ROS-Sgk1-F_1F_o-ATP synthase signaling loop is conserved in human cells. These findings uncover a novel intra-mitochondrial mechanism coupling mitochondrial metabolism changes to ATP production and regulating epithelial cell plasticity.

156 - A high-throughput whole animal screen to identify modifiers of atherogenic lipoproteins

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Metabolic dysfunction is the leading cause of worldwide mortality and exhibits complex etiology. Increased apolipoprotein B (ApoB)-containing lipoproteins (B-lps) is a hallmark of cardiovascular and metabolic diseases. B-lps transport lipids through the plasma to peripheral tissues, and excess plasma B-lps are causative to metabolic syndrome. A single ApoB molecule decorates each B-lp and is functionally essential. However, the cellular mechanisms that regulate ApoB and B-lp production, secretion, transport, and degradation remain to be fully defined. B-lp biology is remarkably conserved in the zebrafish, making it the ideal model to identify novel mechanisms of ApoB modulation. Therefore, the Farber lab generated an in vivo chemiluminescent reporter of ApoB that does not disrupt ApoB function. We performed a high-throughput chemiluminescence whole zebrafish screen of 3000 compounds and identified 48 ApoB-lowering compounds. One hit, enoxolone, is reported to inhibit Hepatocyte Nuclear Factor 4α (HNF4α), an orphaned nuclear hormone transcription factor, and reduces the expression of essential B-lp synthesis genes. Not only does enoxolone reduce ApoB levels in zebrafish, but we also found a pharmacogenetic interaction consistent with HNF4α being the molecular target. These proof of principle studies demonstrate the utility of our whole-animal high-throughput chemical screening approach. Cinnamon oil also reduced zebrafish ApoB in our screen. Using liquid chromatography to fractionate this complex oil, we identified a single chemical, eugenol, that specifically reduces ApoB levels in whole animals and circulation. While our work, and that from others, suggest eugenol affects B-lp levels, the mechanism of action remains unknown. Our future goal is to identify how eugenol affects B-lp levels in zebrafish and confirm this effect in a mammalian model. Ultimately, this research aims not only to identify novel ApoB-modulating therapeutics that would improve outcomes of metabolic disease but would also provide fundamental insights into the regulation and function of ApoB.

157 - trans-Tango: driving gene activity across the vertebrate synapse

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Understanding brain function requires the identification of neuronal connections that mediate cognition and behavior. Conventional labeling methods and reconstruction by serial electron microscopy uncover synaptic partners but do not permit manipulation of identified circuits. *trans*-Tango, first developed in *Drosophila*, is a genetic approach for anterograde labeling across the synapse that not only identifies post-synaptic neurons but also permits their transcriptional regulation. A ligand modified from human glucagon is expressed under Gal4 control and tethered to the pre-synaptic membrane of defined neurons where it activates a signaling pathway in the corresponding post-synaptic neurons. Binding of the ligand to the human glucagon receptor coupled to the QF transcription factor through a protease cleavage site recruits an arrestin-protease fusion protein, that cleaves QF. This allows QF to translocate to the nucleus and promote the expression of any gene under the control of the upstream activating sequence (QUAS) where it binds. We adapted *trans*-Tango for use in the zebrafish nervous system and validated its effectiveness both in transient assays using Tol2 *trans*-Tango plasmids and in stable transgenic lines. We injected the *trans*-Tango ligand, receptor, and arrestin constructs along with Tol2 mRNA into embryos from fish-bearing different neuron-specific Gal4 driver lines and UAS:GFP mated to fish containing *Tg(QUAS:mApple-CAAX)*. GFP labeled pre-synaptic neurons were detected in close proximity to mApple labeled putative post-synaptic neurons in Gal4-dependent patterns. Connectivity was validated by examining known synaptic partners in the retina as well as tectal neurons with known morphology. To confirm that gene expression is regulated through signaling across the synapse, we used optogenetics to selectively activate retinal ganglion cells and *Tg(QUAS:GCaMP6f)* to detect calcium transients in post-synaptic neurons in the optic tectum. These exciting results lay the foundation for genetic approaches to reveal synaptic partners and to access an arsenal of tools to monitor or modulate neural circuits.

158 - Characterization of a novel TDP-43 animal model to identify the first steps in ALS disease pathogenesis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. The key pathological signature of ALS is the aggregation of the Tar-DNA binding protein of 43 kDa (TDP-43). Mutations in TDP-43 lead to ALS, demonstrating that TDP-43 also has a causative role in disease. TDP-43 is a RNA- and DNA-binding protein with diverse functions in RNA metabolism, which shuttles between nucleus and cytoplasm. Its protein levels are tightly regulated by autoregulatory feedback loops. Overexpression of TDP-43 in animal models is therefore likely to generate unspecific toxicity and is only poorly recapitulating the disease state. In disease conditions, mislocalization of TDP-43 from the nucleus to cytoplasm results in cytoplasmic TDP-43 aggregation and a nuclear clearing of TDP-43.

To investigate nuclear loss of function and cytoplasmic gain of function of endogenous TDP-43 *in vivo*, we engineered a zebrafish line (Cyto-TDP line) carrying alanine substitutions in the NLS1 of the bipartite NLS of TDP-43 using CRISPR/Cas9. In this novel ALS animal model, we detected mislocalization of TDP-43 to the cytoplasm by immunohistochemistry staining (IHC). Further analysis showed a strongly correlation between TDP-43 mislocalization and a pigmentation phenotype as well as behavioural abnormalities. In addition, we detect reduced expression and a molecular weight shift by Western blot analysis of cytoplasmic TDP-43, indicating reduced stability and a posttranslational modification of TDP-43 in the cytoplasm. In conclusion, we have established a novel animal model that closely mimics pathological TDP-43, which will allow us to investigate the first molecular and cellular pathways affected in ALS and other TDP-43 proteinopathies.

159 - Identification and Characterization of Tango6 in Zebrafish Development

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The Undiagnosed Disease Network (UDN) is a collection of clinicians and researchers that utilize modern technology to help diagnose individuals with rare or previously uncharacterized diseases. One of the genes that the UDN predicted as causal in developmental disease was Tango6, as a UDN participant with multiple point mutations in Tango6 presented with heart and brain abnormalities including atrial and ventricular septal defects, microcephaly, hydrocephalus, and agenesis of the corpus callosum. Tango6 was originally discovered in *Drosophila*, where it was predicted to play a role in Golgi body organization; it is also required in murine development. In order to understand the role that Tango6 plays in development, we utilized embryonic zebrafish to analyze the quantitative and spatial expression of Tango6. In zebrafish, Tango6 is expressed at low to moderate levels between 24 and 120 hours post fertilization (hpf). In situ hybridization demonstrated that Tango6 is generally present in the head around the midbrain-hindbrain boundary beginning at 48 hpf through 120 hpf. Additionally, it is present in the developing gastrointestinal system from 96 through 120 hpf. Preliminary analysis of mosaically edited Tango6 knockouts generated using CRISPR/Cas9 has found an accumulation of blood in the gut by 96 hpf, suggesting defects in gut morphogenesis or function. Further analysis of the developing gut in the embryos at 96 hpf has shown cellular delocalization, which may be leading to the bloody gut phenotype. In total, these data suggest that Tango6 is involved in brain and gut development, and further analysis of knockouts and spatial expression patterns is underway to determine the precise role of Tango6 in development and disease.

160 - Modeling Noonan Syndrome with SOS1 or SOS2 variants in zebrafish

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Noonan syndrome (NS) is a RASopathy with a prevalence of ~1:2,000 live births. The main characteristics of this disease, generally caused by gain-of-function mutations in the RAS/MAPK pathway, are craniofacial anomalies, growth delay and feeding difficulties, cardiovascular anomalies, and juvenile myelomonocytic leukemia. Numerous genes harboring disease-causing genetic mutations have been identified, including PTPN11, SOS1, SOS2, SHOC2 and RIT1.

Although there are many disease aspects of NS, the prevalence and severity of symptoms varies greatly. It has therefore thus far been nearly impossible to establish a link between the genotype and the (predicted) clinical presentation of a patient. The symptoms of NS are mostly present during child development and decrease as the subject ages. Using the zebrafish embryo as the perfect animal model to study early development on a large scale, we aim to elucidate the genotype-phenotype correlation in NS.

We have used a CRISPR/Cas9 homology directed repair knock-in strategy to generate zebrafish lines with NS-patient associated mutations in SOS1 and SOS2. Preliminary data show that adult fish harboring a SOS2 mutation have a significant growth delay compared to their siblings. Additionally, the offspring of the SOS2 mutant zebrafish have a high prevalence of lymphatic anomalies and consequential edema formation. These results are in line with the most prevalent symptoms of patients harboring the equivalent SOS2 mutation, indicating that this mutant zebrafish line can be a useful model to investigate underlying mechanisms causing lymphedema and other symptoms.

161 - Investigating the role of Vangl2 in the regulation of Wnt/β-Catenin signaling

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The Wnt signaling pathway is one of the oldest and conserved pathways in eukaryotic development playing essential roles to control cell patterning, polarity, migration, and stem cell maintenance. Wnt signaling is commonly split into the Wnt/β-catenin pathway, required for cell patterning and proliferation, and the non-canonical or Wnt/Planar cell polarity (Wnt/PCP) pathway, required for cell migration and polarity. These two pathways share common ligands and receptors and the intracellular scaffolding protein Dishevelled (Dvl), but then diverge downstream of Dvl. In Wnt/β-catenin signaling, β-catenin is a transcriptional co-activator, whereas the transmembrane protein Vangl2 activates the Wnt/PCP pathway. While there is evidence to suggest that Vangl2 can inhibit Wnt/β-catenin, the mechanism remains elusive. Here, we demonstrate that different mutant alleles of Vangl2 predispose the embryo to different Wnt/β-catenin sensitivities during gastrulation. The Vangl2 m209 and m747 alleles have premature truncations near the C-terminus, eliminating two putative Dvl binding domains and the C-terminal PDZ binding domain. In addition to the classical Wnt/PCP phenotype, these alleles are embryonic lethal between YPC and 1dpf when injected with a non-phenotypic dose of Wnt8, a Wnt/β-catenin ligand. Further, the lethality phenotype is rescued when co-injected with Nkd1, a Wnt antagonist for both pathways. In contrast, mutations located more N-terminal, such as the mtk50f allele, have no sensitivity to Wnt8, displaying only the Wnt/PCP phenotype. Both phenotypes are recapitulated with CRISPR-induced alleles, and we are creating different alleles in Vangl2 to narrow down the critical region that provides this Wnt/β-catenin sensitivity. This work will provide valuable insight into the crosstalk between Wnt/PCP and Wnt/β-catenin signaling.

162 - Evolutionary divergence of locomotion in Danionella cerebrum and zebrafish

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Locomotion exists in diverse forms in nature and is adapted to the environmental constraints of an animal. We established an approach to understand how neuronal circuit function diverges in nature by compar-ing larvae of two closely related danionin species, *Danionella cerebrum* (DC) and *Danio rer-io* or zebrafish (ZF), which occupy similar environments and yet exhibit different behaviors. During swimming, we demonstrate that DC utilizes lower half tail-beat frequencies and maximum tail angles to generate a lower thrust which produces a slower and continuous swimming pattern when compared to the burst-and-glide swimming pattern in ZF. We investigated the proximate (cellular and physiological) and ultimate (organismal) causes behind this divergence in their swimming pattern despite the phylogenetic and environmental proximity that the two species share. Importantly, we show a high degree of conservation in the brain anatomy and identified Mesencephalic Locomotion Maintenance Neurons (MLMNs) in DC and ZF to be functionally responsible for the maintenance of the long-lasting swim events in the larvae. Moreover, we propose that the slow and continuous swimming pattern of DC might be a result of adaptation to a combination of factors which include lower availability of dissolved oxygen and delayed inflation of swim bladder. These findings uncover changes in the neuronal circuit and ethology underlying the divergence in the swimming pattern of these two species. In conclusion, we show that two anatomically similar brains with conserved features can produce different behavioral outputs based on functional differences in a subset of neurons. We also suggest selective pressures which could have led to the divergence of the swimming pattern. With the ability to assign behavioral modules to their corresponding genetic and neuronal circuit components in ZF (and other danionins), our work provides a powerful approach in comparative neuroethology to investigate evolution of behaviors and neuronal circuits in vertebrates.

163 - Strip1 coordinates retinal neural circuit formation by promoting retinal ganglion cell survival during development

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In the developing vertebrate retina, an interplay between retinal ganglion cells (RGCs), amacrine, and bipolar cells establishes a synaptic layer known as the inner plexiform layer (IPL). This circuit is essential for visual processing as it transmits information from photoreceptors to visual centers in the brain. However, the molecular mechanisms that underlie its development remain largely unknown. Striatin-interacting protein 1 (Strip1) is a core component of the striatin-interacting phosphatases and kinases (STRIPAK) complex, and it has shown emerging functions in embryonic development. Here, we demonstrate the importance of Strip1 in inner retinal neural circuit formation. Using zebrafish, we show that loss of Strip1 leads to IPL disruption. In *strip1* mutants, RGCs undergo dramatic cell death shortly after birth. Subsequently, amacrine and bipolar cells infiltrate the degenerating ganglion cell layer, resulting in a disorganized IPL. At the molecular level, zebrafish Strip1 interacts with its STRIPAK partner, Striatin 3 (Strn3), and both show similar functions in promoting RGC survival. Furthermore, loss of Strip1 or Strn3 leads to activation of Jun-mediated proapoptotic signaling within RGCs, and Jun knockdown rescues RGC survival in *strip1* mutants. In summary, we propose that Strip1 coordinates inner retinal circuit formation by maintaining RGCs during development, which ensures proper positioning and neurite patterning of inner retinal neurons.

164 - Drivers of Sinoatrial Node Automaticity in Zebrafish: Comparison with Mechanisms of Mammalian Pacemaker Function

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Cardiac excitation originates in the sinoatrial node (SAN) and is driven by a coupled system of transarcolemmal ion currents and intracellular Ca^{2+} cycling. The frequency of SAN excitation determines heart rate (HR) and is under the control of extracardiac (*e.g.*, autonomic nervous system) and intracardiac (*e.g.*, mechanical load) mechanisms. While well studied in mammals, the drivers of automaticity and the effects of vagal nerve stimulation and mechanical preload in the zebrafish SAN are poorly understood. Hearts and SAN were isolated from adult zebrafish (AB, 6-12 months post fertilisation) and rabbits (NZW, ~2 kg). Zebrafish hearts were superfused with antagonists to the principal components driving SAN automaticity in mammals (HCN4, T-/L-type Ca^{2+}, and RyR channels) to assess their individual contribution, and their presence and distribution were assessed by immunofluorescence. The effect of vagal nerve stimulation on zebrafish SAN excitation was visualised with voltage optical mapping. The response of the zebrafish and rabbit SAN to mechanical preload was evaluated by the application of controlled stretch. Antagonists of components of the mammalian membrane- (I_f and $I_{Ca,T}$) and Ca^{2+}- (RyR) 'clocks' reduced zebrafish HR. HCN4 channel expression was greatest in the zebrafish SAN and reduced in the atrium, while RyR was highly expressed in both. The site of initial zebrafish SAN excitation varied between subjects and was shifted with vagal nerve stimulation. Application of mechanical preload increased in HR of zebrafish but not rabbit SAN, and increased the stability of HR in both. We have demonstrated that the cellular mechanisms responsible for SAN automaticity are similar in zebrafish and mammals. Further, we have shown that the principal extracardiac and intracardiac HR control mechanisms operate in a similar manner. Overall, our results support the use of zebrafish as an experimental model for studies of SAN (patho-)physiological function.

165 - Lace plant extracts induce apoptosis of human cancer cells and inhibit tumor growth in zebrafish xenografts

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The lace plant (A*ponogeton madagascariensis)* is an aquatic monocot that forms perforations between longitudinal and transverse veins via developmental programmed cell death (PCD). Newly emerging leaves are filled with anthocyanin pigmentation and the disappearance of anthocyanin in the center of areoles represents the first sign of PCD. In the mature stage of the perforation process, anthocyanin is visibly absent, and perforation is complete. Due to the visible pattern of anthocyanin loss at an early stage (so-called window stage), it is evident that anthocyanin plays an important role in PCD in the lace plant, raising the possibility that it may have anti-cancer properties. Here, we studied the effects of crude anthocyanin extracts from window and mature stages on human cancer cells both *in vitro* and *in vivo*. Extracts were tested on human T-cell acute lymphoblastic leukemia (Jurkat cells) and ovarian cancer cells (OVCAR-8); where the effects were compared to mammary epithelial MCF-10A cells. MTT assay analyses showed treatment with both window and mature extracts reduced cancer cell growth and viability in a concentration-dependent manner without affecting non-tumorigenic cells. TUNEL data confirmed both extracts induced cancer cell apoptosis and resulted in the production of high levels of reactive oxygen species (ROS), a potential mechanism of cell death. To evaluate these extracts *in vivo*, we employed the larval zebrafish xenograft model and transplanted these human cancer cell lines into 48h wholly transparent *casper* larvae. Extracts were tested independently, and the window extract reduced cancer cell proliferation after 2 days of treatment in zebrafish larvae. This work highlights the opportunity of the zebrafish xenograft platform to provide rapid *in vivo* validation of the anti-tumorigenic efficacy of natural products, serving an important role in the preclinical drug discovery pipeline.

166 - Effects of an Acute Increase in Hemodynamic Load on Cardiac Function During Cardiac Development

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Background: Cardiac function adapts on a beat-by-beat basis through extrinsic and intrinsic mechanisms. Stretch is a critical intrinsic modulator of cardiac function, as demonstrated by an increase in heart rate (HR) and stroke volume (SV) with an increase in venous return. In the developing heart, the effects of acute changes in hemodynamic load on cardiac function are not well established but may be critical to the pre-neuronal control of cardiac excitation and contraction.

Methods: Effects of an acute increase in hemodynamic load on cardiac function during development were investigated in vivo using 2-14 days post fertilization (dpf) zebrafish larvae. One advantage of this experimental model is that transparent larvae allow for the measurement of cardiac function in intact animals. We have developed a micro-cannulation technique for in vivo infusion of solution directly into the venous system of zebrafish larvae with a flow-controlled pressurisation system, allowing for acute changes in hemodynamic load. Cardiac volume and contraction are monitored by imaging a genetically-expressed cardiac-specific marker (GFP) and membrane potential and intracellular calcium using functional fluorescent proteins (VSFP-butterfly and GCaMP3). The role of the autonomic nervous system (ANS) and stretch-activated channels (SAC) in observed responses are assessed by pharmacological and micro-surgical interventions.

Results: Results thus far have shown that an acute increase in hemodynamic load at 2dpf causes a decrease in HR, which switches to an increase at 6dpf and is further increased at 14dpf. Interventions have indicated that effects on cardiac function are mediated by both intrinsic (SAC) and extrinsic (ANS) mechanisms.

Conclusions: Our studies are revealing factors involved in the regulation of cardiac function in response to acute changes in hemodynamic load during development. This is essential for understanding cardiac activity in the developing heart and may help us better understand disturbances that occur with congenital heart disease.

167 - Craters in the melanoma tumor serve as hubs of CD8+ T cells in steady state and during immune response

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T cell infiltration into tumors is pivotal for immunotherapy success. We applied long-term, live imaging of an adult zebrafish tumor model to visualize CD8+ cells interactions with tumors in a 3D manner, with continuous imaging under a 2-photon scope for 18 hours. We studied *BRAF;p53*-derived melanoma tumors in *CD8:GFP* transgenic zebrafish. In untreated tumors, CD8+ T cells were primarily located in topological features resembling craters on the tumor surface and at the scale edge. Craters on the melanoma surface had only a few CD8+ T cells, while in craters on the scale edge, CD8+ cells were found interacting with melanoma cells. We observed CD8+ cells migrating from the scale edge to the craters on the tumor surface. We developed an algorithm that renders areas lacking fluorescence signals into measurable objects to quantify CD8+ cell-crater interactions. We then analyzed CD8+ T and dendritic cells (DC) on the melanoma surface after treatment with CpG ODN, a melanoma therapy, and a TGF-β inhibitor, currently in clinical trials. Both immunotherapies introduced a statistically significant increase in CD8+ cell density in craters on the tumor surface rather than tumor parenchyma and an expansion in crater coverage. TGF-β hampers antigen presentation by DC and T cell activation. Its inhibition presented accumulation of CD8+ DCs, proliferating CD8+ T cells and CD8+ T cell clusters contacting CD8+ DCs, all specifically in craters. These observations suggest that the craters are sites for antigen presentation and CD8+ T cell activation. Our findings were corroborated in human nodular melanoma, where craters harboring multiple CD8+ T cells, many expressing PD-1, were found at the major sites of T cell infiltration, i.e., the tumor border and the perivascular spaces. Our study identified specific locations for CD8 T cell activation on the tumor that may facilitate higher efficiency of CD8+ cell infiltration during therapy.

168 - Whole brain imaging for refining tricaine anesthesia in larval zebrafish

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For fish as for all vertebrates, it is required by European law to reduce pain, suffering and distress to the unavoidable minimum in husbandry and experiments. Fast and effective anesthesia is key for refining many procedures. The substance most often used to induce anesthesia in zebrafish is tricaine (MS-222®). When properly prepared and dosed, tricaine causes rapid loss of mobility, equilibrium and reaction to touch. These signs are interpreted as stages of deep anesthesia. Still, its effects on the central nervous system have not convincingly be shown. Therefore, the possibility exists that tricaine acts only on the periphery, resulting in paralyzed instead of anaesthetized fish with severe implications for animals undergoing procedures.

As standard assessment for stages of anesthesia effects on behavior were monitored. In addition, to investigate the effects of tricaine on the central nervous system we used 4-5 days post fertilization (dpf) old zebrafish larvae, expressing panneuronally a calcium transporter Tg(elavl3:H2B-GCAMP6s), that allows to monitor and quantify the neuronal activity. When larvae were treated with the commonly used dose of 168 mg/l tricaine and imaged over time in confocal microscopy, a rapid loss of neuronal activity in the forebrain was observed while other regions.

In conclusion, we saw differences in tricaine susceptibility of different larval brain areas. The effects on the central nervous system are indicative for its anesthetic function and in concordance with behavorial observations. However, because of its weak and slow effects on large brain areas, longer incubation or higher concentrations might be beneficial to reach deep anesthesia.

169 - True bench-to-bedside science: An international pilot study modeling hard-to-treat cancers

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There has been dramatic improvement in treatment outcomes for many pediatric cancers over the last three decades. However, for the 20% of young people with relapsed or refractory cancer, the prognosis remains grim. Canada's PRecision Oncology For Young peopLE (PROFYLE) and Australia's Zero Childhood Cancer (ZERO) programs, leverage nation-wide scientific and clinical oncology expertise to provide personalized precision medicine to children, adolescent and young adult (CAYA) cancer patients who lack treatment options. Beyond improving the lives of young cancer patients nationally, PROFYLE and ZERO have partnered to create an international pipeline for knowledge sharing and sample acquisition.

A key component of both programs is the use of mice for patient-derived xenografts (PDXs). However, long lead times for mouse PDX generation make the timely return of preclinical drug response data challenging. PROFYLE has uniquely incorporated zebrafish larval xenografts, which have the potential to provide comparable information in a clinically actionable timeframe. In a pilot study, we compared retrospective matched ZERO patient and mouse PDX therapeutic response data with prospective zebrafish larval PDX data as a proof-of-principle that drug efficacy signals were maintained across model systems. Three ZERO avatars: high-risk neuroblastoma, Ewing's sarcoma, and anaplastic large cell lymphoma were shipped from Australia to Canada and transplanted into 48h *casper* zebrafish. Strikingly, cancer cell proliferation rates and drug responses to single agents and combinatorial therapy in zebrafish PDXs recapitulated mouse and patient data. Expansion of this pilot project is currently underway to test 11 additional patient samples to further validate the zebrafish drug response platform when mouse PDX data are unavailable. This study demonstrates the robustness and feasibility of the zebrafish larval PDX model as a preclinical tool for patient-specific therapeutic decision-making and highlights the value of international collaboration in improving outcomes of rare childhood diseases.

170 - Molecular roadmaps of brain development and signaling

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Neural cell fate decisions are influenced by many factors including a cell's division history, spatial location and the signaling inputs received. Pioneering studies have shown that cellular signaling is an important mechanism for establishing the embryonic brain framework as it regulates patterning, migration and morphogenesis. However, fundamental questions such as addressing how signaling pathways regulate neural cell fate decisions in the embryo and how their functions expand in later stages are poorly understood since cells receive many inputs during life and measuring the combinatorial effects of all signals remains challenging. We are developing a method that enables the measurement of signaling inputs with single-cell resolution using scRNA-seq. Our system is designed to be activated in a signaling-dependent manner with temporal inducibility control. As a proof-of-principle, we are testing our technology on the Notch signaling pathway in the zebrafish brain. Our goal is to generate in vivo signaling activity atlases of the critical stages of vertebrate brain development, with unparalleled cellular and spatial resolution. When combined with our ongoing work to generate cell diversity catalogs detailing transcriptional landscapes and cell lineage relationships, these atlases will provide an enhanced molecular understanding of the signaling network that underlies neural cell fate decisions.

171 - Mitochondria-endoplasmic reticulum contacts impair mitochondrial transport in models of neurodegenerative diseases

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In the past decade, it has become clear that interactions between mitochondria and other organelles, particularly the ER, are linked to mitochondrial maintenance in neurons. Mutations causing the loss or disruption of these contact sites are tightly associated with neurodegenerative diseases, but it is largely unknown how these contacts affect mitochondria. Work in non-neuronal cells has implicated ER-mitochondrial contacts in mitochondrial fission. Based on this, we hypothesized that ER-mitochondrial contacts may affect mitochondrial density or localization in neurons. In reverse genetic and transgenesis screens where we perturbed the expression of ER-mitochondria contact proteins, we observed no deficits in axonal mitochondrial size or shape. Instead, we identified two proteins, Pdzd8 and VapB, with profound effects on mitochondrial density. Loss of the contact protein Pdzd8 increased mitochondrial density at axon terminals. Expression of a VapB mutant (P56S-ALS8 associated) previously shown to increase ER-mitochondrial contacts also increased mitochondrial density in neurons. We hypothesized that the changes in mitochondrial density could be due to altered mitochondrial transport. While both mitochondrial transport and ER-mitochondrial contacts are important for mitochondrial maintenance, the relationship between these two mechanisms is unknown. Analysis of mitochondrial transport revealed that expression of $VapB^{P56S}$ decreased velocity and distance travelled by motile mitochondria. Furthermore, using a synthetic ER-mitochondria tether protein to irreversibly link these organelles, we can produce deficits in mitochondrial transport similar to $VapB^{P56S}$ overexpression. Together, our data support a model in which ER-mitochondrial contacts regulate axonal mitochondrial transport, affecting density of this organelle in neuronal compartments. Future work will address the impact this altered transport has on the health and function of mitochondria in neurons.

172 - The impact of fecal derived metabolites from autistic versus neurotypical individuals on zebrafish neural development

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There are hundreds of genes associated with autism spectrum disorders (ASDs), but their convergent mechanisms remain largely unknown. The microbiome has been implicated in autism due to the frequent comorbidity of gastrointestinal symptoms in idiopathic ASD, and correlations between gut microbiota metabolites and mRNA processing of the host. There are presently many difficulties investigating the role of microbiota on the host due to the complexity in microbiome taxonomy and variation between individual host microbiomes. In contrast, microbiome metabolites represent the net chemical output of the microbiome, and consequently, may be more conserved in biochemical function. Here, we use 2-day old zebrafish neurodevelopment as a proxy for evaluating the contribution of gut microbe metabolites derived from ASD versus neurotypical (NT) children on neural gene expression, mRNA processing, and sensory system patterning. Germ-free zebrafish were treated with different metabolite samples derived from a collection of age and gender matched NT and ASD children. We identified 275 genes with differential gene expression and 211 genes with differential exon use across treatments between NT and ASD but also differences between two ASD subgroups. Gene ontology analysis strongly suggests a link to RNA binding, including a group of 13 ribosomal protein genes that were upregulated in one of the two ASD subgroups. Investigation at a cellular level demonstrated perturbations in peripheral nervous system development including terminal neuromasts of the posterior lateral line and peripheral nervous system ganglia. Overall, this data provides evidence that germ-free zebrafish are a useful model for understanding the impact and potential mechanism of human gut-derived metabolites on neural development.

173 - Ectoderm is the primary substrate and a modulator of animally-directed lateral mesendoderm migration in early zebrafish gastrula

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At the onset of gastrulation, lateral mesendoderm progenitors undergo cell internalisation at the germ ring margin and migrate collectively between the yolk membrane and the ectodermal layer, first towards the animal pole and then dorsally in direction to the forming embryonic axis. So far, several players that regulate dorsal-directed convergence movements of lateral mesendoderm progenitors have been identified. In contrast, very little is known about how their animal-directed movements are controlled. To gain insight into this process, we have focused on the role of the migration substrate in modulating the extent of animal-directed mesendoderm movements.

Our data show that the ectoderm rather than the yolk cell membrane is used as the primary substrate of lateral mesendoderm cells undergoing animally-directed movement. To explore whether ectoderm acts as an inert substrate or if it affects lateral mesendoderm migration, we performed heterotypic ectoderm tissue transplantation experiments. Remarkably, the extent of animally-directed migration is strongly decreased in the presence of ectoderm from the animal pole but not from lateral regions of the gastrula. Moreover, the inhibitory influence from the animal ectoderm is reduced when the transplanted ectoderm is from donors depleted of BMP signalling. Finally, lateral and animal ectoderm properties can be fully inverted by modulating cell contractility within the ectoderm, either by decreasing or increasing it, respectively. Collectively, these findings point to novel roles of the ectoderm during gastrulation as the primary substrate for lateral mesendoderm migration and in defining the extent of the animally-directed migration. Moreover, they suggest that the modulatory activity of the ectoderm is established by BMP signalling and relies on the contractility of its constituent cells.

174 - Zebrafish models provide novel insights into the disease biology of DNAJC21-mutant Shwachman-Diamond Syndrome

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Shwachman-Diamond syndrome (SDS) is a rare inherited bone marrow failure syndrome (IBMFS) characterized by neutropenia and exocrine pancreatic insufficiency. It is caused by mutations in genes required for ribosome subunit maturation such as *SBDS*, *DNAJC21* and *EFL1*. How the disruption of such ubiquitous cellular processes leads to distinct cytopenic phenotypes is not fully understood. Further, the cumulative risk of developing myelodysplastic syndrome and/or acute myeloid leukemia for SDS patients is 36% by 30 years of age. There are no preclinical models of *DNAJC21*-mutant SDS. Using CRISPR-Cas9 genomic editing, we generated a germline *dnajc21* zebrafish mutant. We observed reduced granulocyte differentiation in mutant larvae by *in situ* hybridization, whereas the erythroid lineage remained unaffected. Further, Dnajc21 loss activated the p53-p21 pathway leading to reduced cell proliferation by pH3 immunostaining. Bulk RNA sequencing of whole larvae at 48 hours post-fertilization identified several metabolic and immune-related genes dysregulated in *dnajc21* mutants. Leukemic transformation in SDS is driven by the acquisition of mutations in additional genes, including the tumor suppressor, *TP53*. To model SDS-AML transformation, we crossed the *dnajc21* mutants with a zebrafish line carrying a *tp53*^{R217H} gain-of-function point mutation that confers anti-apoptotic phenotypes. pH3+ cell counts in *dnajc21-/-/tp53*^{R217H} compound mutants were comparable to wildtype, suggesting restored cell proliferation Compound mutants also exhibited reduced growth and poor lipid accumulation assessed by Oil Red O staining. Flow cytometry on whole kidney marrow (human bone marrow equivalent) isolated from 4-month-old fish revealed reduced myeloid output in both *dnajc21* and *dnajc21/tp53* mutants. In summary, Dnajc21 is required for normal granulocyte differentiation and cell proliferation and is a novel regulator of cellular metabolism. We propose that these zebrafish models can readily serve as *in vivo* platforms to identify therapeutic compounds that restore normal hematopoiesis and prevent leukemic transformation.

175 - Sox2 stabilizes maturing epithelial rosettes in the zebrafish Posterior Lateral Line primordium in part by inhibiting destabilizing Wnt signaling activity

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Protoneuromasts are formed within the migrating primordium, starting from its trailing end, as clusters of cells sequentially reorganize to form epithelial rosettes, each around a central Atoh1a expressing cell specified as a sensory hair cell progenitor. Their formation is initiated in Fgf signaling domains that are periodically established in response to Fgfs produced by cells in an adjacent leading Wnt active zone, where Wnt signaling also inhibits these leading cells from responding to Fgfs and forming protoneuromasts. Fgf signaling-dependent expression of the diffusible Wnt antagonist Dkk1b facilitates establishment of stable Fgf signaling centers in nascent protoneuromasts by preventing potentially destabilizing inhibition from Wnt signaling. Dkk1b also contributes to progressive restriction of the initially broad Wnt signaling domain to a smaller leading zone, as new Fgf signaling-dependent protoneuromasts form in the wake of the shrinking Wnt system. As the leading Wnt system shrinks, Atoh1a expression and epithelial rosette morphogenesis in maturing neuromasts formed earlier, in more trailing parts of the primordium, becomes self-sustaining and independent of the Fgfs signals produced by leading Wnt active cells that initiated protoneuromast formation. However, Dkk1b is not expressed in these maturing neuromasts raising a question about what inhibits potentially destabilizing Wnt signaling in the trailing neuromasts. We now show that Sox2 is expressed in nascent and maturing protoneuromasts in a pattern that is complementary to domains with Wnt signaling activity. Furthermore, Sox2 functions in a partially redundant manner with Sox1a and Sox3, to inhibit Wnt signaling. This helps keep Wnt activity restricted to a leading zone, which we suggest is essential for effective stabilization of maturing protoneuromasts in the trailing zone. Together our observations show how patterning events that initiate protoneuromast formation are followed by changes in regulation requiring SoxB1 family factors that help consolidate neuromast morphogenesis prior to their deposition by the migrating primordium.

176 - Cell fate decisions at the midbrain-hindbrain boundary

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During development, the neural tube gives rise to the brain, spinal cord, and the central nervous system which stem from the ectoderm. Three main brain vesicles develop from the cranial portion of the neural tube, which includes the forebrain, midbrain, and hindbrain. The regionalization and proper signaling coordination of the brain vesicles are important for the form and function of the vertebrate central nervous system. The constricted boundary between the midbrain and hindbrain is known as the isthmus, which acts as the midbrain-hindbrain organizer. Disruptions to the formation of this midbrain-hindbrain patterning can lead to nervous system-related diseases. The isthmus plays a role as a signaling center that patterns the adjacent midbrain and rostral hindbrain, and feedback loops involving isthmus-specific genes and factors, including Fibroblast Growth Factor 8 (*fgf8*), are needed to reenforce this boundary. The cellular heterogeneity of the isthmus region and the roles of factors downstream of known isthmus-related factors is still not yet fully characterized. Here, we conducted single-cell multiomics profiling of the transcriptome and epigenome of zebrafish during the formation of the isthmus (10, 13, and 16 hours post-fertilization) to further establish this regulatory pathway and to distinguish potential subpopulations. To further study single-cell effects on the isthmus, we also blocked the FGF signaling pathway by using the molecule inhibitor SU5402. Preliminary data shows multiple distinct isthmus subpopulations already established at 10 hours. We also identify a subcluster potentially pertaining to rhombomere 0 that has been postulated in the literature but that has not been well characterized previously. Given the significance of the isthmus and its fundamental patterning processes, this research further identifies molecules downstream that maintain this boundary and isthmus-related subpopulations at the single-cell level.

177 - Signaling and Mechanics influence the number and size of epithelial rosettes in the migrating zebrafish posterior Lateral Line Primordium

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Protoneuromasts are formed within the migrating primordium, starting from its trailing end as clusters of cells apically constrict and form epithelial rosettes. Their formation is promoted by Fgf signaling centers that form periodically in the wake of a shrinking Wnt active domain that inhibits epithelial rosette formation and that progressively shrinks toward the leading end of the primordium. However, the precise number and size of epithelial rosettes is not strictly dependent on a prepattern of Fgf signaling activity as it is broadly influenced by the balance of mechanical interactions that promote or oppose formation of epithelial rosettes. When chemokine-dependent migration of leading cells is compromised, the resulting slowing of the primordium is accompanied by the fusion of epithelial rosettes to form fewer larger rosettes. However, such fusion is not observed when Fgf signaling, responsible for migration of trailing cells, is inhibited to slow primordium migration. These observations can be accounted for by a mechanics-based model, where local interactions associated with apical constriction and cell adhesion promote aggregation, while tension along the length of the primordium, influenced by the relative efficacy of leading and trailing cell migration, opposes such aggregation. We describe the development of a Cellular Potts model which allows us to explore how the relative speed of leading versus trailing cells, as well as changes in cell adhesion and mechanical coupling, differentially regulated by Wnt and Fgf signaling, can influence the pattern of neuromast formation and deposition by the migrating primordium. Our studies illustrate how signaling and mechanics cooperate to coordinate self-organization of morphogenesis in the migrating primordium. This work was funded by the intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development 1ZIAHD001012.

178 - Characterization of intestinal development during post embryogenesis

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Zebrafish intestinal epithelium has similar organization to other vertebrates. Due to external development, the digestive system must be functional by the end of embryogenesis. The digestive system, however, continues to mature over the next four weeks of the post-embryonic period into the adult form. In preparation for investigation of genes and signaling involved in intestinal development during the post-embryonic period, we have been characterizing the timing of changes in epithelial proliferation and intestinal looping. During the post-embryonic period, fish develop at widely different rates that no longer correspond strictly to age even when grown in the same conditions. To stage fish during the postembryonic period, fish size has been strongly correlated with a number of externally developing milestones. Here we have begun to link these developmental milestones used in the staging series to events in the development of the post-embryonic intestine. This will provide a more specific staging for when changes occur in the developing intestine during post-embryonic periods.

179 - Rtf1-mediated Transcriptional Regulation of Cardiac Development and Function

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Diverse transcriptional regulatory mechanisms integrate to direct the deployment of cardiac gene networks during development and maintain their expression in the mature heart. Highly evolutionarily conserved families of transcription factors play critical roles in governing the initiation of cardiac gene transcription. However, post-initiation and epigenetic regulation of cardiac gene expression is complex and not yet fully understood. Rtf1 is a multifunctional transcription regulatory protein that modulates pausing and elongation of RNA Polymerase II (RNAPII) and promotes co-transcriptional histone modifications. We found that Rtf1 is essential for cardiac gene expression and cardiac progenitor formation. Zebrafish and mouse embryos lacking Rtf1 activity fail to form a heart and have severely decreased expression of the cardiac transcription factors Nkx2.5 and Tbx20. However, other mesoderm derived tissues do develop in Rtf1-deficient embryos, suggesting that the cardiac lineage is remarkably sensitive to Rtf1 activity. Using knockdown and knockout approaches, we investigated the importance of Rtf1 in neonatal rat ventricular myocytes (NRVMs) and the adult mouse heart. Knockdown of Rtf1 activity in NRVMs, an in vitro cardiomyocyte model, disrupted sarcomere integrity and reduced the expression of genes involved in cardiac contraction, suggesting a failure to maintain the cardiac transcription program in the absence of Rtf1 activity. Similarly, loss of Rtf1 in the adult mouse heart resulted in dilated cardiomyopathy. To investigate the mechanism by which Rtf1 regulates cardiac gene transcription we examined RNAPII density in zebrafish embryos using ChIP-seq. Intriguingly, we found that loss of Rtf1 activity decreased promoter-proximal pausing of RNAPII, and inhibition of pause release in Rtf1-null embryos partially rescued pausing of RNAPII at cardiac genes and restored cardiac progenitor formation and heart tube elongation. Our findings suggest that Rtf1's primary function in cardiac gene regulation is to promote promoter-proximal pausing, and that pausing positively regulates cardiac gene expression during development.

180 - Valproic Acid Affects Neuronal Specification and Differentiation During Optic Tectum Development of Zebrafish Embryos

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Valproic acid (VPA) is a commonly used drug to treat epilepsy, bipolar disorder, and schizophrenia. Fetal exposure to VPA has been associated with an increased risk of Autism Spectrum Disorder (ASD). The mammalian superior colliculus and its zebrafish homolog, the optic tectum (OT), have been purported to be involved in the ASD phenotype. To assess the effect of VPA on proper OT development, zebrafish larvae were continuously treated with 250uM VPA from gastrulation to 5 days post fertilization (dpf). Using this treatment paradigm, we found that while proliferation of the neuroepithelium continued, neuronal specification stalled, as visualized by the delayed onset of tagRFP expression driven by the *neuroD1* enhancer, a neuronal specification marker. This delay in specification was partially remediated when VPA was administered after 3dpf when initial neuronal specification was well underway. Additionally, single-dose administration of VPA at 0dpf or 1dpf was found to have a lasting effect through 5dpf, thus identifying a critical window for VPA exposure on OT development. After initial neuronal specification began, photoconversion of Kaede in individual OT neurons showed a decrease in neurite length in VPA treated embryos, resulting in little to no neuropil formation. This suggests that in addition to neurogenesis, VPA treatment has an additional effect on axonogenesis and dendritogenesis. Lastly, recent findings have implicated oxidative stress as a mechanism of VPA alteration on neuronal specification, as well as the ability of genes downstream of the stress-response transcription factor *nrf2* to protect against these effects *in vitro*. However, pretreatments with D3T, an inducer of the *nrf2* stress-response, revealed no protective effect in the zebrafish OT following 250uM VPA treatment. In conclusion, our results identify a critical period for the effect of VPA on OT development, as well as a role for VPA in altering neurogenesis and neurite outgrowth in the zebrafish OT.

181 - A novel zebrafish-based in vivo model of Zika virus infection unveils NS4A as a key viral determinant of neuropathogenesis

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Infection of pregnant women by Zika virus (ZIKV) can cause neurodevelopmental defects in newborns known as congenital Zika syndrome, which includes microcephaly. Murine models studying ZIKV neurovirulence have several limitations in terms of cost, time, ethics, cell imaging, and genetic manipulation. Thus, alternative animal models more conducive to the study of early development of the ZIKV-infected brain *in vivo* are required.

Zebrafish is a powerful tool for studying human neurological and infectious diseases. Optically transparent, it is ideal for imaging labelled specific neural cell populations. Considering this, we aimed to develop a zebrafish in vivo model of ZIKV infection to study viral neuropathogenesis.

Eighty percent of zebrafish larvae infected with ZIKV infectious particles exhibited developmental defects ranging from curved spinal cord to ovoid morphology. TUNEL assays on whole animals showed an increased apoptosis in the brain following ZIKV infection. This correlated with a decrease in head size and in neural progenitor cell abundance, as well as drastic mobility impairments. Importantly, these defects were reversed when the larvae were treated with the flaviviral polymerase inhibitor NITD008, which decreased viral loads more than 150-fold, unambiguously demonstrating that ZIKV replicates in zebrafish. Whole animal immunostaining of viral proteins revealed infection foci in the hindbrain and in the spinal cord, strongly supporting that ZIKV replicates in the central nervous system. Furthermore, expression of viral protein NS4A alone recapitulated morphological defects, demonstrating that this viral protein is a key determinant in ZIKV neurovirulence.

Overall, our data unveil the zebrafish larva as a model for ZIKV infection with neurological phenotypes comparable to the defects observed in humans. This model will enable rapid antiviral drug testing *in vivo* and a better understanding of host determinants required for ZIKV neuropathogenesis given its flexibility for genetic manipulations.

182 - Hindbrain Defects Induced by Di-butyl Phthalate (DBP) in Developing Zebrafish Embryos

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Di-butyl phthalate (DBP) is a globally used plasticizer found in alarmingly high concentrations in soil and water ecosystems. As phthalates are non-covalently bound to plastic polymers, phthalates easily leach into the aquatic environment. The effects of DBP on aquatic organisms is concerning, most notably, studies have focused on the endocrine-disrupting effects. However, reports on the developmental neurotoxicity of DBP are rare. Using the zebrafish vertebrate model system, we treated pre-gastrulation staged embryos with 2.5µM DBP, a concentration environmentally noted. We find that general hindbrain structure and rhombomere patterning is disrupted at 72 hours post fertilization (hpf). We investigated hindbrain specific neural patterning of cranial motor neurons and find defects in branchiomotor neuron patterning and migration. Furthermore, defects in r4 specific Mauthner neuron development were also noted. Thus, we conclude that DBP exposure during embryonic development induces defects to the hindbrain and concomitantly the neurons that are born and differentiate there.

183 - Control of meiotic chromosomal bouquet and germ cell morphogenesis by the zygotene cilium

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Meiosis is a cellular program essential for haploid gamete production. A hallmark of meiosis is chromosomal pairing via meiotic Cohesins and synaptonemal complex proteins. However, chromosomal pairing also depends on cytoplasmic mechanical forces. In meiosis, chromosomal pairing is facilitated by unique telomere dynamics. Telomeres bind Sun/KASH proteins on the nuclear envelope (NE), associating them with perinuclear microtubules that emanate from the centrosome. This facilitates telomere rotations on the NE that shuffle chromosomes, providing their homology searches. Ultimately, telomeres are pulled towards the centrosome and cluster on the NE, looping their chromosomes to the other side, forming a configuration called the zygotene chromosomal bouquet. The bouquet is universally conserved, was discovered in 1900, and is essential for chromosomal pairing and fertility. However, how cytoplasmic counterparts of bouquet formation are mechanically regulated is unknown. Here, we identified a novel cilium in zebrafish meiosis, that specifically connects to the bouquet centrosome and constitutes a cable system as the cytoplasmic bouquet machinery. Through analyzing multiple ciliary mutants, we demonstrate that the zygotene cilium is essential for chromosomal bouquet formation, germ cell morphogenesis, ovarian development and fertility. We further show that the zygotene cilium is conserved in male meiosis as well as in mammalian oogenesis. Our work uncovers a novel concept of a cilium as a newly identified player in meiosis. We propose a new cellular paradigm that cilia can control chromosomal dynamics.

184 - Novel genetic interactions between cep290 and kif7 in a zebrafish ciliopathy model

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Ciliopathies are disorders caused by mutations in ciliary genes that disrupt cilia function. Cilia are organelles found in many organs and function mainly via signal transductions and/or movement. Such ciliated organs are susceptible to ciliopathies. However, many ciliopathies' mechanisms are unclear, and most involve a single gene. Our group recently identified a novel cilium in the zebrafish ovary. To investigate the role of cilia in the ovary, we use fish mutants in Cep290, a ciliary basal body required for cilia formation and function, and Kif7, a ciliary kinesin that organizes the ciliary tip. We found that *cep290-/- ;kif7 -/-* double mutants exhibit a more severe loss of cilia than each of the single mutants alone, suggesting genetic interactions between *cep290* and *kif7*. However, while mutations in single genes have been extensively investigated in generating ciliopathies, genetic interactions between known ciliopathy associated genes are less known. We hypothesize that the loss of function of both *cep290* and *kif7* will cause more severe pleiotropic ciliopathic phenotypes than typically observed in single ciliopathic mutants, raising *cep290-/- ;kif7 -/-* double mutant as a novel model to understand ciliopathic genetic interactions. We investigate multiple ciliated organs to uncover and characterize such genetic interactions. Here I demonstrate in adult zebrafish that *cep290-/- ;kif7 -/-* fish develop more severe pathological kidney cysts compared to each single mutant, as well as synergistically lead to severer scoliosis, two major ciliopathic phenotypes. These results unravel genetic interactions between two key ciliary players, and in the underlying mechanisms of ciliopathies, establishing a new ciliopathy model to study and later treat ciliopathies.

185 - Metabolic Adaptation Through the Lens of the Anoxic Transcriptional Signature

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While cells harness most cellular energy (ATP) through oxidative phosphorylation, hypoxia, a known environmental stressor, limits this means of ATP production and can cause cellular and organismal death. Many organisms, including zebrafish, have evolved adaptive responses to withstand prolonged periods of hypoxia, which typically involve metabolic suppression of energy-demanding processes (e.g. transcription) to preserve ATP. Identifying novel signaling molecules that mediate such responses can provide insight on therapeutic approaches to mitigate hypoxic injury that occur in illnesses such as chronic kidney disease. Here, we performed an RNA-seq analysis to screen for genes differentially expressed in hypoxia (3% O2) and anoxia (0% O2). We hypothesized that hypoxia would result in widespread changes of metabolic genes implicated in glycolysis and oxidative phosphorylation, and that anoxia would result in increased expression of fewer genes essential for survival. Surprisingly, anoxia resulted in differential expression of over 2800 genes, only 87 of which were shared with hypoxia. Among the anoxia-induced genes were known hypoxia responsive genes, such as *ndrg1* and *hif3*, in addition to novel candidates such as cell cycle regulators and circadian genes. Validation of *ndrg1a* through qPCR revealed that it is robustly upregulated and whole-mount *in situ* hybridization revealed expression of *ndrg1a* transcript in tissues in which it is not expressed under normoxia. Functional characterization of *ndrg1a* further revealed a role in the downregulation of the sodium-potassium pump, a response that is reversible upon reoxygenation. The *ndrg1a*-dependent response to downregulate this pump is likely part of an effort to reduce ATP usage during anoxic stress. The work presented is funded by the Department of Defense (W81XWH-16-1-0466), National Institute of Health/NICHD (R21HD089476), National Institute of Health /NIGMS MARC U*STAR (T34 HHS 00001), CBI (NIGMS/NIH T32 GM066706), and IMSD (NIGMS/NIH T32 GM055036).

186 - Kinesin light chain 4 (KLC4) has roles in mitochondrial function and endosome transport during neuronal development

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Neurons must develop highly polarized and complex morphologies to assemble neural circuits. Proper development of neuron morphology as well as neuronal function are critically dependent on tightly controlled transport of vesicle and organelle cargos to specific cell locations. Kinesin-1 is an indispensable microtubule-based motor protein responsible for anterograde axonal transport of multiple cellular cargos, including endosomes, synaptic vesicle precursors, lysosomes, and mitochondria. The mechanisms by which kinesin-1 selects specific cargos are not well understood, however the cargo-binding kinesin light chain (KLC) subunits are likely involved in specificity. Vertebrates have four *klc* genes, but our knowledge of the different roles played by individual KLCs in neurons is very limited. KLC4 has been implicated in mitochondria health and function in carcinoma cells, and mutation in human *klc4* causes a type of hereditary spastic paraplegia that manifests in early childhood. However, little is known about cellular functions of KLC4 in neural development. Our lab has identified roles for KLC4 in sensory axon outgrowth and branching, and in regulation of microtubule dynamics. To further characterize the mechanism by which KLC4 regulates these processes, we are using high-speed live imaging of endosomal transport, mitochondria, and microtubule dynamics in *klc4* mutants during axon outgrowth and branching. We find that mitochondria are smaller in *klc4* mutant embryos compared to wild type, suggesting that mitochondria are more fragmented and that KLC4 may have roles in regulating mitochondrial fusion or homeostasis. In addition, mutants display altered endosomal transport. Defects in either of these processes could potentially lead to the defects in axon morphogenesis.

187 - Deep learning-based approach for analyzing the organization and formation of the germline cyst in zebrafish oogenesis

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In gametogenesis, germ cells organize in a cellular hub called the germline cyst, which is common to both sexes and is conserved from insects to mammals. In oogenesis, the cyst forms by incomplete cytokinesis of mitotic oocyte precursors called oogonia, resulting in interconnected germ cells that are clustered compactly and surrounded by somatic cells. Differentiating oocytes continue to develop within the cyst, before leaving it to form the primordial follicle. The cyst serves as a hub for key events in early oogenesis, which in humans occur in the developing prenatal ovary, and by birth determine the number and quality of oocytes for the entire female life span. However, while the cyst has been extensively characterized in Drosophila, its formation and function in vertebrates, including mammals, remain unclear. We present a deep learning-based high throughput analysis pipeline combining in-situ imaging and high-resolution live imaging microscopy. Through our analysis, we will uncover the cyst cell division pattern and features such as the connectivity of cyst cells and their spatial arrangement in vivo. In addition to these, we expand our analysis, in-vivo modulating the various cellular components to analyze their effect on the germline cyst development and maintenance utilizing high-resolution real-time live imagining. These methods will pave the way for investigating the cyst cellular organization. Our findings will expand the toolkit for the analysis of early oogenesis in zebrafish, and shed new light on its underlying cell and developmental biology.

188 - Silencer regulatory elements control tissue regeneration

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Recent studies have demonstrated the existence of dedicated enhancers that direct gene expression upon injury or during tissue regeneration. Regeneration programs also include reductions in gene expression that might be explained by lost presence of a transcriptional activator from an enhancer during regeneration, or increased presence of a transcriptional repressor facilitated by a silencing element. DNA silencer elements are more technically challenging to validate and are less studied than enhancers, but they potentially have profound biological importance in the context of regeneration. Here, we assessed RNA-seq and ATAC-seq datasets from regenerating zebrafish fins for genes that reduce expression during regeneration and have a distal sequence in the vicinity that increases in chromatin accessibility. We incorporated dozens of these sequences into an assay that challenges sequences to silence injury-induced expression in larvae after fin fold amputation, identifying several candidate silencer sequences. A sequence distal to *smarca1*, a gene that sharply decreases expression during regeneration, repressed the activity of multiple promoters in stable transgenic lines and could diminish expression of nearby genes in stable knock-in lines. HiC-seq analysis for DNA interactions identified multiple regeneration-dependent associations of this region with nearby genes that reduce expression during regeneration. Deletion of this silencer from the genome increased expression of these genes and reduced the efficacy of regeneration after fin amputation. Our findings reveal a functional role for silencer regulatory elements during complex tissue regeneration.

189 - Regeneration and Developmental Enhancers Are Differentially Compatible with Minimal Promoters

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Gene transcription is controlled by multiple types of *cis*-regulatory elements, including enhancers and promoters. Enhancers are activated in a cell type-, tissue-, or condition-specific manner to stimulate promoter function, which initiates RNA synthesis. Enhancer activity *in vivo* is typically determined via transgenic enhancer assays, in which an enhancer is coupled with a minimal promoter. Zebrafish are a powerful animal model to examine enhancers derived from various species. However, the efficiency of minimal promoters and their compatibility with multiple developmental and regeneration enhancers have not been systematically tested in zebrafish. We assessed the efficiency of six minimal promoters and comprehensively interrogated the compatibility of the promoters with developmental and regeneration enhancers. We found that the mouse *fos* promoter and *Drosophila* DSCP promoter yielded high rates of leaky expression that may complicate the interpretation of enhancer assays. Notably, the adenovirus *E1b* promoter, the zebrafish *lepb* 0.8-kb *(P0.8)* and *lepb* 2-kb *(P2)* promoters, and a synthetic promoter combining elements of the *E1b* and *P0.8* promoters drove little or no ectopic expression, making them suitable for transgenic assays. We also demonstrated significant differences in compatibility among specific combinations of promoters and enhancers. Our study provides guidelines for transgenic enhancer assays in zebrafish to aid in the discovery of functional enhancers regulating development and regeneration.

190 - Cytoskeletal Dynamics of Tissue-Resident Macrophages Following Cutaneous Axon Degeneration

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Neuronal cell death and axon degeneration commonly occur during development, in neurodegenerative disease states, and following injury. Phagocytes must engulf the neuronal debris left behind during these events to prevent inflammation and promote homeostasis. The axons that innervate peripheral tissues, such as skin, undergo significant remodeling during development and injury, but until recently the relevant phagocytes remained unidentified. We discovered that Langerhans cells, a skin-resident immune cell, engulf axonal debris following skin injuries. These cells possess long, dynamic cellular protrusions to navigate their complex three-dimensional environment to engulf axonal debris. The cytoskeletal mechanisms that regulate these protrusions and subsequent engulfment events remain poorly understood. Here, we establish transgenic tools that specifically label actin and microtubules in Langerhans cells and other macrophages. Combining our new reagents with live-cell imaging approaches allows for the unprecedented visualization of macrophage cytoskeletal dynamics in a native tissue environment. Our new transgenic tools demonstrate that Langerhans cell protrusions are richly populated with both actin and microtubules. We characterized the dynamics and lifetimes of Langerhans cell protrusions during axon degeneration; surprisingly, we found that Langerhans cells do not change their behavior following axon degeneration. Perturbing actin dynamics with Latrunculin B completely inhibits Langerhans cell protrusive behavior and debris engulfment. Furthermore, we found that the actin regulators RhoA/ROCK are required for normal protrusion behavior. Upon addition of Y-27632, a ROCK inhibitor, we found that Langerhans cells increase their total protrusion length, number, and lifetime. In conclusion, our new tools allow for the real-time investigation of the cytoskeletal dynamics required by tissue-resident macrophages to navigate diverse organ environments and engulf neuronal debris.

191 - The MEK-ERK signaling pathway promotes maintenance of cardiac chamber identity

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The vertebrate heart is comprised of two types of chambers, ventricles and atria, which exhibit unique structural and contractile properties. Effective cardiac function depends upon the distinct characteristics of ventricular and atrial cardiomyocytes. Intriguingly, despite the early specification of ventricular and atrial lineages, chamber-specific features need to be actively reinforced even after myocardial differentiation is underway. Our prior studies in zebrafish have found that sustained FGF signaling is required for ventricular cardiomyocytes to maintain their ventricular identity: upon inhibition of FGF signaling, these cells extinguish expression of the ventricular gene *vmhc* and initiate ectopic expression of the atrial gene *amhc*. However, the genes that mediate ventricular maintenance downstream of FGF signaling remain unclear. Here, we show that MEK-ERK signaling plays an important role in promoting ventricular identity maintenance. Inhibition of the MEK1/2-ERK1/2 pathway following cardiomyocyte differentiation results in ectopic *amhc* expression as well as reduced *vmhc* expression in ventricular cells. Similar to the phenotype resulting from FGF inhibition, inhibition of the MEK-ERK pathway causes cells in the inner curvature of the ventricle to readily initiate ectopic *amhc* expression, whereas cells in the outer curvature are more resistant. Inhibition of either the FGF or MEK-ERK pathways is most potent in inducing ectopic *amhc* during the same developmental stages; these results suggest that FGF and MEK-ERK signaling are required for ventricular maintenance during a similar timeframe, during which we can detect phospho-ERK signals in both the myocardium and endocardium. Additionally, expression of a constitutively active form of MEK1 partially rescues the ectopic ventricular *amhc* phenotype induced by FGF inhibition, such that fewer embryos exhibit ectopic *amhc* in the outer portions of the ventricle. Together, our data suggest a model in which MEK-ERK signaling acts downstream of FGF signaling to enforce the maintenance of ventricular chamber identity.

192 - Zebrafish as a Model for Studying Embryonic Development and Behavior in Bipolar Disorder

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Bipolar disorder (BD) is defined primarily by recurrent manic and depressive episodes. In addition to mood symptoms, disturbances in affective stability, energy levels, locomotor activity, and circadian rhythms are hallmarks of the disorder. Models based on clinical characteristics of the disease may provide great insight into shared and distinct disease-related mechanisms. The specific objective of this project is to create a BD zebrafish animal model by knockdown of a key gene -*clock*- that regulates biological rhythms. *We hypothesize that morpholino knockdown of clocka and clockb genes in zebrafish results in ineffective modulation of the circadian timing system which affects embryonic development and behavior.* This hypothesis is based on salient literature in mouse models (m*Clock*Δ19) that exhibit behavioral profiles similar to BD mania. We compared morphological embryonic phenotypes between *clocka*^{KD}, *clockb*^{KD}, *clocka*^{KD}:*clockb*^{KD} and WT zebrafish at different periods of embryogenesis using ZEISS Axio Zoom Imaging System. DanioVision Observation Chamber and EthoVision tracking software were used to compare behavior patterns of WT versus *clock*^{KD} morphant zebrafish. Behavior phenotypes were assessed using Locomotor Activity, Light-Dark, Startle Response, and Extinction of Adaptation Response Tests. Behavioral endpoints of *clocka/b* morphants and WT groups were compared using t-test (two-tailed, unequal variance) analyses. Preliminary results suggest *clocka*^{KD}:*clockb*^{KD} morphants exhibit developmental delay and differential behavioral phenotypes compared to WT including increased locomotor activity, decreased anxiety, and disrupted circadian behavioral phenotypes. Future studies using CRISPR-Cas9-mediated techniques to knockout exon regions in zebrafish genes *clockaΔ20* and *clockbΔ17*, homologous to m*ClockΔ19*, will enable us to examine the role of the circadian timing system in the developmental, anatomical, and behavioral mechanisms that underlie BD and allow for high-throughput therapeutic screenings for the treatment of BD.

193 - Optimized generation and application of conditional knock-in alleles in zebrafish

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[1]Umassmed

Advances in CRISPR technology now allows routine gene knockout in the zebrafish. However, generating alleles for definitive cell- or stage-specific gene knockout remains challenging. Here, we present optimized approaches to generate and apply zebrafish bearing loxP-flanked knock-in alleles for conditional knockout. Our initial approach relied on that of Hoshijima et al., (Dev Cell, 36:654-67, 2016) using double-stranded template released from plasmid with a single site-specific nuclease to stimulate homology directed repair (HDR). Using this approach to generate a conditional *gata2a* allele, we experienced numerous pitfalls and identified points for improvement. As in the previous study, we found it essential to match homology sequence in the HDR template to that of the target locus in the fish used for injection. Further, we used two Cas12a RNPs simultaneously targeting the sequence where loxP will be inserted. We found it essential to incorporate careful validation of transient knock-in efficiency in individual somatic genomes to confirm and optimize injection conditions. We also incorporated a marker to screen for non-homologous targeting along with Southern blot analysis to confirm single on-target insertions in F1 fish. Using our optimized protocols, we generated conditional alleles for *foxc1a* and *rasa1a*, each in a single generation. In both cases, we identified founders and progeny bearing single copy targeted alleles. Importantly, injection of *cre* mRNA into homozygous embryos bearing conditional alleles exhibited expected phenotypes at full penetrance. However, using cell-specific inducible Cre (CreERT) transgenes gave milder phenotypes, which correlated with lower recombination rates. Our current efforts focus on optimizing cell-specific recombination, including effects of CreERT copy number and using cell-specific transgenes expressing constitutive Cre or zebrafish codon-optimized Cre. Taken together, we believe our efforts will provide the foundation for routine generation and application of conditional alleles in the zebrafish community.

194 - Loss of dlx5a alters Wnt signaling and impair Meckel's cartilage morphology

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The *dlx* family of transcription factors are conserved across vertebrates and important in the development of various tissues including craniofacial elements. Previous work through morpholino-mediated knock-down or hypomorphic mutants of *dlx* genes showed reductions in *dlx* expression lead to malformations of first and second pharyngeal arch structures and reduced cranial neural crest cell survival. To further characterize the role of *dlx* genes during craniofacial development, single and compound mutants of *dlx1a*, *dlx2a*, *dlx5a* and *dlx6a* were produced by CRISPR-Cas9 genome editing. Analysis of cartilage and calcified bone development were performed at 5 and 14 days post fertilization (dpf) and revealed alterations in the size and shape of the first and second pharyngeal arch structures. Additionally, the ceratohyal cartilage, a derivative of the second pharyngeal arch is mispositioned in *dlx5a* mutants. Loss of *dlx5a* also resulted in increased cell proliferation and increases in *sox9a* and *col11a2* expression. Interestingly, *dlx5a*$^{-/-}$ larvae also displayed altered expression of *wnt5b* and *ror2* at 2dpf and 3dpf, suggesting loss of *dlx5a* affects Wnt signaling responses in cranial neural crest cells of the first and second arches resulting in abnormal Meckel's cartilage morphology. This is the first time an association between *dlx* function and Wnt signaling during craniofacial development was observed and may provide some clues to how similar neural crest cell identities lead to different facial structures. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

195 - Sensory axons induce epithelial lipid microdomain remodeling and determine the distribution of junctions in the epidermis

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Epithelial cell properties are determined by the polarized distribution of membrane lipids, the cytoskeleton, and adhesive junctions. Epithelia are often profusely innervated, but how contact with neurites affects the polarized organization of epithelial components is poorly understood. We previously found that basal keratinocytes in the larval zebrafish epidermis wrap around axons to enclose them in ensheathment channels sealed by autotypic cell junctions. To characterize the morphogenetic mechanisms that promote ensheathment channel formation, we used live imaging to characterize how sensory axons remodel cell membranes, the actin cytoskeleton, and adhesive junctions in basal keratinocytes. At the apical surface of basal keratinocytes, axons promoted the formation of lipid microdomains quantitatively enriched in reporters for PI(4,5)P2 and liquid-ordered (Lo) membranes. Lipid microdomains supported the formation of cadherin-enriched F-actin protrusions, which wrapped around axons, likely initiating the formation of ensheathment channels. Lo reporters, but not reporters of liquid-disordered (Ld) membranes, became progressively enriched at axon-associated membrane domains as autotypic junctions matured at ensheathment channels. In the absence of axons, cadherin-enriched lipid microdomains still formed on basal cell membranes, but were not organized into the contiguous domains normally associated with axons. Instead, these isolated domains formed ectopic heterotypic junctions with overlying periderm cells, a distinct epithelial cell type in the epidermis. Thus, axons inhibit the formation of epithelial heterotypic junctions by recruiting cadherin-rich lipid microdomains to form autotypic junctions at ensheathment channels. These findings demonstrate that sensory nerve endings dramatically remodel polarized epithelial components and regulate the adhesive properties of the epidermis.

196 - Banp is an essential transcription factor that regulates DNA damage response and chromosome segregation during the cell cycle

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Btg3 associated nuclear protein (Banp) is a tumor suppressor that was first discovered as a nuclear matrix associated protein. Banp binds to the CGCG element containing motif enriched near the transcription initiation site of CpG island promoters, namely the Banp motif, promotes transcription in a DNA methylation dependent manner, and regulates metabolic genes in pluripotent stem and differentiated neuronal cells. However, physiological functions of Banp are still unknown. Here, we identified zebrafish *banp* mutants and found a novel role of Banp in cell-cycle progression of retinal progenitor cells (RPCs). In *banp* mutants, RPCs show the activation of DNA replication stress, leading to a tp53-dependent DNA damage response, which in turn induces mitotic defect and apoptosis. We found that tp53 knockdown greatly inhibits apoptosis, but not mitotic defect and DNA double strand breaks, implying that Banp is necessary for DNA replication and DNA damage repair upstream of tp53. Our ATAC- and RNA-seq analysis revealed that chromatin accessibility was reduced near the transcription start site of genes down-regulated in *banp* mutants. Furthermore, the Banp motif was often found in their chromatin-closed region, implying that Banp directly promotes transcription of these targets via the Banp motif. Interestingly, these *banp* targets include two chromosomal segregation regulators, Cenpt and Ncapg, as well as a DNA replication fork regulator, Wrnip1, so Banp promotes chromosome segregation during mitosis and DNA replication stress repair through the transcriptional regulation of these genes. Thus, our findings suggest that Banp functions as a hub of the transcription network that regulates multiple cell-cycle regulators.

197 - Multiple genes in the neurodevelopmental risk locus 22q11.2 have convergent roles in zebrafish behavior and brain development

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Deletion of a region of chromosome 22q11.2 encompassing 45 protein-coding genes greatly increases risk for several neurodevelopmental disorders and is the greatest known genetic risk factor for schizophrenia. While a few candidate genes within the region have been postulated to contribute, the molecular mechanisms underlying this substantial risk are incompletely understood. Further, it is generally thought that interactions between genes within the region may drive risk though few studies have tested this. We have capitalized on the many strengths of the zebrafish system to systematically dissect the individual and combinatorial contributions of genes within the region to behavior and brain function. Specially, we have generated individual mutant lines for all conserved protein-coding genes within the 22q11.2 region as well as combination mutant lines that disrupt syntenic genes and have submitted them to a deep-phenotyping paradigm including larval behavioral analysis and targeted whole brain imaging. Out of 55 mutant lines tested, eight lines, representing five individual genes, display reproducible behavioral phenotypes and implicate mitochondrial function, gene expression regulation, and membrane trafficking in pathogenesis. Whole brain activity and morphology imaging of mutant lines with behavioral phenotypes reveals partially overlapping changes suggesting these genes may be involved in the regulation of similar biologic processes. Supporting this notion, double mutant behavioral analysis suggests genes may function in both shared and parallel pathways. Together, these studies have identified biologic processes involved in 22q11.2 deletion pathogenesis and have begun to identify gene by gene interactions within the region that may underlie neurodevelopmental disorder risk and warrant further studies.

198 - How is axon caliber determined locally?

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Neurons are extremely long, thin cells, and like most cells, their shapes are central to their function. The extensions that make up most of the cell's length – axons and dendrites (collectively, neurites) – carry out signaling and transport that are influenced by their calibers. Despite the well-known importance of neurite caliber, little is known about changes to and regulation of neurite caliber on a developmental timescale. Using touch-sensing neurons in the zebrafish larvae, we characterized variation in caliber of axons in vivo. We found that axons can taper, becoming thinner after branching. Additionally, in this system, axons often widen over development, though tapering is still observed. Work from others found that blebbistatin, a drug that inhibits myosin, widens axons. This finding was recapitulated in our in vivo work; however, treatment with blebbistatin showed that axons treated with blebbistatin had the same or more pronounced tapering than control neurons. These findings suggest that myosin is not responsible for axon tapering, and they provide fundamental insights into how touch sensory systems develop and how neurite diameter is determined.

199 - Mechanism of apoptotic cell extrusion in zebrafish

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When apoptosis occurs in a small number of cells within epithelial tissues, apoptotic cells are apically extruded by cell-cell communication between the apoptotic cells and the nearest neighboring cells. Thus, the apoptotic cell extrusion is crucial for maintaining epithelial barrier functions and homeostasis and is regulated by actomyosin contraction and lamellipodial crawling of the nearest neighboring cells. However, the underlying molecular mechanism and contribution of distal cells still remain elusive. Here we showed that when apoptosis is induced by femtosecond laser irradiation in a cell within zebrafish embryonic epithelia, calcium wave propagates from the laser-induced apoptotic cell to distal cells beyond the nearest neighboring cells. Although intercellular calcium wave is driven by gap junction and extracellular messengers including ATP and S1P, it was not the case in apoptotic cell extrusion in zebrafish: IP_3 receptors and mechanosensitive calcium channels were involved in the propagation of intercellular calcium wave. We also showed that the calcium wave leads to the collective migration of distal cells toward apoptotic cells through the formation of cryptic-lamellipodia (c-lamellipodia). Our *in vivo* force measurement method revealed that the calcium wave generates approximately 1kPa of force toward extruding apoptotic cells. Furthermore, inhibition of calcium waves by pharmacological and genetic approaches resulted in defects in c-lamellipodia formation and force generation, leading to a failure of apoptotic cell extrusion. Therefore, our study revealed that intercellular calcium waves regulate apoptotic cell extrusion through force generation, and highlighted a previously unidentified mechanism of intercellular calcium wave propagation.

200 - Relationship between organ size and function in lateral line organ in zebrafish

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Each organ forms at an appropriate size during development. Organ size regulation is required for acquiring and maintaining organ function, but whether organ function affects organ size remains unclear. To investigate this, we used the lateral line organ named neuromast as a model. During zebrafish development, functional neuromasts are formed by 6 days post fertilization (dpf), and they are composed of approximately 15 hair cells, which sense water flow, and approximately 40 support cells. Depending on the bending of the cilia of hair cells by water flow, mechano-electrical transducers (METs, tmc2a and tmc2b) on the cilia open and induce the influx of extracellular cations (mainly Ca^{2+}), indicating that MET channels are the initiator of neuromast function. To investigate whether organ function affects organ size in neuromasts, we inhibited MET channels by genetic (double knockout of *tmc2a* and *tmc2b*) and pharmacological (curare treatment) approaches. The loss of function conditions of neuromasts resulted in the 1/3 reduction of hair cells (from 15 to 10 cells) but not support cells, eventually leading to the formation of small neuromasts. Immunostaining of either cleaved caspase 3 or phospho-Histone H3 (Ser10) revealed that the reduction of hair cells is not caused by growth arrest but apoptosis of hair cells. Furthermore, evaluation of MET channel ability using fluorescent dye showed that when hair cells lose their function, apoptosis preferentially occurs in the cells that had high MET channel ability, suggesting that neuromasts have a quality control system that removes aberrant hair cells. Therefore, our results uncovered that organ function affects organ size in neuromasts, and proposed that the quality control system in hair cells contributes to proper organ size regulation in neuromasts.

201 - Different olfactory stimuli evoke different forms of adaptation in zebrafish olfactory system

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Sensory neurons adjust their sensitivity to allow for continued efficient transfer of sensory information to the brain. Recent studies have suggested that many sensory neurons not only depress (decrease their response to sensory stimulation) but some neurons exhibit sensitization: a small initial response followed by increase in response amplitude. Such opposing forms of neuronal plasticity have been characterised in the mammal and other vertebrate visual system and zebrafish lateral line but it is not clear whether they exist in other sensory modalities.

Here we used 2-photon calcium imaging of the zebrafish olfactory bulbs and telencephalon to define whether depression and sensitization to application of behaviourally relevant olfactory stimuli exist in the olfactory system. We observed a range of sensory adaptations in response to repeated presentations of a food odorant, with 52.1% of responding voxels displaying depression, 18.9% of voxels displaying no adaptation and 15.8% showing sensitisation to the stimulus. GABAA receptor antagonist pentylenetetrazole caused a reduction in the number of sensitising responses and an increase in non-adapting cells, suggesting that the sensitization in the olfactory system may result from inhibitory input from GABAergic interneurons, granule cells. Surprisingly, repeated presentations of the fear-evoking alarm substance, schreckstoff, showed a significantly greater depression as well as a reduced diversity of responses in comparison to the food odorant, with almost all responding cells exclusively responding to the first presentation only. This demonstrates that different olfactory cues exhibit differing levels of adaptation across the olfactory network. Our results further highlight the importance of opposing forms of plasticity in the computation of sensory information across different sensory modalities and the importance of differing adaptations in sensory computation.

202 - Lifetime Single Cell Atlas Network Biology of the Enteric Neuron System in Zebrafish

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Neural crest cells (NCC) are embryonic stem cells that are transient, highly migratory and multipotent that give rise to different cell types within many critical tissues; being one of them the Enteric Nervous System (ENS). Sox10 is expressed in NCCs during their cell fate specification and migration, and *sox10*-expressing NCC give rise to subpopulations of enteric neural progenitors that mature into enteric neurons (ENs). Recently, several separate scRNA-seq projects in zebrafish have profiled *sox10*-derived cells during different embryonic, larval and adult stages, capturing the development of enteric progenitors transitioning to ENs. Integration across the previous mentioned datasets projects might discover additional ENs and its progenitors. In this study, using the previously published datasets, we generated a combined *sox10* lifetime transcriptional atlas spanning several zebrafish stages to identify novel gene signatures of ENs that allow us to annotate cellular identities of the ENS. Initially we subset clusters based on the major neural-neuronal annotated cellular types. A closer analysis into the time window of 68 -120 hours post fertilization (hpf), where ENs classes expect to emerge and differentiate, was selected to identified conserved markers. Specifically, clusters with colocalized cells from 68 to 120 hpf detected markers related with neuronal differentiation and modulation. Additional sub-clustering of 68 -120 hpf were subjected to single-cell network biology, to infer regulatory programs using STRING database. Network analysis showed biological processes, such as neuron development, regulation of neurotransmitter and modulation of synaptic transmission. Specially, for ENs clusters, Reactome Pathways were enriched based on neurotransmitter release. Differences in the network analysis also were noted between 68 and 120 hpf for the ENs cellular types. Target functional enriched node genes were identified for future loss of function analysis to understand ENs development and differentiation. This work provides a foundation upon which future investigations of ENS development may be proceed.

203 - Behavioral profiling in zebrafish identifies a new translatable anesthetic with analgesic properties.

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The overarching goal of my research is to discover neuroactive molecules including new analgesics and anesthetics. To accomplish this goal, we developed a high throughput *in vivo* behavioral screening paradigm in conjunction with a computational analysis pipeline to identify new behavior modifying compounds. Recently, we discovered a highly specific anesthetic related behavioral profile. This behavioral signature was subsequently leveraged in a 10,000 small molecule screen to identify a cohort of new compounds that cause anesthesia like behaviors in both larvae and adult zebrafish. Those that were effective in the adult zebrafish were found to also induce loss of righting reflex (LORR, a hallmark of anesthesia) in rodent models. The discovery of these primary hit molecules translating into mammals motivated a structure activity relationship campaign aimed to optimize primary hit molecules for solubility and efficacy. Adult zebrafish LORR assays were used as an initial filter to identify analogs with potential to translate into the mouse. From these efforts we have identified our new lead molecule, Nidradine. This compound has been shown to induce both LORR and loss of withdrawal reflex, demonstrating a new chemical agent that can induce a surgery ready state in mammalian animal models. Interestingly, at sub-anesthetic concentrations, Nidradine can also act as an analgesic in a rodent model of pain. Future studies will determine if the analgesic and anesthetic effects of this class of small molecule can be uncoupled.

204 - Spatiotemporal dynamics of gene expression during an inflammatory response in the zebrafish epithelium

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Inflammatory responses are controlled by signaling factors secreted at the sites of wounds and infections, and their interactions with tissue and immune cells. Understanding how these many factors combine to set the time and length scales of inflammatory responses remains a major challenge. One of the hurdles to addressing this problem is the difficulty of observing cellular responses to inflammatory challenges in their native tissue contexts. Here, we use a combination of single-cell RNA sequencing, *sm*FISH, and live fluorescence imaging to characterize the spatiotemporal dynamics of gene expression changes in the zebrafish tail fin in response to bath LPS exposure. In addition to activation of tissue-resident immune cells, we observe stochastic activation of several pro-inflammatory genes in basal epithelial cells. Surprisingly, for the master immune regulator *il1b*, this activation leads to the emergence of a spatially-patterned state, despite the uniform bath stimulus. This suggests that spatially-structured inflammatory activation can emerge from internal dynamics and interactions within tissue even in the absence of spatially-structured stimulation.

205 - Multiple factors promote the arrangement of microridge protrusions on epithelial cell surfaces

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Microridges are actin-based protrusions arranged in maze-like patterns on the surface of mucosal epithelial cells. To characterize how microridges mature and rearrange, we imaged microridges in the skin of zebrafish larvae. Microridges initially form from the coalescence of short precursors called pegs. After their initial formation, microridges continue to lengthen and become progressively more aligned and regularly spaced. Live imaging demonstrated that as they become more well ordered, microridges rearrange dynamically by fission and fusion. Imaging F-actin and Non-Muscle Myosin II (NMII) revealed that microridge rearrangements were associated with local NMII activity in the apical cortex. High-resolution imaging revealed that cortical NMII minifilaments are connected to protrusions and drive their fission and fusion. Inhibiting NMII activity blocked rearrangements, reduced microridge density, and altered microridge spacing. Increasing NMII activity by overexpressing Plekhg6, a Rho GEF, increased microridge density and alignment and decreased spacing variability. Preliminary data suggests that increasing NMII contraction by other means, such as by treatment with calyculin A, a phosphatase inhibitor, and by knocking down actin-binding proteins such as Supervillin and Filamin, has similar effects, altering microridge alignment and spacing. These findings show that multiple factors temporally and spatially regulate NMII contraction to create biomechanical conditions conducive to microridge protrusion formation and patterning.

206 - The Cardiomyocyte-Specific Kinase Tnni3k Influences Heart Regeneration Through Modulation of Inflammation.

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Myocardial infarction (MI) is one of the leading causes of death worldwide. The main consequence of an MI is the irreversible loss of cardiomyocytes (CMs) and their replacement by a permanent fibrotic scar. By contrast, the zebrafish heart efficiently regenerates after injury through cardiomyocyte proliferation and progressive fibrosis resorption. Therefore, identifying the mechanisms by which the zebrafish overcomes fibrosis and achieves myocardial regrowth are cornerstones for designing novel regenerative therapies.

The CM-specific kinase Tnni3k has been proposed as a regulator of myocardial regeneration. Loss-of-function polymorphisms in *Tnni3k* increase the frequency of diploid CMs and improve the outcome in murine heart failure models. Importantly, Tnni3k is among the most upregulated genes in the heart of patients with end-stage dilated cardiomyopathy. Elevated Tnni3k has been associated with CM polyploidization and impaired proliferation in mice. However, the underlying mechanisms by which Tnni3k influences regeneration remain elusive. To dissect the role of Tnni3k in myocardial regeneration, we generated a new zebrafish mutant strain that lacks the entire *tnni3k* locus, as well as several gain-of-function lines. We found that Tnni3k levels do not significantly influence CM ploidy nor proliferation after injury. However, we discovered that Tnni3k levels affect the formation and regression of the fibrotic scar. Using transcriptomic profiling followed by detailed histological studies, we learned that elevated Tnni3k induces lymphocytic infiltration associated with fibrosis at baseline. This exacerbated and chronic inflammation hinders the resorption of the fibrotic scar following injury. Our data highlights a unique inflammatory signature characterized by macrophage and T cell infiltration specific to *tnni3k* overexpression.

Collectively, our findings suggest that Tnni3k expression influences the inflammatory environment and hampers the resorption of fibrosis following cardiac injury. Identifying the mechanisms by which Tnni3k instructs changes in the cardiac environment will unveil candidate targets that could promote fibrotic scar resorption in humans.

207 - Migratory cells in the zebrafish epidermis may regulate epithelial cell properties

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Early in development, the vertebrate epidermis consists of two epithelial cell layers: the superficial periderm layer and the inner basal cell layer. Basal stem cells give rise to multiple specialized cell types, and proliferate to form the stratified epidermis later in development. We found that a reporter for basal epithelial cells (ΔNp63:Gal4 BAC) also drives expression in an abundant migratory cell population between 24- and 36-hours post-fertilization. Imaging these cells with an actin reporter revealed that they are highly polarized, making dynamic filopodial protrusions at their leading edge. High magnification imaging revealed that these cells migrate exclusively between periderm and basal cells. Intriguingly, the peripheral axons of touch sensory neurons also grow in this ECM-poor region between the two epithelial cell layers. Imaging a nuclear reporter revealed that crawling migratory cells mechanically deform the nuclei of basal cells. Strikingly, these migratory cells often migrate over basal cells just before they undergo cytokinesis. To characterize the migratory paths of these cells, we expressed a photoconvertible fluorescent protein with the ΔNp63 driver, photoconverted patches of skin, time-lapse imaged migratory cells crawling into and out of these regions over 12 hours, and traced their paths. In the head, cells migrated primarily from ventral to dorsal, whereas in the trunk, they migrated both dorsally and ventrally. By 36 hpf, many migratory cells appeared to become stationary, likely integrating into the epithelium. Given their presumed origin from ΔNp63-expressing precursors, their migratory paths, and their integration into the epithelium, we speculate that these cells are developing ionocytes and/or mucus-secreting goblet cells, and are currently testing this hypothesis genetically. These results illustrate the complexity of early epidermal development, and motivate us to explore the interactions between epithelial cells, sensory neurons, and basal stem cells.

208 - Distribution pattern of dark spots on the head of medaka for individual identification over time

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Thanks to recent advances in genome editing technology, various human disease models have been generated in medaka and zebrafish. For late-onset diseases, such as neurodegenerative diseases, it is important to conduct a long-term observation and a behavioral test on a disease model of adult fish. In addition, it is desirable to track each individual model fish as the disease progress. In the follow-up study, individual identification is required. Tags attachment and tattooing are difficult to be applied to small fishes since they cause behavioral disorders. As for zebrafish, a stripe pattern could be used as an individual-specific marking, but medaka does not have such a distinct pattern. In this study, we proposed and evaluated a new individual identification method for medaka using a distribution pattern of dark spots on the head.

We acquired digital camera images of dark spots on the heads of six inbred medaka in an intact state at six time points over a 34-week period. Comparing the distribution patterns of the six individuals at each time point, all individuals had different distribution patterns. Observing in time series, we could connect the distribution patterns at each time point with those of the same individual at the previous time point, although part of the patterns changed in some cases.

Then images of dark spots of 30 individuals were acquired twice at a four-week interval. At both time points, the distribution patterns of all individuals were clearly distinguishable. The correspondence between the first and second distribution patterns could be also made. These two sets of images were presented to three examinees as a blind pairing test, and all achieved a 100% identification rate.

These results suggested that the distribution pattern of dark spots on the head of medaka could be used as an individual-specific marking for individual identification.

209 - Zebrafish pancreatic β cell clusters undergo stepwise regeneration using Neurod1-expressing cells from different cell lineage

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Pancreatic β cell cluster, which produce Insulin, play a central role for glucose homeostasis. Regenerative capacity of mammalian β cells is limited so that loss of β cells causes diabetes. In contrast, zebrafish has high regenerative capacity of β cell cluster through its life, making them an attractive model for the study of β cell cluster regeneration. However, fundamental questions remain, such as how zebrafish β cell clusters regenerate, when regeneration process is completed, and what is the main cellular source of regenerating β cells.

Here we showed using some transgenic lines that all regenerating pancreatic β cells arose from Neurod1 expressing cells. In addition, pancreatic β cell cluster regeneration was complete by 13 days after β cell ablation through a two-step regeneration process: first, their β cell clusters regenerated functionally under the conditions of a small number of β cells; then, after blood glucose levels became normal, their β cell clusters regenerated morphologically by creating new N1 cells to fill in the missing cells.

Altogether, my results shed light on the fundamental cellular mechanisms underlying β cell cluster regeneration.

210 - Investigation of a col6a2 related congenital muscle dystrophy model in zebrafish

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Collagen 6 congenital muscular dystrophy (Col6-CMD) is a rare spectrum disorder, that is caused by dominant-negative and recessive mutations in *COL6A1*, *-A2*, *-A3* and in rare cases by mutations in *COL12A1.* The phenotypic spectrum includes Bethlem Myopathy (mild) and Ullrich congenital muscular dystrophy (severe) that are connected by a continuum of intermediate phenotypes. The disease particularly affects the skeletal muscle and connective tissues, but collagen 6 (COL6) plays also a role in the extracellular matrix of skin, tendon, cartilage, intervertebral discs, lenses, inner organs and blood vessels. The functions of COL6 are important in COL6-CMD, but pleiotropic effects have not yet been systematically studied.

The objective of this study is to understand tissue-specific phenotypes and the development of a COL6-CMD zebrafish disease model using morpholino injections. Morphants were assessed morphologically by live-imaging with the Acquifer imaging device followed by histological examination and *in situ* hybridization. Additionally, a functional analysis of larval locomotion using the ZebraBox, an automated analysis chamber for observing zebrafish, is planned.

In situ hybridization provided insights into the role of col6α2 expression in different tissues, such as jaw muscles, gut, heart and cartilage during embryonic development. A broad spectrum of phenotypes was observed in *col6α2* morphants (n=139), ranging from mild (n=45) to moderate (n=29) to severe (n=65) phenotypes, which resembles the human COL6 CMD phenotype. In histological examinations, the mutant zebrafish showed alterations in the swim bladder development and eye size.

In conclusion, we investigated *col6α2* MO injected zebrafish larvae which reflect the pathogenesis of the disease in early developmental stages. Furthermore, we were able to obtain first insights into the expression of Col6*α*2 in different tissues of *col6α2* morphants.

211 - Fras1 and the Fraser Complex support an environment for basal epidermis and osteoblast integrated tissue morphogenesis underlying fin skeletal patterning

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Fraser Syndrome is a rare autosomal recessive disorder variably affecting multiple organs and characterized by disrupted associations between epithelia and adjacent mesenchyme. Syndactyly, including missing and fused digit bones, is a commonly associated malformation. We explored if zebrafish *fraser extracellular matrix complex subunit 1 (fras1)* mutants could model Fraser Syndrome-associated syndactyly. Approximately 10% of *fras1* mutants survived to adulthood with striking and variable fin abnormalities. Caudal fins exhibited endochondral bone fusions, ectopic cartilage, disrupted fin symmetry, and fewer rays, many which were unbranched. We evaluated regenerating fins to further explore Fras1 contributions to ray patterning. *fras1* was specifically basal epidermis-expressed within a scRNA-Seq atlas of regenerative outgrowth, while other Fraser Complex protein-encoding transcripts were elevated in the distal growth zone and expression verified by *in-situ* hybridization. Fras1 and Frem2 proteins accumulated along the basal side of distal-most basal epidermal cells. *fras1* mutants regenerated fins to their original size. However, ray branching and fin symmetry defects worsened in *fras1*$^{-/-}$ regenerates. Extensive sub-epidermal blistering was associated with a poorly organized basal epidermal layer. Fras1 was absent while Frem2 was greatly diminished and mislocalized. Ray branching requires Sonic hedgehog signaling between distal basal epidermis and adjacent mesenchymal pre-osteoblasts. However, *shha* expression monitored by *Tg(-2.4shha:gfpABC)* and Shh/Smo activity reflected by upregulated T*g(ptch2:Kaede)* remained intact in *fras1* mutants. We propose the Fraser Complex supports a robust matrix environment for integrated tissue morphogenesis involving the basal epidermis and osteoblasts. We conclude zebrafish fin development and regeneration provides an accessible context to explore mechanisms of Fraser Syndrome-associated syndactyly and Fraser Complex function. Funded by NIH/NIGMS F31GM139343 (AER) and R01GM127761.

212 - Exercise disrupts the fibroblast-lineage injury response and alters skeletal patterning during zebrafish fin regeneration

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Mechanical strain experienced during rehabilitative exercise increases bone mass and decreases recovery time from traumatic bone injuries. However, whether for athletes or “regular” patients, rehabilitation practices largely depend on trial-and-error. Resolving how exercise-induced mechanical strain influences distinct cell behaviors acting at different phases of skeletal repair would facilitate optimized rehabilitation modalities and therapies. We use the zebrafish caudal fin, a leading vertebrate regeneration model, to evaluate how swimming exercise impacts skeletal regeneration. Caudal fins have a bony ray scaffold, with fibroblasts within and between rays, all surrounded by a stratified epidermis. Injury “activates” intra-ray fibroblasts and initiates dedifferentiation of wound-adjacent osteoblasts, which then migrate distally as mesenchyme and pre-osteoblasts to establish an organized blastema. Throughout the subsequent outgrowth phase, mesenchyme and pre-osteoblasts proliferate and re-differentiate to progressively restore fin tissue, including the branched ray skeleton. We use a swim tunnel system to show exercise initiated during blastema establishment negatively impacts the robustness of the regenerative response. Long-term imaging of fluorescently marked cells during regeneration shows swimming predominantly perturbs the fibroblast/mesenchyme lineage with likely secondary effects on osteoblasts and other cells. Further, continued exercise throughout regenerative outgrowth alters ray branching morphogenesis. Our results suggest exercise-induced strain modulates the early fibroblast-lineage fin injury response and later skeletal patterning. We are now pursuing links between biomechanical forces and specific cell behaviors to understand these exercise impacts on fin regeneration. Funded by F32GM140712 (VML) and the Wu Tsai Human Performance Alliance and the Joe and Clara Tsai Foundation.

213 - Toward novel therapeutic interventions for STXBP1-associated disorders

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Approximately 25-30% of people suffering from epileptic seizures do not respond to available antiseizure drugs (ASDs). Additionally, ASDs tolerated well by adults may cause severe side effects in children. Thus, there is a high need for models to test novel therapeutic options. Especially rare forms of epilepsy, where evaluation of intervention may be challenging due to the limited number of subjects, will benefit from animal models suited for rapid drug screening.

Focusing on STXBP1-associated disorders, we developed two drug-screening protocols for potential ASDs: one based on behavioural analysis and another based on morphological alterations. Combining these screening methods with various genetic and chemical zebrafish epilepsy models, we mean to identify novel potential ASDs from libraries of FDA-approved compounds and natural products. Top hits from these initial screens will be confirmed through EEG recordings, and evaluated for their effects on neuronal morphology and additional disease comorbidities such as Schizophrenia- and Autism Spectrum Disorder-like features.

214 - Cardiomyocyte proliferation in the developing and regenerating heart is dependent on Histone deacetylase 1 activity

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Unlike mammals, the zebrafish retains its ability to proliferate cardiomyocytes throughout adulthood. Understanding how this is achieved is an important means to develop novel therapeutic strategies to prevent scar formation in patients experiencing a heart attack. However, neither the molecular mechanisms that orchestrate proliferation of embryonic cardiomyocytes during development of the heart, nor during adult heart regeneration after cryo-injury are sufficiently understood. We identified the recessive, embryonic lethal zebrafish mutant *baldrian* in a forward genetic ENU-mutagenesis screen. *Bal* mutants show a severely impaired cardiac growth due to decreased cardiomyocyte proliferation. Through positional cloning we have identified a missense mutation in the zebrafish *histone deacetylase 1* (*hdac1*) gene resulting in protein instability and subsequent loss of Hdac1 function *in vivo*. Hdac1 inhibition using Mocetinostat, a class I HDAC inhibitor, significantly reduces cardiomyocytes proliferation, and thereby phenocopying the *bal* mutant phenotype. To assess whether the role of Hdac1-associated mechanisms during cardiomyocyte proliferation are conserved, we analyzed regenerative cardiomyocytes after cryo-injury of adult zebrafish hearts. Inhibition of Hdac1 by Mocetinostat lead to a decreased rate of proliferating cardiomyocytes in the wound boarder zone, whereas revascularization was unalterd. Interestingly, also the wound resolution was unaffected 30 days post injury. Preliminary results in neonatal ventricular rat cardiomyocytes suggest a conserved function of HDAC1 also in mammalian cardiomyocyte proliferation. In summary, our findings suggest an important and evolutionary conserved role of histone deacetylase 1 in developmental as well as adult regenerative cardiomyocyte proliferation.

215 - Looking again at neural crest cell fate restriction: low-level co-expression of fate-specification factors reveals retained broad multipotency of migrating neural crest cells in vivo.

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Neural crest cells (NCCs) are multipotent and widely studied as a model for understanding stem cell fate restriction. Two contrasting mechanisms for neural crest (NC) fate restriction exist: progressive fate restriction (PFR) proposes restriction through stereotypical intermediate progenitors that follow distinctive migratory pathways, whereas direct fate restriction (DFR) proposes that fully multipotent cells directly adopt a single cell type. Using Nanostring transcriptional profiling of zebrafish single trunk NCCs we were unable to identify the expected intermediate progenitors of pigment cell fates, but instead identified a highly multipotent progenitor (HMP) and differentiated pigment cells, arguing against the previously-favoured PFR scenario. Here we extend our evaluation of the *in vivo* state of developing NCCs using highly sensitive RNAscope to examine the overlaps and locations of expression of neuronal (*phox2bb*), and pigment (melanocyte (*mitfa)*, iridophore (*tfec* and *ltk)*, xanthophore (*pax7b*)) cell fate specification markers, using co-expression as a *minimal* indication of potency). In agreement with our Nanostring data, pre-migratory NCCs express all pigment cell markers, with co-expression retained also in migrating NCC. In contrast to observations using traditional ISH, we see co-expression of xanthophore and iridophore genes on both lateral and medial migration pathways. In addition to displaying pigment cell potential, some early and migrating NCCs show neuronal potential (*phox2b* with *ltk* or *mitfa* or *pax7b*), again revealing their unexpectedly broad multipotency. Together, our data confirm that *in vivo* NCCs retain a broad multipotency even in late stages of NCC migration and that there are no fate-restricted intermediate progenitors. Our data are consistent with our recently proposed Cyclical Fate Restriction model in which a HMP cell moves dynamically through a series of substates, each primed for the adoption of a single cell fate and differentiation, allowing environmental signals to influence the ‘dwell-time’ in each substate and hence to bias eventual fate selection.

216 - Slits and BMPs orchestrate opposing properties on cell morphology during tissue patterning

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The zebrafish larval fin is a simplified system for understanding how migrating cells invade and pattern themselves within a tissue. As the larval fin grows, paraxial mesoderm cells migrate in and adopt a gradient of different morphologies from highly polarised and branched apically, to isometric proximally. They are also subject to cell tiling which arranges these mesenchymal cells at regularly spaced intervals. How these graded cell geometries and spacings are instructed is not clear. We have uncovered opposing signalling systems involved in this process. Through mapping the fin mutant, *stomp,* we have demonstrated that the axon guidance molecule, Slit3, signals from the mesenchyme to Robo receptors in the Apical Ectodermal Ridge to establish their own polarity. This requires generation of AER derived Sphingosine-1-Phosphate, which signals back to the mesenchyme. Mutation of an S1P receptor gives a phenotype indistinguishable from *slit3* mutants in which cells are separated but fail to polarise. This signalling echo system represents a novel location system for cells to adjust their shape based on proximity to a boundary. We have also found that loss of BMP signalling generated the opposite phenotype, where cells are polarised, but fail to separate and tile. Thus, through forward and chemical genetics, we have determined two opposing mechanisms patterning mesenchyme cells in an epithelium.

217 - Feeding zebrafish with specific oils rich in polyunsaturated fatty acids: a tool to investigate the impact of specific fatty acid enrichment on disease development.

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Different types of polyunsaturated fatty acids (PUFAs) including conjugated linolenic acids (CLnAs) have shown an increasing interest in terms of health impact. We developed an approach allowing the enrichment of zebrafish (*Danio rerio*) in specific fatty acids, in order to allow deeper investigations regarding the mechanisms by which changes in PUFAs body composition affects the development of diseases.

Four purified diets were formulated to meet the essential nutritional requirements of zebrafish and provide an enrichment in a specific lipid source such as linseed oil rich in alpha-linolenic acid (ALA) (51,8% of fatty acids in the diet) and fish oil rich in eicosapentaenoic acid (EPA ; 9,15%) and docosahexaenoic acid (DHA) (9,56%) for the omega-3, pomegranate seed oil rich in punicic acid (PunA) (48,15%) and sunflower oil rich in linoleic acid (LA) (39,04%), an omega-6 fatty acid. Those four diets were given to 6 months old zebrafish in normal dose (25-30 calories per fish per day) for 8 weeks.

We demonstrated that after 8 weeks of experiment, our fatty acids of interest were incorporated to various extend into zebrafish tissues. Small amounts of LA and ALA were bio-converted into longer fatty acids, although those conversions were not efficient. It was also observed that fish fed with pomegranate seed oil had higher concentration of 18:4 n-3, which could indicate an effect of PunA on Δ6 desaturase. In addition, rumenic acid accumulated in the fish body, indicating the presence of a Δ13 reductase activity in zebrafish. Moreover, no significant differences in terms of weight, size and BMI were observed between the different diets.

218 - Development of a versatile, automated and high-throughput drug screening platform for zebrafish embryos to find novel therapeutics for AML

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Zebrafish provide a unique opportunity for drug screening in living animals, but the limited availability of imaging and analysis platforms that offer speed and flexibility has limited their use. We have developed a fast, easy-to-use automated screening procedure suitable for high-throughput drug screens of live zebrafish, which we are utilising in a small-molecule screen for synthetic lethality in haematopoietic stem cells (HSCs) carrying driver mutations for acute myeloid leukaemia (AML).

We have utilised the Wiscan Hermes High Content Imaging System to rapidly acquire images of embryos at 2-4dpf. In collaboration with IDEA Bio-Medical, an Artificial Intelligence-driven application was developed to automatically detect -fish in brightfield images, identify anatomical and physical structures in the fish, and select fish in the desired side-orientation. To analyse HSCs in the caudal haematopoietic tissue (CHT), this was combined with analysis of fluorescence images to count GFP-HSCs in the tails of embryos, which correlated with manual counts (r=0.844). We further validated this system in high content to assess the effects of x-ray radiation and genetic mutations on HSCs.

Following validation, we have generated zebrafish modelling a common AML driver mutation (DNMT3a) and are undertaking a drug screen using a library of 1120 biologically active compounds, comparing the HSC counts in treated wildtype and mutant fish to assess for synthetic lethality in the mutant animals.

Given the flexibility of the system, we were also able to further validate the platform for other assays including cell death, hair cell development, angiogenesis, eye size, and the simultaneous analysis of multiple cell types using dual fluorophores.

In summary we demonstrate the broad applicability and rapidly customisable applications of the Wiscan Hermes and Athena software as a platform for high content drug screens in zebrafish, and applied the platform for drug screening to develop novel therapeutics for AML.

219 - Identification and functional analysis of angiocrine factors during zebrafish heart regeneration

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Coronary vessels supply the cardiac muscle with nutrients and oxygen and their occlusion leads to myocardial infarction. Contrary to adult humans, zebrafish have the unique ability to regenerate after cardiac injury, and fast revascularization is key to this process. Endothelial cells produce so called angiocrine factors that regulate tissue homeostasis and repair in a paracrine (angiocrine) manner. Angiocrine factors have been shown to be critical for regeneration of alveolar epithelial cells and hepatocytes. However, their role during cardiac regeneration remains largely unknown. We hypothesize that regenerating coronaries regulate several key aspects of cardiac regeneration by releasing angiocrine factors. To test this hypothesis, we performed a detailed transcriptomic profiling of regenerating coronary endothelial cells (cECs) after cardiac injury. We intersected these datasets with a matrisome dataset and identified a panel of candidates encoding potential angiocrine factors. Amongst these genes, *plxdc2* and *gpnmb* were strongly induced in regenerating cECs. We found that *plxdc2* and *gpnmb* expression peaks at 7 days post cryoinjury, a time point of vigorous revascularization and cardiomyocyte proliferation. To further investigate their role during cardiac regeneration, we are generating gain- and loss-of-function reagents. Using these tools, we are currently investigating the role of Plxdc2 and Gpnmb during heart regeneration. This study will contribute to our understanding of the mechanisms activated in an adult regenerative heart after injury and might help to devise more efficient therapies for the mammalian heart.

220 - Identifying Novel Drivers of Clonal Selection in CEBPA-mutated AML

Alexandra Lubin[1], Catherine Hockings[1], Yvette Hoade[1], Lucy Copper[1], Phoebe Dace[1], Sam Wain[1], Androniki Giallouri[1], Abel Ruiz Jaimez[1], Elspeth Payne[1]

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CCAAT/enhancer binding protein alpha (CEBPA) is a transcription factor mutated in 10-15% of acute myeloid leukaemia (AML), a haematopoietic malignancy with high mortality. CEBPA mutations are also observed in rare cases of germline predisposition to AML. CEBPA mutations show a distinct pattern of distribution; in-frame C-terminal mutations (C-term) or frame-shift N-terminal mutations (N-term). These mutations have differing effects on myeloid and stem cell development, and CEBPA-mutated AML typically involves one of each of these mutations suggesting a selective pressure from each mutation to develop the other.

We have developed a zebrafish model for CEBPA-mutated AML, with mutant lines modelling both C-term and N-term mutations. All biallelic mutant combinations show defective myeloid development, but distinct phenotypes were also seen between the genotypes highlighting their functional differences. Strikingly, biallelic mutants with two cebpa N-term mutations showed an increase in haematopoietic stem and progenitor cells (HSPCs) expressing c-myb, while by contrast, biallelic mutants with two cebpa C-term mutations showed a decrease in HSPCs expressing c-myb.

To further understand the relative role of C- and N-terminally mutated cebpa in developing HSPC and the transcriptional drive behind the differences we undertook RNA-seq from each mutant genotype combination. We identified a large number of genes differentially expressed between each of the biallelic mutants and the wildtype, with significant overlap in these genes between the groups. However, there were also a number of genes distinct to each genotype, which may provide insight into what is driving the difference in phenotype, and the selective pressure from one mutation to develop the other. These differences include a number of cytokine receptors, with the colony stimulating factors that regulate the differentiation of HSPCs showing distinct patterns between the genotypes. The genes identified may be involved in driving the phenotypic differences, and the clonal selection observed in the development of AML.

221 - Wnt and Notch signaling regulation in pre-hemogenic endothelium control hematopoietic stem and progenitor cells heterogeneity.

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Definitive hematopoietic stem/progenitor cells (HSPCs) are produced from a specialized population of endothelial cells in the embryonic aorta called hemogenic endothelial cells. This process, known as the endothelial-to-hematopoietic transition (EHT), is conserved across vertebrates, and is followed by HSPC differentiation in all blood lineages. Single-cell methods have uncovered substantial heterogeneity in lineage priming within nascent HSPCs from the aortic endothelium, however it is currently unknown how this diverse differentiation capacity is conferred. Such understanding could have important ramifications for bone marrow transplantation where heterogeneity in HSPC behavior is observed. Here we found that microRNA (miR) loss of function zebrafish mutant, miR-128 (miR-128-/-), has increased EHT, resulting in supernumerary lymphoid and erythroid primed HSPCs and relative mature cells. Correlatively, miR-128-deficient endothelium derived from human pluripotent stem cells show similar defective EHT, supporting miR-128's functional conservation in human. Transcriptomic analysis of endothelial cells from wild type and miR-128-/- embryos revealed putative miR-128 target genes, *cskn1a* and *jag1b,* involved in the inhibition of the two master EHT signaling pathways, Wnt and Notch respectively. Additionally, responsive transgenic lines and single cell RNA sequencing revealed that Wnt and Notch signaling activity are decreased specifically in pre-hemogenic endothelium of miR-128-/-. Chemical inhibition of Wnt or de-repression of *cskn1a* promoted HSPCs with erythroid lineage commitment, while lymphoid cells number increased after de-repression of *jag1b* or blockage of Notch activity at the onset of EHT.

This study sheds light on how the HSPC heterogeneity in lineage priming is programmed prior to endothelial transdifferentiation by miR-128-co regulation of Wnt and Notch.

222 - miR-125a establishes the circle of Willis arterial network function

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The circle of Willis (CW) is the conserved arterial ring at the base of the brain and recognized as a compensatory system in case of arterial occlusion. Malformation of the CW, including abnormal stenosis, dilation, and tortuosity, is the hallmark of many cerebral diseases such as dementia, stroke, and aneurysm. Unfortunately, there is little understanding of how CW malformations arise and contribute to cerebrovascular dysfunctions.

Here, I identified miR-125a as a novel regulator of the CW development. Adult miR-125a loss-of-function mutants show anatomical CW absence or stenosis, while mutant embryos develop arteriovenous hyper-connected and tortuous CW in early angiogenesis. We dynamically visualized the cellular events controlled by miR-125a and found excessive endothelial proliferation in early vascular bed and disordered migration during CW forming. Transcriptome profiling of zebrafish miR-125a mutant and wild-type cranial endothelial cells identified mitochondrial biogenesis master regulator *pgc1a* as a candidate miR-125a target. *pgc1a* miR-125a responsive element loss of function phenocopies the CW malformations observed in miR-125a mutants. Besides, single-cell RNASeq analysis revealed reactive oxygen species as the metabolic signature in mutant arterial endothelial cells. Different from glycolysis function in endothelial energy regulation, mitochondrial metabolism is a key factor for endothelial angiogenesis and vascular homeostasis, especially the hyperbaric oxygen environment in brain. Altogether, this work aims to identify how CW malformation development could influence the etiology of cerebrovascular diseases such as stroke and aneurysm, and how regulating endothelial metabolism could prevent such aberrations.

223 - Pulses of RhoA Signaling Stimulate Actin Polymerization and Flow in Protrusions to Drive Collective Cell Migration

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Cells migrate collectively to form organs, close wounds, and in the case of disease — metastasize. To accomplish this, cells need to generate force to propel themselves forward. The motility of singly migrating cells is driven largely by an interplay between Rho GTPase signaling and the actin network. Whether cells migrating as collectives use the same machinery for motility in vivo is unclear. To address this question, we are using the zebrafish posterior lateral line primordium as a model for collective cell migration. The primordium is a tissue of about 140 cells that migrates directly under the skin of the zebrafish embryo from behind the ear to the tip of the tail. Using Rho GTPase localization sensors, we found that active RhoA clusters on the basal sides of the primordium cells while active Rac/Cdc42 localizes fairly uniformly at the membranes of the cells without clustering. We further found that pulses of RhoA activation contract the actomyosin network in the primordium as shown by the pulses of Myosin II and F-actin clusters. Combining drug inhibitors and tissue-specific expression of genes targeting RhoA/ROCK signaling, we found active RhoA and actomyosin pulses are required for primordium motility. We further revealed positive and negative feedbacks that ensure the pulses of RhoA and actomyosin in the primordium. These pulses of RhoA signaling stimulate actin polymerization at the tip of the protrusions, myosin II-dependent actin flow and protrusion retraction at the base of the protrusions, and deform the basement membrane underneath the migrating primordium. This suggests that RhoA induced actin flow on the basal sides of the cells constitutes the motor that pulls the primordium forward, a scenario that likely underlies collective migration in other contexts.

224 - An Inexpensive Kit to Support a Remote, Inquiry-based Undergraduate Developmental Biology Laboratory Course

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One of the challenges during the pandemic was to maintain high-quality scientific experiences for undergraduate students doing their laboratory courses at home and remotely. To meet this challenge, we created a kit for a senior level developmental biology course that included three reliable, inquiry-based experiments that required the students to provide only a computer/digital phone and water. These laboratories included (1) Regeneration and Reproduction in Planaria, (2) Cell Migration in Slime Mold and (3) Plasticity of Morphogenesis in Plants. These laboratories were reliable even in the variabilities of the students' home environments and flexible enough to offer the students a wide range of freedom in their experimental designs. In a positive twist, students were able to observe steps in development that are normally missed because they occur between laboratory periods. One of the main strengths of in-person, on-campus developmental biology laboratories is the chance to build microscopy skills, take publication quality images, and use these images to build figures that tell the story of the experiment. To preserve this strength, we compared microscopes in a price range that would make it possible for each student to have their own. We found that endoscopes (40-1000X magnification) could take high quality images and movies of samples of sizes from a few millimeters to several centimeters, which includes zebrafish embryos. We expect there is a place for these portable laboratories even when laboratory classes are in person. In particular, the ability to take high quality images using a phone or computer brings up the possibility of adapting the protocols developed for these laboratories for community outreach events, for experiments in the field, and for students unable to access a regular laboratory classroom.

225 - Live imaging and degradation mediated manipulation of an endogenous fluorescent Vangl2 fusion protein in zebrafish

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Planar cell polarity signaling (PCP) coordinates the orientation, structure and movement of cells within a plane of a tissue during development. PCP activity is based on the asymmetric localization of its core components on cell membranes. Understanding pathway dynamics has been challenging, as embryos are sensitive to exogenous fluorescent PCP reporter levels. To decipher how asymmetric component localization is translated into polarized cell behavior, and to study endogenous PCP activity in living zebrafish embryos, we have used CRISPR/Cas9 gene editing to target a superfolder GFP linker cassette (sfGFP) onto the N-terminus of Vangl2, a core PCP regulator. Fish trans-heterozygous for *sfGFP-vangl2* and loss-of-function alleles are slightly shorter but morphologically normal as embryos, and viable and fertile as adults, demonstrating that the sfGFPl-Vangl2 fusion protein is functional. We have analyzed Vangl2 dynamics during neural tube morphogenesis, which is defective in multiple PCP mutants. In neuroepithelial cells, sfGFP-Vangl2 shows polarized anterior membrane localization, which was previously observed using transgenic Vangl2 reporter constructs. However, we have performed detailed single-cell level analyses using cell transplantations, which have revealed dynamic Vangl2 localization into smaller membrane domains. We have also taken advantage of zGrad, a GFP-specific protein degradation tool, to manipulate sfGFP-Vangl2 protein levels. Transgenic ubiquitous degradation of sfGFP-Vangl2 phenocopies the *vangl2* mutant phenotype. Moreover, targeted degradation specifically in floor plate cells, after basal body (BB) polarization, disrupts BB positioning. This suggests that Vangl2 is required to maintain floor plate cell polarity. Furthermore, conditional degradation of Vangl2 in multiciliated cell lineages gives rise to embryonic axial curves and adolescent idiopathic scoliosis, highlighting the role for PCP in spine homeostasis. In the future, conditional Vangl2 protein degradation strategies will permit analysis of PCP function across diverse embryonic, juvenile and adult contexts.

226 - Zebrafish tp53 R217H and R242H Mutants Recapitulate Li-Fraumeni Syndrome Phenotypes and Develop Tumors

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Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome associated with a highly penetrant and diverse tumor spectrum characterized by germline mutations in the *TP53* tumor suppressor gene. In addition to a high incidence of cancer, individuals with LFS experience early tumor onset, often during childhood, with a cumulative lifetime cancer risk of 68% in males and 93% in females. LFS is a complex disease where current surveillance protocols are not tailored to a patient's genotype for early tumor detection. Preclinical animal models representing the genomic landscape observed in LFS offer the promise of defining specific tumor-driving mechanisms and provide a platform for testing molecularly targeted therapies.

We have generated two *tp53* zebrafish point mutants, R217H and R242H, (representing common human LFS constitutional lesions, R248H and R273H) and obtained a *tp53* null mutant line (Langenau lab). These point mutants recapitulate LFS phenotypes: reduced expression of p53 target genes; resistance to p53-induced apoptosis; and displayed higher levels of proliferation following p53 pathway induction compared to *casper* zebrafish. As adult fish, they develop tumors beginning at 6 months post-fertilization with the majority showing histological similarities to human sarcomas. *tp53* R242H mutants experience an earlier tumor onset and greater tumor incidence than *tp53* R217H and null mutants, suggesting that this mutation is more aggressive. Additionally, the R217H and R242H mutants present with a divergent tumor location distribution and skewed sex ratios, indicating different tumor-driving mechanisms.

RNA sequencing and DNA methylation analyses are in progress to elucidate mutation-specific mechanisms and pathways, which can be leveraged to identify targeted therapeutics. Finally, the *tp53* R217H and R242H mutants will be utilized as a preclinical platform for *in vivo* characterization of potential therapeutic compounds that restore normal p53 function (using the restoration of p53-induced apoptosis as a readout) which may delay/prevent tumor onset.

227 - Ionic regulation in zebrafish – recent progress on the transport and compensatory mechanisms

Raymond Kwong[1]

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Zebrafish inhabit a hypo-ionic environment and therefore are challenged by a continual loss of ions to the water. To maintain ionic balance, they actively take up ions (e.g., sodium, calcium, chloride) from the water and reduce passive ion loss. Ion-transporting cells (ionocytes) are thought to be the major cell types responsible for ion uptake. The current model for ion uptake in zebrafish involves multiple subtypes of ionocytes which coordinate the absorption of specific ions. In this presentation, I will discuss our current understanding of the functional involvement of various ionocyte subtypes in zebrafish. I will also discuss recent progress on understanding the flexibility of the ion transport systems and the potential pathways involved in the compensatory regulation of ions during exposure to environmental stressors.

228 - Unbiased High-Throughput Phenotyping of Danio Rerio for Developmental and Genetic Screening

Maxwell Zheng[1], Pratik Bokadia[1], Gregor Horstmeyer[1], Aurelien Begue[1], Paul Reamey[1], Veton Saliu[1], Jennifer Bagwell[2], Mark Harfouche[1], Robyn Tanguay[3], Michel Bagnat[2], John Lam[3]

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Due to its transparent nature, large clutch size, and genetic malleability, the zebrafish (Danio rerio) is a robust developmental model organism. Live imaging of zebrafish at an early stage of development (1-5 days post fertilization) is essential in obtaining many developmental metrics. However, high-throughput screening in different imaging modalities (infrared, visible brightfield, and fluorescence) is limited by a system's overall resolution, field-of-view, and physical compatibility with standard 96-well-plates that are commonly used for screening zebrafish in these early developmental stages. Here we demonstrate how the recently developed micro-camera-array-microscope (MCAM) imaging system overcomes these limitations. By leveraging the combination of a micro-camera-array, multimodal illumination, and an integrated software suite, MCAM performs rapid screenings, such as the embryonic photomotor response (EPMR) assay and fluorescence quantification, of wild-type zebrafish in a 96-well-plate within seconds. The MCAM simultaneously acquires high frame-rate, configurable between 20-120 frames per second, and high resolution video over an entire well-plate while managing the control of the illumination subsystem to ensure that the embryos are imaged using infrared light (850nm), and excited using visible light. MCAM's software suite then analyzes embryonic activity on a per-well basis to automatically quantify common metrics such as tail coiling and twitching in a manner that is compatible with both chorionated and dechorionated embryos. Programmable controls permit users to trigger the activation of illumination and dynamically change between imaging modalities, thus streamlining the ability to run multiple phenotyping assays on MCAM. For fluorescence screening, MCAM can be configured to illuminate an entire well-plate with 440nm or 590nm LEDs and capture the emitted fluorescence signal between 510-560nm and 600-600nm in a few seconds. This technology allows for large scale fluorescent and brightfield time lapse imaging studies that will provide researchers with unprecedented spatial and temporal control, large sample sizes, integrated software, and high resolution data.

229 - Tissue-specific contribution of the unfolded protein response to the chronic ER stress-mediated apoptosis and abnormal phenotype in AXER knockout medaka fish

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The unfolded protein response (UPR) is activated to cope with the endoplasmic reticulum (ER) stress induced by the accumulation of unfolded/misfolded proteins in the ER. This activation leads to the maintenance of homeostasis in the ER. However, in chronic ER stress conditions, the UPR induces apoptosis. Previous research has studied the ER stress-mediated apoptosis but how much each pathway of the UPR contributes to this phenomenon is still unclear.In this study, we analyzed the role of each pathway of the UPR against chronic ER stress in a tissue-specific manner by using medaka fish. We generated ATP/ADP exchanger in the ER membrane (AXER) gene knockout medaka fish to evoke chronic ER stress and activate UPR pathways. We also visualized physiological ER stress and apoptosis in medaka fish and found chronic ER stress-mediated apoptosis in AXER knockout medaka fish during the developmental stage with abnormality in different tissues. Then, we investigated the effect on apoptosis and abnormal phenotypes by constitutively activating the particular pathway of the UPR.As a result, we found that the constitutive expression of XBP1(S), a transcription factor induced from the IRE1 pathway of the UPR, inhibited apoptosis and rescued the heart-specific abnormal phenotype. Interestingly, constitutive expression of the ATF6a pathway of the UPR rescued brain-specific abnormal phenotype, not in the heart. These results indicate that UPR pathways have specific roles against chronic ER stress in different tissue. This time, we will discuss the tissue-specific contribution of the UPR mechanism, and we suggest the possibility that regulation of the UPR leads to a therapeutic strategy for various chronic ER stress-mediated human diseases.

230 - pyHeart4Fish: A novel tool to identify cardiovascular phenotypes.

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In the last two decades cardiovascular diseases (CVDs) were the leading causes of death worldwide. Congenital heart diseases are the most common type of human birth defects with 1 in 100 live-born infants being affected. Consequently, it is of utmost importance to find new treatments and novel therapeutic targets for CVDs. High-throughput drug screening is a promising approach to test for novel therapeutic targets using commercially available compounds.

In this study we used a phenotype-based high-content screening approach in zebrafish to identify clinically applicable cardiovascular modulators. Using the 'Acquifer Imaging Machine' we screened a total of 1280 compounds for angiogenesis and heartbeat phenotypes. Here, we identified a major target cluster for heartbeat modulators, which targeted the glucocorticoid receptor Nr3c1, a ligand-activated transcription factor, that is involved in inflammation, metabolism and stress response. The cluster of seven Nr3c1 agonists significantly increased heart rate in zebrafish larvae at 48 hpf. In line with our results, several genome-wide association studies (GWAS) showed *NR3C1* to be associated with atrial fibrillation (AF). While previous studies already demonstrated a role of *nr3c1* in zebrafish heart development, its role in heartbeat alterations and arrhythmia remained unclear. Therefore, we developed the novel Python-based tool 'pyHeart4Fish' to quantify heart rates and contractility of atrium and ventricle independently. For each zebrafish videos of fluorescent hearts were segmented into atrium and ventricle using the AV-band. Then, changes in fluorescence intensity were fitted to two averaged sine functions using interleaved iterative approximation as implemented in the Python SciPy module (optimize.curve_fit). pyHeart4Fish offers a great advantage for studying cardiovascular function, as shown by targeting Nr3c1, which caused chamber-specific arrhythmia in zebrafish.

231 - Characterization of a novel heart-specific isl1 regulatory region in zebrafish embryos

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Zebrafish cardiogenesis relies on migration and differentiation of cells derived from two lateral heart fields. The first heart field (FHF) contributes to the main heart tube relatively early in embryonic development, while subsequent formation of chambers, cardiac conduction system as well as outflow and inflow tracts rely on continuous addition of second heart field (SHF)-derived cardiac progenitors (CPs). The *isl1* transcription factor is expressed in a variety of embryonic tissues, however in the growing heart its expression is limited to the SHF and the structures derived from this progenitor pool.

In order to find enhancers that could drive *isl1* expression specifically in the SHF, we referenced evolutionary conservation and ChIP-seq datasets deposited in public repositories. We identified 4 regions in the vicinity of the murine genomic *Isl1* locus conserved in zebrafish, and 6 more regions with active enhancer (H3K27ac) marks in the zebrafish *isl1* locus at the relevant developmental stages. To determine their ability to drive tissue-specific expression, we performed an in vivo enhancer assay. Of the 7 putative enhancers tested, 1 (I3) drove tissue-specific reporter expression in the heart in transient assay. I3 is located in an intron of the zebrafish *isl1* locus (chr5:40730686-40732102).

In a stable transgenic line, the I3 enhancer exhibited an expression pattern consistent with SHF-derived structures at 24 and 48 hpf. To further ascertain the identity of the GFP expression domain, we sorted the cells using FACS and performed a qPCR analysis. We found that at 24 hpf this cell population is enriched in *isl1* and *nkx2.5* transcripts as well as *tal1*, a vascular fate marker, which suggests its identity as SHF. I will present our ongoing functional analysis of this enhancer as our initial steps to investigate the contribution of SHF progenitors to various structures of the heart.

232 - Semi-Automated High-Throughput Cardiac Function Analysis in Larval Zebrafish (Danio rerio)

Jack Bechtel[1], Aurelien Begue[1], Gregor Horstmeyer[1], Jed Doman[1], Jennifer Bagwell[2]

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Ecotoxicology, drug toxicity, and cardiovascular disease studies regularly analyze cardiac system function in the embryos and larvae of the widely researched zebrafish (*Danio rerio*). Analysis of cardiovascular function in these small, transparent, vertebrates allows for improved understanding of the effects of environmental toxins, genetic perturbations, and pharmaceuticals on cardiac health and development.

Rapid, large-scale research using zebrafish, however, has been limited by bottlenecks in imaging of numerous zebrafish larvae making broad spectrum comparative studies and screens time consuming and expensive. Traditionally, when collecting heart-rate data, zebrafish are individually examined and heart beats are manually counted on a per fish basis, or videos of the heart are recorded at frame rates of 30 frames per second (fps) or greater, for 15-30 seconds per fish. Software exists to help automate heart-rate analysis on videos of single fish, but these programs generally are optimized for specific sample positioning and imaging modalities. These orientation restrictions require anesthesia (with tricaine) of larvae older than 3.5 dpf, and may fail to detect heart-rate in chorionated embryos (2-3 dpf). Subsequently, experimental results can be skewed, especially for more complex analyses. Sample constraints imposed by use of current cardiac analysis software can lead to discarded results, loss in accuracy, and increased data collection time to reach statistical power.Here we describe a method for high-throughput cardiac data collection using micro-camera-array-microscope (MCAM) imaging technology and semi-automated artificial intelligence assisted analysis of larval zebrafish cardiovascular metrics including heart-rate, stroke volume, and cardiac output. By combining MCAM technology and automation in analysis, our methodologies substantially increase data collection speeds by simultaneously imaging up to 24 wells and reduce both user subjectivity in analysis and total data analysis time.

233 - Investigating the role of notochord defects in the idiopathic scoliosis-like phenotype in ptk7a zebrafish mutants

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Idiopathic scoliosis is a prevalent spinal deformity affecting around 3% of children. Its etiology is multifactorial and remains poorly understood. Zebrafish has been widely recognized as a powerful model for studies of IS mainly because of its similarity to humans concerning the mechanical loading along the spinal column. One particular mutant at a planar cell polarity gene called *ptk7a* developed 3D curvature in the absence of apparent vertebral malformations resembling human IS. The pathogenesis of IS in these mutants was demonstrated to be caused by defective CSF flow, a disorganized Reissner fiber and dysregulated downstream Urp signaling.

We created a new *ptk7a* CRISPR knockout that developed an IS-like phenotype in zygotic homozygotes as previously described. Careful analyses of this mutant revealed a shorter body axis, a wider notochord and curvature defects as early as 3dpf before vertebrae formation. The notochord serves as the axial skeleton of the embryo and as a scaffold for spine formation. The pressure exerted by notochord vacuoles on the surrounding tissues is essential for lengthening and the straightening of the body axis. We hypothesized that loss of function of *ptk7a* leads to a decrease in notochord pressure that would contribute to the curvature defects detected in *ptk7a* mutants. To investigate this hypothesis, we are currently conducting a kinematics study using the open source toolbox called DeepLabCut to indirectly measure the body stiffness in *ptk7a* mutants at 2dpf. The trunk movements pattern at a specific frequency was comparable between different embryos. More data samples and further analyses are being performed to better characterize morphological and temporal patterns as well as the dynamic of body movements in *ptk7a* mutants.

Our study will help uncover a novel pathogenic mechanism affecting notochord development in IS and hence provide novel mechanistic insights into the pathogenesis of human IS.

234 - Fate-biased regeneration – ascl1a disruption accelerates retinal ganglion cell regeneration kinetics

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Regeneration has largely been studied using paradigms requiring multiple tissues or cell types to be replaced. In these contexts, the regenerative process is often said to “recapitulate development”, exhibiting many parallels with tissue histogenesis. In contrast, paradigms involving the selective ablation of discrete cell types can elicit “fate-biased” regenerative processes, where progenitor cells preferentially give rise to the lost cell type. How “fate-biased” regeneration is regulated is unknown. To begin to address this question, we are performing a CRISPR/Cas9-enabled reverse genetic screen to identify factors that promote or inhibit retinal ganglion cell (RGC) regeneration following selective RGC ablation. Interestingly, disruption of the basic helix-loop-helix transcription factor *ascl1a* – a gene previously shown to be required for regeneration following widespread retinal cell loss or traumatic injury – resulted in accelerated RGC replacement kinetics. This is in keeping with Ascl1 acting to inhibit RGC fate during development in mice and suggests that loss of *ascl1a*, in the context of selective RGC ablation, serves to bias retinal progenitors toward RGC production during regeneration. In an effort to identify transcription factors associated with RGC fate bias, we are using single-cell RNA sequencing to compare retinal progenitor profiles in control and *ascl1a*-disrupted retinas following selective RGC ablation. Preliminary results from these studies will be discussed.

235 - Nkx3.1 is a transcriptional driver of pericyte differentiation

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Pericytes wrap around microvasculature and have been shown to impart vasoactivity from early stages in development. While there is some knowledge about the differentiation markers of pericytes, only little is known about the drivers that specify multipotent cells towards the pericyte lineage. Here with the help of a transgenic reporter line we show that, *nkx3.1* expressing cells are associated with brain vasculature and co-express pericyte differentiation marker *pdgfrβ.* Early ablation of these *nkx3.1*$^{+ve}$ cells leads to brain hemorrhage, lack of pericytes and mispatterned cerebral vasculature. Brain vessel-associated *nkx3.1*$^{+ve}$ cells migrate and divide like pericytes. Lineage tracing experiments show that *nkx3.1*$^{+ve}$ cells have origins in both mesoderm and neural crest. Interestingly, *nkx3.1* gain-of-function during early developmental stages leads to an increase in the number of cerebral pericytes, while its maternal loss-of-function leads to lack of pericytes and malformed brain vasculature, hinting towards the role of Nkx3.1 as an early driver of pericyte cell fate. Taken together, we describe a novel player in the pericyte developmental cascade that may regulate transcription in precursor cells from different germ layers to become brain pericytes. Understanding the lineage and gene expression in pericytes will contribute towards a better understanding of vascular and stroke disorders associated with pericyte malfunction.

236 - Bnip3lb regulated mitophagy maintains the embryonic pool of hematopoietic stem cells by protecting them from ROS induced apoptosis

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In contrast to the established detrimental effect that elevated Reactive Oxygen Species (ROS) has on adult hematopoietic stem and progenitor cells (HSPCs), our previous work has shown that ROS promotes HSPC formation in the dorsal aorta. In order to elucidate the mechanism and developmental timing of this shift in the role of ROS in HSC biology we sought to examine the initiation and regulation of mitophagy in these cells, this being a key process by which adult HSCs regulate ROS by removing and recycling damaged mitochondria. Our recent data shows that oxidative stress begins to limit HSPC numbers immediately upon their colonization of the secondary hematopoietic niche, the caudal hematopoietic tissue (CHT, the zebrafish analog of the fetal liver), and live imaging with Tg(ubi:mitoGR), a fluorescent transgenic reporter for mitophagy, shows that this coincides with the onset of mitophagy in HSPCs. Mitophagy can be induced via multiple pathways but our scRNAseq and in situ-hybridization analyses suggest that it is induced in these cells via the *bnip3lb* receptor, a NIX homolog. We validated the role of *bnip3lb* via morpholino directed knockdown and found that mitophagy was reduced alongside HSPC marker expression in the CHT, whereas induction of mitophagy by small molecules or heat shock inducible ΔOTC mutants elevated detection of HSPC markers runx1 and cmyb by WISH, and CD41:GFP by flow cytometry. Mechanistically, we found that *bnip3lb* knockdown increases ROS levels, and that the reduction in HSPC numbers can be rescued by chemically reducing ROS. Finally, we demonstrate that the enhancement in ROS levels caused by reduced mitophagy appears to increase apoptosis in the CHT region and alter HSPC fate. We therefore propose that developmentally programmed mitophagy directed by *bnip3lb* is responsible for protecting proliferative embryonic HSCs from the harmful effects of oxidative stress while the HSC pool expands.

237 - The extracellular matrix proteins Fibronectin and Laminin promote anterior neural tube closure in zebrafish

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During primary neurulation, a flat neural plate folds to form a closed tube, which then develops into the brain and spinal cord. In humans, failures in neurulation cause life threatening neural tube defects such as anencephaly and spina bifida. In all vertebrates, there is an extracellular matrix between the mesoderm and the basal side of the developing neural tube. Mesoderm is required for anterior neural tube closure in mice and zebrafish. We found that the extracellular matrix is required for anterior neural tube closure in zebrafish and that biosynthesis of the extracellular matrix proteins *fibronectin (fn)* and *laminin* (*lam*)may be one of the roles for mesoderm in anterior neurulation. Embryos deficient for either *fn1a* or *lamc1* alone had closed anterior neural tubes. In contrast, approximately 50% of *fn1a;lamc1* double mutants had an open forebrain neural tube. In the hindbrain of wildtype embryos and most *fn1a*;*lamc1* double mutants, *ephrin receptor A4a* (*ephA4a*) was expressed in rhombomeres 1, 3, and 5. Consistent with the neural tube abnormalities found by Araya et al. (*Developmental Dynamics* 245:580–589, 2016), *ephA4a* expression revealed that a small subset of double mutants displayed a “twisted brain” phenotype. This twisted brain phenotype is also found in mesoderm-deficient embryos such as those that lack Nodal signaling. A survey of expression of *fn* and *lam* genes during neurulation stages found expression in the axial mesoderm and to a lesser extent in the neuroectoderm. Together, these data are consistent with a model in which mesoderm is the major source of two key components of the extracellular matrix that lies between the mesoderm and the neuroepithelium. This extracellular matrix promotes closure of the anterior-most region of the neural tube and normal morphology of the developing brain. This project is supported by grant 2R15HD068176-02 to JOL.

238 - Model of human FAM177A1 deficiency reveals defects in ER-Golgi transport pathway

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The Undiagnosed Diseases Network (UDN) is an NIH-supported research collaborative that seeks to provide answers for patients and families affected by unknown conditions. A 5-year-old UDN patient presented with global developmental delay, macrocephaly, diffuse hypotonia, and autism spectrum disorder soon after birth, with seizures appearing later. The hypothesis in this case is an autosomal recessive condition, as the patient harbors a compound deletion removing large portions of *FAM177A1*, encoding a small protein of unknown function. Thus, we are deploying zebrafish to determine if *FAM177A1* is a human disease gene. We first overexpressed FAM177A1 and Fam177a1a fused to mNeongreen in zebrafish embryos and found that the proteins are localized in the Golgi apparatus and possibly endoplasmic reticulum (ER), suggesting that the protein is involved in the function of ER and the Golgi complex. Given that the human mutations likely represent null alleles, we generated two geneless alleles, removing large portions of the coding region. Since there is a *zgc:153383/fam177a1b* as a paralogue of *fam177a1a* and the two genes could be functionally redundant, we also generated two geneless alleles of *fam177a1b* in the *fam177a1* homozygote background. Although both *fam177a1a* and *fam177a1a;fam177a1b* double homozygotes are viable and fertile, MZ*fam177a1a*mutants have 9% shorter and the MZdouble mutants have 15% shorter body length compared to wild-type embryos at 1 day post fertilization (dpf). The shortened body phenotype persists at 5 dpf even though this phenotypic difference decreases with time. [LSK1] We have also performed bulk RNAseq and unbiased metabolomic analyses using the mutants. Current analyses support misregulation of apoptosis, inflammation, and cholesterol pathways. Therefore, our studies provide that FAM177A1 is a novel human disease gene and we hypothesize its molecular function is critical for the ER-Golgi transport system, which will be an important therapeutic target.

239 - Disruption of grin2B, an ASD-associated gene, produces social deficits in zebrafish.

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Autism Spectrum Disorder (ASD), like many neurodevelopmental disorders, has complex and varied etiologies. Advances in genome sequencing have elucidated multiple candidate genes associated with ASD, including dozens of missense and nonsense mutations in the NMDAR subunit GluN2B, encoded by *GRIN2B*. NMDARs are glutamate-gated ion channels with key synaptic functions in excitatory neurotransmission. How alterations in these proteins impact neurodevelopmental is poorly understood, in part because knockouts of GluN2B in rodents are lethal. Here, we establish zebrafish as a model to study GluN2B, as zebrafish GluN2B displays similar structural and functional properties to human GluN2B. Using CRISPR-Cas9, we generated fish lacking all functional GluN2B, notated as *grin2B*$^{-/-}$, and surprisingly found that they survive into adulthood. Given the prevalence of social deficits in ASD and the viability of the zebrafish model, we assayed social preference in the *grin2B*$^{-/-}$ fish. As found previously, wild-type fish showed a strong social preference by 3 wpf, qualitatively described as spending more time near an age-matched conspecific. In contrast, *grin2B*$^{-/-}$ fish exhibited significantly reduced social preference. Lack of GluN2B, and not just general NMDAR dysfunction, is driving this phenotype, as frameshift mutations in other NMDAR subunits do not generate social deficits. To test whether the lack of GluN2B resulted in a broader disruption of neurodevelopment, we assayed other basic behaviors. *grin2B*$^{-/-}$ larvae do not show alterations in spontaneous or photic-evoked movements, are capable of prey capture and exhibit learning capabilities. Whole-brain imaging of *grin2B*$^{-/-}$ larvae did not show extensive changes in brain size, nor amounts of excitatory or inhibitory neurons. However, the greatest changes in brain size and inhibitory neurons were found in the subpallium, a region linked to ASD in humans. Together, these findings are an initial step in understanding the role of GluN2B in ASD etiologies and establish an *in vivo* model for future studies.

240 - Electricity of the Embryo: Visualizing bioelectric patterns during early embryonic development in Danio rerio

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Changes in the membrane potential of non-excitable cells, known as bioelectric signaling, areemerging as a “potentially” important regulator of regeneration, yet less is known about its rolеduring vertebrate embryogenesis. Using both voltage sensitive dyes and a new transgenicreporter, we investigated whether changes in membrane potentials could be discerned acrossdistinct regions of the zebrafish embryo, prior to and during axis development stages. Weconducted live imaging of the differential patterns of depolarization and hyperpolarization duringgastrulation by leveraging Bruker’s MuVi-SPIM fluorescent light-sheet microscope. First, wetested two fluorescent voltage reporter (FVR) dyes, DiBAC and Rhodamine 6G, whichdemonstrated distinct cell populations exhibit either depolarized or hyperpolarized membranepotentials, respectively. Depolarized, DiBAC labeled cells were present in the epiblast of thegastrula with a maximal intensity at the shield that diminishes toward the ventral side of theembryo. In contrast, Rhodamine 6G labeled hyperpolarized cells were restricted to the cells ofthe involuting marginal zone and were retained within the internalizing mesendoderm cells. Wedeveloped a stable transgenic line that ubiquitously expresses the genetically encoded voltageindicator (GEVI) Marina, whose relative fluorescence increases in response to membranedepolarization. Marina transgenic embryos showed the most significantly intense depolarizationsin those cells immediately at the dorsal-most and ventral-most sites of ingression, and this GEVIline also confirmed an overall graded pattern of depolarization across the dorsoventral axis of theepiblast. This work demonstrates that distinct bioelectric patterns do exist across the gastrula andit sets the foundation for testing the functional relevance of these patterns to axis determination.Supported by the National Institute of Health (NIH) [HD060023] R15, the Arnold and MabelBeckman Foundation for advanced light sheet microscopy, and Smith College.

241 - Critical period plasticity in a zebrafish sensorimotor circuit

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Critical periods are developmental windows when sensory experience is essential for normal circuit structural and functional development. Here we demonstrate a novel visual critical period in larval zebrafish that shapes the performance of a visuomotor behavior. Most sensory modalities exhibit a postnatal interval of critical period plasticity, and disruption to sensory input during this time can lead to sustained defects in circuit function. Despite the biological and biomedical importance of critical period plasticity, the neuron level changes in circuit structure and function remain incompletely understood, and major obstacles to addressing these gaps in established mammalian models include the need for invasive neurophysiological and vast circuit complexity. In zebrafish, we have shown that the loss of environmental illumination triggers a light-search that utilizes persistent same direction turning, which an individual's preference is sustained as a consistent motor asymmetry. What remained unknown is how an individual's motor asymmetry was imposed. We now demonstrate that visual experience shapes behavioral performance by determining an individual's turn direction during light-search. This plasticity is dependent on visual experience and is developmentally restricted, showing hallmarks consistent with well-established mammalian critical period models. Functional imaging of genetically defined neurons in a rostral lobe of the posterior tuberculum show asymmetric light driven responses, consistent with motor asymmetry, establishing a potential neuronal basis for sensory driven plasticity. Last, we show that GABAergic signaling maintains critical period duration. This new model for critical period plasticity provides robust behavioral readouts for sensory modulation and tools for in vivo characterization. Using this model, we can determine how sensory experience modulates neural structural and functional development.

242 - Canonical Wnt signaling regulates the development and regeneration of lateral line hair cells.

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The zebrafish lateral line system has proven to be an invaluable model for the study of mechanosensory hair cell biology. The morphological and genetic profiles of lateral line hair cells are striking similar to the mechanosensory hair cells of the auditory and vestibular systems, with the advantage of being easily accessible to analysis and manipulation. In addition, lateral line hair cells in the zebrafish possess a robust ability to regenerate throughout the lifespan of the animal, which contrasts with the lack of regeneration seen in the mammalian inner ear. The lateral line system is composed of sensory organs, called neuromasts, which contain mechanosensory hair cells and surrounding populations of support cells, which proliferate and differentiate to replace damaged cells. Work from multiple labs has demonstrated that Wnt signaling is critical for lateral line development and regeneration. To further examine the role of the Wnt pathway in the lateral line, we used two zebrafish mutant lines which differentially alter the Wnt pathway. A mutation in the transcription factor *lef1* results in decreased Wnt activity and a mutation in the receptor *kremen1* leads to increased Wnt signaling. Together, we are using these mutant lines to refine our understanding of the role of the Wnt pathway in regulating how hair cells initially develop in the lateral line and how they are replaced following damage. This work will allow us to better understand the biology of mechanosensory hair cells, and how regeneration might be promoted following damage.

243 - lrwd1 is involved in the control of locomotion during zebrafish early development

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The LRWD1 (Leucine-Rich repeats and WD repeat domain containing 1), which the expression was low in testis tissue of patients with spermatogenic dysfunction, was also found responsible for the microtubule polymerization during spermatogenesis.

The locomotion of the zebrafish embryo requires coordination between neurons and skeletal muscle. Besides, microtubule polymerization has also been reported to be required for the development of these two tissues. Therefore, we are interested to see if lrwd1 plays a role in controlling locomotion during zebrafish early development.

We generated lrwd1 mutant lines by CRISPR/Cas9 technique and detected several locomotion markers. Total distance moved, the velocity, cumulative duration as well as mobility were found significantly decreased after lrwd1 knockout.

We are continuing to investigate the detailed mechanisms of how lrwd1 is involved in regulating locomotion at the level of development both in the neuron and skeletal muscle.

244 - Coronary regulation of cardiac regeneration

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Alterations in coronary network formation and deficient perfusion of the cardiac muscle lead to myocardial injury and dysfunction. Efficient revascularization after cardiac damage is essential to support tissue repair and limit scarring. Contrary to the non-regenerative adult human heart, the zebrafish heart exhibits a remarkable ability to regenerate. The injured zebrafish heart activates a rapid and efficient coronary revascularization response.

We found that regenerating coronaries form a vascular scaffold that supports cardiomyocyte replenishment. Transcriptomic analyses of coronary endothelial cells allowed us to identify angiocrine factors regulating different aspects of cardiac regeneration.

Our results highlight the importance of coronaries during heart regeneration beyond their role as a transport system and identify pro-regenerative angiocrines. Simulating these processes in the injured mammalian heart should help its healing.

245 - DNA REPAIR DEFICIENT ZEBRAFISH MODELS FOR THE INVESTIGATION OF NEUROLOGICAL DISEASE

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An individual cell acquires approximately 1 million DNA lesions per day. Most lesions are resolved with little or no negative effects. Nonetheless, mutations that render important DNA repair constituents non-functional can result in debilitating diseases. Despite the advancements made through cell culture analysis, animal models are required to enable the study of redundancy and interactions at an organismal level. The small size of the zebrafish, rapid reproduction and ease of genetic manipulation has led them to be a less expensive alternative for mammalian systems.

A new zebrafish DNA break repair model, an RNaseH2a mutant, is expected to have a defect in the removal of ribonucleotides from DNA. They are also likely to be defective in the removal of R-Loops, which are speculated to be the drivers of neurodegenerative diseases such as Aicardi Goutières Syndrome (AGS).

Highly surprisingly, we found that homozygous mutants are phenotypically normal at adulthood, unlike previous AGS *in vivo* models. However, their resulting offspring show reduced development, increased ribonucleotide incorporation and upregulation in key inflammatory markers, resulting in both maternal and paternal embryonic lethality. Despite remaining without any apparent phenotype, homozygous adults still show an accumulation of ribonucleotides in both the brain and testes, that is not present in early development.

It is hypothesized that the homozygotes resulting from heterozygous parents may have a compensatory mechanism that allows them to survive, without the function of RNaseH2a. Such a mechanism may not be activated or is overwhelmed by the inherited ribonucleotides in their offspring. We are aiming to identify such compensatory mechanisms to enable a greater understanding of, and treatment potential for, patients with AGS.

246 - A role of pannexin-1 channels in an experimental zebrafish model for Parkinson’s Disease

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Pannexins (*Panx*) are a group of channel proteins abundantly expressed in the central nervous system and many vertebrates' tissues. A family member, *Panx1*, plays an essential role in ATP and glutamate release. The *Panx1* channel is implicated in epilepsy, stroke, trauma, inflammation, or pain. A role in Parkinson’s disease has been proposed, but details thus far are lacking. Here, the locomotor activity and visual-motor response of wild-type Tupfel longfin and *panx1a* knockout zebrafish larvae were tested after treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is known to induce Parkinson's disease-like effects in the zebrafish by blocking the Complex I in mitochondria. Short-term treatment with MPTP caused a decrease in the movement of the larvae. Locomotor activity worsened after incubation with *Panx1* inhibitors probenecid and mefloquine. The analysis of biomarker expression levels demonstrated the transcriptional upregulation of genes in *panx1a* knockout larvae with known roles in mitochondrial health and dopamine synthesis. A transcriptome analysis authenticated a broad dysregulation of metabolic processes, including those affecting mitochondrial health. It was concluded that *Panx1a* channels play roles in maintaining metabolic homeostasis in the zebrafish. We propose that loss-of-function of *Panx1a* contributes to the severity of outcomes in the MPTP model of Parkinson's disease.

247 - The A-type lamin, LaminL3, maintains genome stability by coordinating DNA replication timing with mitosis

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Reduced expression of Lamin A, a major component of the nuclear matrix, is associated with increased malignant potential of tumor cells. The anti-tumorigenic role of Lamin A has been attributed to its contribution to nuclear stiffness, which protects nuclei from mechanical rupture and consequent DNA damage. We tested whether shear stress is necessary for genome instability in the absence of the A-type Lamin expressed in early zebrafish embryo. Mutant embryos lacking LaminL3 were analyzed during cleavage, a period of mitotic divisions that simply subdivide the embryo in the absence of gene expression, cell movement, or mechanical deformation of cells. Whereas nuclear envelopes are functionally intact in mutant embryos, chromosome replication is delayed and continues even as mitosis proceeds, leading to bridged and fragmented chromosomes that produce micronuclei. LaminL3 is required to coordinate DNA replication timing and mitosis. In the absence of nuclear deformation or mechanical stress, loss of this A-type Lamin in replicating cells leads directly to genome instability, a hallmark of cancer.

248 - A SNP on Chromosome 25 in synaptotagmin 7a is tightly linked, but not causative, for the acoustically hypersensitive phenotype in escapist zebrafish mutants

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Behavioral thresholds are the point at which stimuli are sufficient to elicit a response from an organism. Proper establishment of baseline behavioral thresholds during development is critical for proper responses to the environment, including for threat detection. The acoustic startle response is a highly conserved behavior that demonstrates a species specific, baseline threshold as well as acute regulation of the threshold. Dysregulation of startle threshold is a hallmark of a variety of neurodevelopmental disorders, but despite its clinical and biological significance, the genetic and circuit mechanisms underlying establishment of the baseline startle threshold are unknown. Zebrafish are an excellent model organism for studying establishment of the acoustic startle threshold due to the conserved behavior and underlying circuits of the acoustic startle response among vertebrates. Through a forward genetic screen, the Granato lab identified five zebrafish mutant lines that exhibit a lowered baseline acoustic startle threshold (Marsden et al, 2018). Using RNA sequencing linkage analysis, we identified a SNP on Chromosome 25 that is tightly linked to the *escapist* acoustic hypersensitive phenotype. Using the CRISPR/Cas9 system, we generated independent predicted null mutations in *synaptotagmin 7a*, the gene in which the identified *SNP* resides. Through complementation testing, we conclude that *syt7a* is unlikely to be the gene affected resulting in the *escapist* hypersensitive phenotype. Further characterization of the *escapist* line using the *SNP* to identify mutants and siblings reveals that mutants do not exhibit behavioral hypersensitivity in other visual, auditory, or kinematic behaviors tested at 6dpf, suggesting the phenotype is specific to the regulation of the acoustic startle threshold. Currently, I am utilizing whole brain activity mapping to identify brain regions that are up or down regulated in *escapist* mutants. Overall, this project will provide insight on potential molecular and circuit mechanisms underlying establishment of the acoustic startle threshold.

249 - Function and plasticity of perivascular fibroblasts in development and regeneration

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Fibroblasts play an important role in maintaining tissue integrity by secreting components of the extracellular matrix and initiating response to injury. Recent advances in single-cell RNA sequencing have revealed a high level of heterogeneity in the fibroblast population from different tissues. However, the function and plasticity of fibroblasts remain poorly understood. Using zebrafish as a model, we have identified sclerotome-derived perivascular fibroblasts as a novel population of blood vessel associated cells. Combining live imaging, cell ablation and genetic mutants, we show that perivascular fibroblasts play dual roles in vascular stabilization where they establish the ECM around nascent blood vessels and function as pericyte progenitors. To examine the function of perivascular fibroblasts beyond early vascular development, we develop a tendon regeneration model. Cell lineage tracing reveals that laser-induced tenocyte (tendon fibroblast) ablation can be quickly regenerated by collagen-expressing fibroblasts. Using live imaging and single cell clonal analysis, we demonstrate that perivascular fibroblasts are actively recruited to the injury site where they generate new tenocytes. Strikingly, other neighboring fibroblasts derived from the same sclerotome lineage, including uninjured tenocytes, show no regenerative response, highlighting the functional heterogeneity within fibroblast populations. Moreover, pericytes do not respond to tenocyte ablation, suggesting that perivascular fibroblasts lose their regenerative capacity upon further differentiation. Together, our work demonstrates that perivascular fibroblasts initially function to stabilize nascent blood vessels during development and are later retained as tenocyte progenitors to facilitate tissue injury repair.

250 - A Tale of Two Tecti: Characterizing Cell Types in the Optic Tectum of Larval Zebrafish

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Located within the dorsal midbrain, the optic tectum (OT) in zebrafish is responsible for receiving multimodal sensory input and directing complex behaviors as early as 5-7 days post fertilization (dpf). Despite excellent genetic and functional characterization efforts, a comprehensive OT cell catalog during this oft-studied behavioral timeframe is largely unavailable. Using single-cell RNA sequencing, we surveyed 13,320 tectal cells (7dpf) and identified 25 molecularly distinct populations, many of which have not been previously described. We further characterized the cell type, neuronal identity and potential circuitry (if applicable), and developmental state of each identified population. At 7dpf, mature tectal neurons primarily demonstrate GABAergic activity, although several glutamatergic populations are also present. Additionally, through the expression of *slc6a9* (GLYT1), we identified a small population of glycinergic neurons, which were previously considered absent from the OT. Interestingly, the tectum is often considered functionally mature at 7dpf; however our results, which combine RNA velocity, Gene Ontology, and differential expression analysis, show it is still developing at this stage. To corroborate this, we also describe a population of *her4*$^{+}$/*robo4*$^{+}$ cells that we hypothesize to be intermediate neural progenitors. Therefore, despite the complex behavior displayed during this larval stage, we note developmental finetuning of the tectum is still underway. Finally, we provide the transcriptomes of all populations, and demonstrate the ability to infer genetic profiles of previously described tectal cells, as well as inform future targeted genetic studies. To our knowledge, this is the first single-cell RNA sequencing study that exclusively characterizes the optic tectum.

251 - Expanding the zebrafish community Cre resource: endogenous hand2 and mpeg1.1 Cre/CreERT2 drivers for mesoderm and macrophage lineages

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Until recently, methods to create gene-specific lineage reporters and functional genomics tools were limited by our ability to molecularly define and clone gene-regulatory elements. With CRISPR-Cas9, we now can unlock previously inaccessible cell lineages by targeted integration of Cre into any gene of interest. To expand the zebrafish community's Cre/*lox* resources, we used our GeneWeld CRISPR-Cas9 knockin strategy to generate *2A-cre/creERT2* driver lines in *hand2* and *mpeg1.1*, markers of select mesoderm and macrophage/microglia lineages, respectively. *2A-cre/creERT2* with a linked eye lens-specific secondary marker was targeted to two CRISPR gRNA sites in each gene. The first site was located early in the 5' region of the coding sequence, the second site at the 3' end before the translation stop codon. Independent 5' and 3' *2A-cre* and *2A-creERT2* targeted integration lines were established for *hand2*. Preliminary analysis of Cre recombinase activity using ubiquitous *loxP*-based reporters showed that *hand2-2A-cre* faithfully labeled the expected cardiac, branchial arch, and mesothelial lineages in the embryo. In larva, *hand2-2A-cre* marked hepatic stellate cells in the liver and visceral peritoneum in the intestine. Treatment of the *hand2-2A-creERT2* line with 4-OHT lead to efficient recombination and refined lineage labeling that mirrored the pattern observed with *hand2-2A-cre*, with no recombination in the absence of 4-OHT. RT-qPCR of homozygous *hand2-2A-creERT2* embryos indicated the 3' integration did not significantly affect *hand2* transcript levels. Initial analysis of a 5' target site *mpeg1.1-2A-cre* line revealed labeling of embryonic macrophages and microglia at different stages. Our latest results validating and characterizing the activity of the *hand2* and *mpeg1.1 2A-cre/creERT2* lines will be presented. Together, this set of Cre and CreERT2 drivers establishes critical new tools to investigate mesodermal and immune cell lineages and provides a framework to generate additional drivers of utility to the community.

252 - Maternal control of developmental progression from the egg through the first cell cycle in zebrafish.

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Maternal-effect genes encode factors necessary for developmental events from fertilization to the first cell divisions during embryogenesis. Using a forward genetic screen, we have isolated three mutations of particular interest whereby females produce eggs that activate development but fail to develop beyond the one-cell stage. Mutants *atomos* and *indivisible* have mutations that are predicted to generate truncations in the ubiquitin protein ligase Ubr3 and the serine protease Tmprss4b, respectively. Eggs from *atomos* females can be fertilized, but the first mitotic cell cycle is arrested at metaphase, suggesting that Ubr3 is necessary for progression from metaphase to anaphase at this stage. Eggs from *indivisible* females exhibit irregularities in fertilization, and those that are fertilized divide abnormally. Interestingly, the human ortholog for the affected gene in *indivisible, Tmprss4*, has been implicated in processing the SARS-CoV-2 spike protein. This suggests that Tmprss4b influences a broad array of biological functions throughout the lifespan of vertebrates. Eggs from a third maternal-effect mutant, *volcán,* are not fertilized. These unfertilized eggs generate irregular dynamic protrusions, producing grape-like clusters of spherical aggregates that emanate from the animal pole, reminiscent of polar bodies. These eggs also contain numerous ectopic spindle-like microtubules, which resemble acentriolar spindles. This suggests that the gene affected in *volcán* inhibits the production of acentriolar microtubule spindles in the egg. We are currently working on identifying the mutant gene for *volcán*. Together, our mutant analyses are revealing novel regulators of the transition from egg to embryo in vertebrates.Funding: Penn Provost’s Postdoctoral Fellow, NIH R21HD094096

253 - Pharmacogenetic and whole-brain activity analyses uncover integration of distinct molecular and circuit programs that drive learning

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Our sensory environments are incredibly complex: we must decide, on a moment to moment basis, which stimuli require our attention, and which should be ignored. We are able to successfully navigate that complexity through appropriately establishing, maintaining, and modulating our sensory thresholds. In particular, habituation is a simple behavioral plasticity mechanism through which animals dynamically increase their response thresholds for repetitive sensory stimuli. Although it is a very simple form of plasticity, forward genetic and chemical screens using the larval zebrafish have identified a multitude of molecular pathways and neurotransmitter signaling mechanisms crucial for habituation. A central question now is whether distinct molecular mechanisms act independently or intersect with one another at the molecular, cellular, or circuit level. To address this question, we used the larval zebrafish system in combination with a set of five genetic mutants and three neurotransmitter receptor antagonists that impair habituation. We conducted pharmacogenetic interaction analyses to determine whether individual molecular regulators of habituation act by regulating signaling through NMDA, dopamine, or glycine receptors. Through this approach, we clustered our eight habituation regulatory mechanisms into modules: we identified two molecular regulators that act through the suppression of dopamine signaling, one that positively regulates NMDA and dopamine signaling, and two that act independently of the three tested neurotransmitter systems. Interestingly, these results highlight that dopamine may bidirectionally modulate habituation learning. Finally, we performed whole-brain activity mapping to examine similarities and differences between the brain activity signatures associated with each pathway module. Combined, our results define a core set of distinct modules that act in concert to regulate learning-associated plasticity and provide compelling evidence that even simple learning mechanisms in a compact vertebrate brain are regulated by a complex and overlapping set of molecular and circuit pathways.

254 - Defining the role of retinoic acid in regulating Cx43 oscillations during skeletal patterning.

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Patterning of the vertebrate skeleton is a complex process, regulated by signaling pathways and small molecules. To better understand the pathways responsible for skeletal patterning, we utilize the zebrafish as a model system due to its robust ability to regenerate the bony structures in the caudal fin. Prior research revealed that depletion of the gap junction protein Connexin43 (Cx43), as well as blocking gap junctional intercellular communication (GJIC) by small peptides, leads to premature joint formation and the shortening of bony fin ray segments. These findings indicate that Cx43-GJIC suppresses joint formation. Consistent with this interpretation, levels of *cx43* mRNA oscillate during regeneration and the lowest levels coincide with initiation of joint morphogenesis. Pathways controlling the *cx43* oscillations are poorly understood. One likely candidate in controlling *cx43* oscillations is the morphogen retinoic acid (RA). Here we aim to investigate the relationship between RA and the regulation of *cx43* oscillations. Preliminary data suggest that *aldehyde dehydrogenase1a2* (*aldh1a2*), responsible for the synthesis of RA, is reduced preceding the decrease in *cx43* during regeneration, suggesting that RA may positively control *cx43* expression. *In situ* hybridization of *ald1a2* in the *cx43*-depleted mutant *sof-*^{b123} does not exhibit a significant difference compared to wild-type, suggesting RA synthesis is independent of *cx43* levels. Additionally, morpholino-mediated knockdown of *aldh1a2* leads to decreased bone segment and regenerate length. Interestingly, knockdown of the retinol-binding protein Crabp2b (responsible for mediating RA-dependent gene expression) has no effect. Taken together, these findings suggest that RA influences *cx43* expression independent of its transcriptional role. These and future studies will provide important insights into the oscillatory nature of *cx43* expression, and therefore, into the nature of skeletal patterning in the regenerating fin.

255 - Predicting modifiers of genotype-phenotype correlations in craniofacial development

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Most human birth defects are phenotypically variable even when they share a common genetic basis. Our understanding of the mechanisms of this variation is limited. Loss of the transcription factor Gata3 associates with the highly variable human birth defects HDR syndrome and microsomia, and can lead to disruption of the neural crest-derived facial skeleton. We have demonstrated that zebrafish *gata3* mutants model the variability observed in humans, including craniofacial defects. In this study, we bioinformatically identified potential modifiers of *gata3* mutant phenotypes. We performed RNA-seq on neural crest cells isolated from zebrafish across control, Gata3 loss-of-function, and Gata3 rescue groups. Differential expression analyses revealed 551 potential targets of *gata3*, and GO enrichment analysis suggested multiple distinct mechanisms, including Wnt signaling and RNA polymerase function. We used the LINCs L1000 database to identify small molecules predicted to modulate *gata3* mutant phenotypes based on differentially expressed genes. The predicted effector molecules reflected our bioinformatic analyses suggesting that Wnt signaling and polymerase activity were affected by Gata3 function. Of these predicted molecules, we found that vinblastine and clofibric acid enhanced the *gata3* phenotype, while daunorubicin and triptolide suppressed it. Our study illustrates multiple potential pathways for Gata3 function, and demonstrates a systematic, unbiased process to identify modifiers of genotype-phenotype correlations.

256 - Advances in genome editing: Mapping RNA polymerase occupancy with an epitope-tagged RNA Pol II

Kazuyuki Hoshijima[1], David Grunwald[1]

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Our lab continues to explore approaches to precise genome editing in the zebrafish. We will discuss methods that have improved efficiency. We describe Homologous Recombination approaches we have used to modify genes encoding transcription factors (TF) so that they express antigen-tagged versions of the TFs from native loci. Here we describe use of Cut and Tag methods to map the occupancy of epitope-tagged RNA Polymerase II in zebrafish embryos.

257 - Characterizing developing behaviours in chd8 and shank3b autism-risk allele mutant zebrafish lines

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Autism Spectrum Disorder (ASD) is amongst the most prevalent neurodevelopment disorders in the world, approximated to affect 1 in 58 people. The etiological basis of ASD has a genetic bias, with 50-80% of the disorder being attributed to genetic factors. Using animal models to study ASD is key to unraveling etiological genetic influences, allowing structured study of ASD risk genes and their consequences on neurodevelopment and behaviour. In this study, we aim to characterize autism-like behaviours in *shank3* and *chd8* mutant zebrafish lines, genes implicated in ASD according to the Simons Foundation Autism Research Initiative gene database. Here, we hypothesize that zebrafish with germ-line mutations in *chd8* or *shank3b* will display differential behaviours from wild-type zebrafish at both larval (5-, 7-, and 10-days) and adult (8+ months) ages. To examine behaviour, *chd8*$^{+/-}$ or *shank3b*$^{+/-}$ zebrafish are bred, and larval (ages 5-, 7- and 10-days post-fertilization) and adult (8+ months post-fertilization) behaviours are assessed using the the ZebraBox® and ZebraCube® recording chambers, respectively. We observed that in light, adult *shank3b*$^{+/-}$ zebrafish travel a shorter distance overall, travelling higher distances at a low speed and lower distances at a moderate speed, suggesting overall that they are less active. In the light, distance travelled of adult *chd8*$^{+/-}$ fish is not significantly different than wild-type fish. In adult novel-tank assays, *chd8*$^{+/-}$ fish exhibited more exploratory behaviours than wild-type fish. In an operant conditioning assay, we observed that both wild-type (TL) and *chd8*$^{+/-}$ adult fish were able to associate a conditioned stimulus (food) with and unconditioned stimulus (colour red), whereas *shank3b*$^{+/-}$ adult fish could not. Our findings suggest that fish with mutations in autism-risk genes have abnormal locomotive and cognitive behaviours in adulthood, and we aim to characterize these behaviours at earlier timepoints (larval and juvenile) to understand behavioural changes over a life-span.

258 - Evidence for a possible feedback loop influencing Cx43 expression during fin regeneration

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Mechanisms for determining the location of joints in the skeleton remain poorly understood. We use the zebrafish regenerating fin as a model to address this fundamental question. The fin is comprised of 16-18 fin rays, and each ray is comprised of bony segments separated by joints. Skeletal precursor cells commit to either the osteoblast lineage (producing segments) or to the joint forming cell lineage (producing joints). The protein Connexin43 (Cx43) plays an important role in this cell fate decision. For example, ß- catenin has been shown to function downstream of Cx43 to suppresses joint formation. Furthermore, *cx43* mRNA has been shown to be transiently reduced at the initiation of joint formation. Thus, we suggest that *cx43* oscillations control the timing of joint formation in the regenerating fin rays. Recent studies indicate that ß-catenin, in addition to acting downstream of Cx43, may also influence *cx43* levels. First, pharmacological activation of ß-catenin signaling reduces *cx43* mRNA. This suggests the possibility that elevated ß-catenin inhibits *cx43* transcription. This in turn transiently reduces Cx43, thereby permitting joint formation. However, pharmacological inhibition of ß-catenin signaling similarly causes reduced *cx43* mRNA. Continuing studies are exploring the nature of how ß-catenin interacts with *cx43*. If validated, these findings suggest tight coordination between Cx43 and ß-catenin function, and provide novel insights into how skeletal patterning is regulated.

259 - Modelling NAA15-mediated congenital heart disease in a zebrafish

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A variety of congenital heart diseases (CHDs) such as tetralogy of Fallot, hypoplastic left and right heart syndrome, and arrythmias have been linked by whole exome sequencing of parent-offspring trios to loss-of function mutations in *NAA15*. *NAA15* encodes an auxiliary component of the N-terminal acetyltransferase A (NatA) complex that functions to acetylate nascent peptides during translation to affect stability. Although N-terminal acetylation is a prevalent modification, the critical targets of NatA in cardiomyocytes and the potential cardiac phenotypes caused by *NAA15* loss-of-function have not been defined in any vertebrate model. I created null mutations in two *naa15* orthologues in zebrafish, *naa15a* and *naa15b*. 100% of double knock-out embryos die within 7 days post-fertilization (dpf) from pleiotropic defects that include cardiac malformations. Specifically, *naa15* mutant animals have a hypoplastic ventricle with severely compromised pump function and intermittent arrythmias at 4 dpf. The reduction in ventricular size is caused by a significant decrease in cardiomyocyte numbers due to failed proliferation. Although sarcomere organization appears largely preserved in naa15 mutant cardiomyocytes, we documented compromised Ca^{2+} transients. Although *naa15* expression is relatively ubiquitous, cardiomyocyte-specific overexpression of Naa15a partially rescues ventricular size and function, demonstrating that the cardiac phenotypes are largely intrinsic to the heart. Injection of human *NAA15* mRNA rescues ventricular function in *naa15*-deficient zebrafish, highlighting conservation of molecular function in the heart. Currently, I am testing whether *NAA15* variants identified in patients with different forms of CHD can rescue pump function in our zebrafish model and identifying protein targets of NatA-mediated N-terminal acetylation using *naa15a* proximity labeling in zebrafish. Targets will be prioritized based on cellular function and tested in hypothesis-driven follow-up studies. Ultimately, the outcomes of these studies will inform future therapeutic and diagnostic options for patients with *NAA15*-mediated CHDs.

260 - Intestinal epithelial cells exhibit plastic responses to commensal and pathogenic microbes

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Intestinal stem cells (ISCs) respond to signals from their niche and the gut lumen to generate specialist cell types that maintain the epithelial barrier against microbial invasion. While an appropriate ISC response to environmental cues is critical to host health, little is known of ISC contributions to gut homeostasis following exposure to commensal or pathogenic microbes. The zebrafish is an increasingly used model for intestinal host-microbe interactions since zebrafish possess significant functional and genetic overlap to mammals. However, a paucity of genetic markers, including ISC markers, have been identified in the zebrafish intestine, hampering investigation of ISC responses to microbial colonization or pathogen challenge. To bridge this knowledge gap, we performed single-cell RNA sequencing of larval zebrafish intestines under conventional and germ-free conditions, as well as adult intestines following mock or *Vibrio cholerae* infection. We observed extensive heterogeneity in the intestinal epithelium including previously undescribed cells with known mammalian homologs, such as tuft-like cells, Best4/Otop2 cells, and candidate ISCs. Examination of candidate ISCs in these datasets revealed genetic regulators of microbe-dependent growth and secretory cell differentiation, as well as a transcriptional shift toward goblet cell development following *V. cholerae* infection, highlighting the plasticity of candidate zebrafish ISCs. To interrogate putative ISCs *in vivo*, we generated a reporter for *tnfrsf11a*, a candidate ISC marker in both larval and adult zebrafish single-cell datasets. *Tg(tnfrsf11a:GFP)* fish exhibited reporter expression in an epithelial cell subset at the base of intestinal folds, consistent with ISC localization. We are currently investigating the identity of *tnfrsf11a*-positive cells and analyzing these cells under conventional and infection conditions. This work advances the use of zebrafish as a model of intestinal host-microbe interactions by highlighting extensive cellular similarity between fish and mammalian intestines, and uncovers cell-type-specific regulators of the host response to both commensal microbes and a deadly human pathogen.

261 - Neurological defects in a zebrafish model of CHARGE syndrome

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Mutations in the ATP-dependent chromatin remodeller chromodomain, helicase, DNA binding (CHD) 7 are the primary cause of CHARGE syndrome (CS) and have been associated with autism spectrum disorder (ASD). CHARGE is an acronym for the most characteristic features presented by patients: coloboma of the eye, heart defects, atresia chonae, retardation in growth and development, genital abnormalities, and ear defects. Although not included in the diagnostic criteria CS features often include neurological and behavioural problems such as hyperactivity. Little is known about the molecular mechanisms that underlie these neurological symptoms. Further, there is no known treatment yet either for patients with CS or for the neurological symptoms shared between CS and ASD. To investigate this, we generated a novel CRISPR/cas9 zebrafish chd7−/− model. chd7 knockout zebrafish larvae exhibit a small head phenotype, defects in craniofacial cartilage development and display aberrant axonal network development. Further the chd7 mutants have less GABAergic neurons and exhibit a hyperactivity behavioural phenotype. Using an unbiased whole transcriptomic approach, we found that the GABAergic neuron defect was at least in part due to the downregulation of a CHD7 target gene, paqr3b and the subsequent upregulation of the MAPK/ERK signalling pathway, which is also dysregulated in CHD7 mutant human cells. We further show a novel role of chd7-paqr3b axis in neurogenesis. Through a phenotype-based screen in chd7−/− zebrafish and *C. elegans*, we show that the small molecule ephedrine restores MAPK/ERK signalling and improves both GABAergic defects and behavioural anomalies. This work provides insight into the neuropathogenesis in CS and identifies a promising compound for further preclinical studies.

262 - Exploring axial mesoderm regulation in zebrafish with CellOracle prediction and CRISPR/Cas9 validation

Blerta Stringa[1]

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During embryogenesis, cell fate specification is governed by Gene Regulatory Networks (GRN) that represent the underlying complex and dynamic regulation of gene expression. To overcome some of the limitations of exploring GRNs regulating vertebrate embryogenesis, we deployed CellOracle machine learning-based approach to perform *in silico* perturbations of candidate transcription factors (TF) regulating axial mesoderm development and predict their developmental outcomes in zebrafish embryos. Combination of CRISPR/Cas9 and scRNA-seq analyses of *noto/flh* mutants and crispants, not only confirmed CellOracle predictions of the well-studied notochord deficiency but also revealed a previously unreported increase of prechordal plate population. To further validate our *in silico* prediction, we induced genome editing in *lhx1a*, *sebox* and *irx3a* genes prioritized by CellOracle and analyzed resulting transcriptomes at 10 hours post fertilization with scRNA-seq. Although no morphological changes were observed, *lhx1a*_crispant transcriptome showed early axial mesoderm differentiation arrest accompanied by a reduction of *nog1* expression, which was confirmed by orthogonal methods. Marker of prechordal plate and notochord were quantitatively evaluated in both control and crispant with qRT-PCR and HCR. In the future, stable single and compound mutants for each TF predicted by CellOracle will be used to build a GRN for the axial mesoderm development in zebrafish.

263 - Craniofacial dysmorphism mediated by serotonin receptor Htr2b in a zebrafish model of CHARGE syndrome

Maximilian Breuer[1, 2], Maximilian Rummler[3], Priyanka Jamadagni[1], Jaskaran Singh[1], Charlotte Zaouter[1], Bettina Willie[3], Kessen Patten[1]

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CHARGE syndrome is a severe multisystemic developmental disorder that is most commonly caused by mutations in the ATP-dependent chromatin remodelling enzyme CHD7. To understand the function of CHD7, we generated a chd7 knockout in zebrafish by CRISPR/Cas9 mediated mutagenesis. This model has proven highly efficient in replicating the characteristics observed in CHARGE syndrome including craniofacial defects. In our studies we could show that chd7$^{-/-}$ larvae show severe craniofacial dysmorphism. Using MicroCT analyses, we also characterized structural abnormalities in adult chd7$^{-/-}$ fish. The skull and spine show altered morphology with highly variable bone mineral density and bone volume.

Using an unbiased transcriptomic analysis (RNA-Seq), we identified a significant downregulation of the 5-hydroxytryptamine receptor 2b (Htr2b) in these chd7$^{-/-}$ zebrafish. Interestingly, this member of the serotonin receptor family is closely associated with developmental defects in heart and jaw development. We found that *htr2b* is expressed in the branchial arches, giving rise to the jaw, via *in situ* hybridization. Chd7 mutants present with a loss of col2a1a in the craniofacial regions shown by *in situ* hybridization and immunohistochemistry. Targeting htr2b with specific inhibitors downregulates expression levels of *col2a1a* in the craniofacial regions. Furthermore, the inhibition of Htr2b results in abnormal development of the palatoquadrate in the jaw as determined by alcian blue staining, with high similarity to the morphology observed in chd7 mutants. Our results indicate a high dependency on Htr2b for successful palatoquadrate development, which may underlie the craniofacial phenotype in CHARGE syndrome.

Our study is first to elucidate the mechanisms underlying craniofacial and spinal development in both larvae and adult chd7$^{-/-}$ zebrafish resulting in a loss of col2a1a. Furthermore, our data show a strong implication of dysregulated HTR2B functions upon loss of function of CHD7 in CHARGE syndrome pathogenesis and suggest that HTR2B may serve as a novel potential therapeutic target.

265 - The CRL4 E3 Ligase Links Cohesinopathies to Thalidomide Teratogenicity

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[1]Lehigh University

Cohesins promote enhancer-promoter interactions required for proper gene expression during development. Cohesin pathway mutations lead to developmental syndromes called cohesinopathies, including Roberts Syndrome (RBS) and Cornelia de Lange Syndrome (CdLS), that exhibit severe organ malformations, craniofacial abnormalities, phocomelia, and intellectual disabilities. RBS is caused by mutations in ESCO2, an acetyltransferase that activates cohesins. CdLS is caused by mutations in cohesin subunits or auxiliary factors, like NIPBL, which loads cohesins onto DNA. Severe developmental abnormalities closely resembling those of RBS/CdLS, also arise due to *in utero* exposure to the drug Thalidomide, a potent teratogen used to treat morning sickness in the 1950s. Thalidomide teratogenicity acts through direct binding to Cullin-4 Ring Ligase (CRL4). CRL4 is a multi-subunit E3 ubiquitin ligase that targets substrates for degradation. Previously we used zebrafish to test a novel hypothesis that RBS/CdLS birth abnormalities arise, in part, through dysregulation of a CRL4 component. Our findings revealed that *ddb1*, an essential CRL4 subunit, is indeed downregulated in cohesinopathic models (Esco2 or cohesin subunit knockdown) in zebrafish embryos. Importantly, increasing *ddb1* mRNA via microinjection significantly impacted phenotype severities that otherwise occur in Esco2 or cohesin subunit knockdown embryos. Here, we report on new findings in Nipbl knockdown zebrafish embryos, that support our hypothesis that CRL4 and cohesin share a molecular pathway. Given the role of CRL4 in protein degradation, we hypothesize that a subset of accumulated proteins is responsible for developmental abnormalities. We performed mass spectrometry on zebrafish embryos individually knocked down for either Ddb1, Esco2, or cohesin subunit to identify proteins downstream of CRL4. We identified 30 proteins, present at elevated levels, in common between all treatments compared to control embryos. Together, these findings support a link between thalidomide and cohesinopathies, providing a novel mechanism through which developmental maladies such as RBS and CdLS may be approached.

266 - Zebrafish models of neurodevelopmental disorders set the stage for future treatments

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The goal of the Thyme lab is to uncover the molecular basis for complex neurodevelopmental disorders and ultimately to develop drug treatments. We use the larval zebrafish model, taking advantage of new high-resolution and large-scale approaches for neural phenotyping and genetic analysis. Previously, we assessed whole-brain activity, brain morphology, and behavior of zebrafish loss-of-function mutants for orthologs of 132 human schizophrenia-associated genes. Here, I will describe in-depth molecular studies of one mutant from this study and additional screens of genes involved in other disorders. We have ongoing screens characterizing mutants for many genes involved in neurodevelopment disorders such as autism and childhood-onset schizophrenia. Characterizing these mutants is the first step in identifying screenable phenotypes and critical proteins that could be targets of drug discovery. In parallel to our zebrafish work, we have built a new computational method for in silico drug discovery. This method uses information from published structural data to guide the prediction and is superior to published docking methods. Our ongoing studies of the basic biology of the genes linked to neurodevelopmental disorders will yield the protein targets for computational drug discovery and neural phenotypes to prevent or reverse. Testing several hundred lead compounds in larval zebrafish is feasible and many proteins are highly conserved, making it an ideal model for compound screening and testing new tools and methods. Our proposed pipeline of integrating computation, whole-organism screening, and in-depth mechanistic studies promises to yield potentially therapeutic molecules that impact diverse mechanisms underlying neural development and function.

267 - Studying cardiovascular defects in two fast aging progeria models in zebrafish

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Aging is the major contributor to the etiology of cardiovascular diseases. Yet, the molecular mechanisms causing aging of the cardiovascular system are not well understood. Here, we have undertaken a genetic approach to generate rapid-onset aging mutants in zebrafish to study the molecular and cellular changes of the cardiovascular system during aging. The rapid aging disorders, Hutchinson-Gilford Progeria Syndrome (HGPS), an early onset progeria caused by a mutation in the nuclear lamina protein *lamin a* (*lmna*) and Werner Syndrome (WS), a late onset progeria caused by mutations in the *Wrn RecQ like Helicase* (*wrn*) are frequently characterized by premature and fatal cardiovascular complications in patients. Effective *in vivo* models to understand these cardiovascular defects are currently unavailable.

We have characterized the expression patterns of the homologous genes of *lmna* and *wrn* during zebrafish embryogenesis by whole-mount *in situ* hybridization. While *lmna* localizes to craniofacial regions, heart and myotome, *wrn* is expressed in gut and swimbladder at 5dpf. To further investigate the roles of these genes during zebrafish cardiovascular development, we used a CRISPR/Cas9 knockout strategy to generate a *lmna* null and HGPS (Progerin) overexpression mutant. We used a small compound inhibitor which selectively inhibits the Helicase function of Wrn, which subsequently resulted in lipid metabolism changes during early embryonic development.

At the meeting, we will also present data based on the analysis of a HGPS (Progerin) overexpression transgenic line. This model is based on a particular mutation in the *lmna* gene, which results in a protein accumulation that weakens nuclear lamina stability. We will also present data related to the effects of HGPS-related rapid aging on cardiovascular development and metabolism *in vivo.* Characterizing these transgenic and mutant lines in zebrafish will be of great value to understand the underlying mechanisms in fast-aging disorders and aging itself.

268 - Characterization of Kupffer Vesicle centrosome behavior during cilia formation

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[1]Syracuse University

An essential process for cilia formation during epithelialization is for the centrosome to move and dock with the cell's forming apical membrane. Our studies goal was to identify mechanisms of centrosome positioning during mesenchymal to epithelial transition using *Danio rerio's* left-right organizer (Kupffer's Vesicle, KV) as a model. We found that while KV mesenchymal cells were rearranging into epithelial cells, the cells moved their centrosomes from random intracellular positions to the forming apical membrane. During this process, these cells centrosomes were constructing cilia intracellularly and this construction occurred only when centrosomes reached a certain position in relation to the forming rosette center. Once the centrosome with associated cilia reached the rosette center, they remained intracellular until the lumen expands to a set size. Using optogenetic strategies we identified that the small GTPase, Rab11, regulates not only cilia formation, but centrosome movement towards the forming apical membrane, whereas Rab8 only seemed to modulate cilia elongation once the cilia were already extending into the KV lumen. We propose a model that during KV cell epithelialization and KV lumenogenesis, the KV cell centrosome, while moving towards the forming apical membrane, is constructing a cilium in a Rab11 dependent fashion. Once the centrosome and associated cilium are at the apical membrane the cilium waits to protrude from the cell once the KV lumen is at a set size.

269 - Screening for Maternal-Zygotic Mutants

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Gastrulation is a critical early morphogenetic process, during which the three germ layers; mesoderm, endoderm and ectoderm, are formed and shaped into a body plan. Gastrulation is initiated during, a process that entails both activation of the zygotic genome and downregulation of the maternal transcripts. Whereas genomic studies indicate that gastrulation requires the maternal-to-zygotic transition and is largely controlled by the zygotic genome, limited genetic studies point to a significant contribution of the maternal genome. To test this, we initiated an ENU-based F3 forward genetic screen for recessive maternal and maternal-zygotic mutations affecting embryogenesis. To identify maternal-effect mutations, we intercross F3 siblings and analyze the resulting F4 progeny during gastrulation and at the end of embryogenesis, and identify the mutant loci by whole exome sequencing (WES). Upon screening ca. 500 F3 mutagenized families, we found over 40 maternal-effect mutants, including novel as well as previously reported phenotypes. As expected, we found many *strict maternal* mutations, impairing oocyte polarity, early cleavages, and gastrulation movements. We also found strict maternal-zygotic (MZ) mutants, whereby the phenotype is manifest only when both maternal and zygotic function are impaired. WES of *stl785*, a strict maternal-effect mutation characterized by embryonic arrest at sphere stage and lysis during gastrulation, mapped it to chuck/poky locus on Chromosome 13 encoding Inhibitor of NFkB Kinase 1 (ikk1), previously identified by Mullins and Wagner labs (Dev Bio 346, 272-283, 2010) as required for differentiation of the zebrafish embryonic epidermis. We will present our RNA-seq analyses revealing transcriptomic changes in this mutant. We will also present additional mutants identified in our screen.

270 - Disruption of interleukin 4 signalling models aspects of multiple sclerosis in zebrafish

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Multiple sclerosis (MS) is a common cause of neurological disability among young adults. Brain and spinal cord inflammation and destruction of the myelin that insulates nerves are central features of MS pathology. Available animal models do not fully recapitulate the disease. Interleukin-4 (IL-4) is an anti-inflammatory cytokine that can suppress or delay the development of neurological symptoms in murine models of MS. In this work, we show that disrupting IL-4 signalling by knocking down the IL-4 receptor (IL-4r) in zebrafish leads to defects in the developing brain and spinal cord and an altered mRNA signature consistent with decreased myelination and activation of pro-inflammatory pathways. Morphants in which il-4r was knocked down by morpholino oligonucleotide injection had a smaller head and presented spontaneous lesions in the spinal cord. Quantitative PCR revealed decreased expression of RNAs encoding for myelin proteins and increased expression of RNAs encoding for inflammatory proteins. Our results suggest an essential function of IL-4 signalling in the brain and spinal cord, and that its disruption models key aspects of MS in zebrafish. The model will allow the investigation of disease mechanisms contributing to MS.

271 - Comparing nondestructive larval genotyping of Danio rerio for nuclear and mitochondrial DNA genetics

Shannon Holmberg[1], Ankit Sabharwal[1], Stephen Ekker[1]

[1]Mayo Clinic

The advent of both nuclear and mitochondrial genomic editing tools demonstrates the need for larval genotyping of *Danio rerio.* Two methods have recently been described for individual, nondestructive rapid genotyping. These new methods work to address the deficiencies of the previously established method of larval genotyping in a small water bubble followed by microsurgery of the larval fin, a tedious and often lethal procedure. For zebrafish researchers, these protocols enable greater efficiency in husbandry and assays, accelerating research using this popular animal model- overall improving sustainability. These noninvasive techniques include a limited digestion in proteinase K and use of the Zebrafish Embryo Genotyper (ZEG), a shaking device with rough slides. Both methods involve extracting epithelial cells containing DNA that can then be PCR amplified and used for genotyping. We aim to further characterize the impact of these assays on larvae through survival, behavioral and transcriptomics data as well as optimizing assay conditions for differential amplification. In testing and troubleshooting nondestructive methods, we note pros and cons to both physical and chemical techniques as well as exploring whether these methods can be used for nuclear and mitochondrial genotyping. RNAseq analysis will be performed using Basepair Tech software. Behavior assays will be conducted with Zantiks machines. Use of the ZEG on several loci shows that it yields modest amounts of DNA resulting in a need for primer optimization. The enzyme method yields higher DNA concentrations that are more tolerant of differential amplification. Modifications to the previously published enzyme protocol have enabled stronger survival compared to the initial protocol. Ongoing transcriptomics studies and behavioral assays for both methods of genotyping should highlight the gene response to mechanical stress, if any.

272 - Cellular and molecular mechanisms regulating Muller glial morphogenesis

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Glial morphogenesis is a key developmental process for establishing close contacts between glia and the nearby neurons. These close contacts are necessary for the glia to impart their supportive functions and to ensure the maintenance of the nervous system. The retina is a relatively simple neural tissue composed of six main neuron types and a single principal glial type, the Muller glial cell (MG). MG tile the neural tissue and are elaborately shaped at sub-cellular domains to contact specific neuron types, synapses and the vasculature. Using time-lapse confocal microscopy on transgenic zebrafish we have characterised the dynamic cell behaviours of MG at five distinct sub-cellular regions throughout the entirety of development. We show that MG send out dynamic protrusions that sense neighbouring cells before stabilising at their correct targets in the mature retina. We have manipulated the composition of the retina to remove specific partner neurons and show that MG are directed by signals nearby neurons at each cellular layer of the retina. Finally, we show, using custom glial morphogenesis image analysis software and CRISPR/Cas9, that specific cell adhesion molecules in the retina guide MG processes to recognise and elaborate within the synaptic neuropil. In conclusion we have characterised the cellular behaviours and identified molecular mechanisms that shape MG during their morphogenesis in the developing retina.

273 - Investigating actin and microtubule cytoskeletal interactions in the yolk cell during zebrafish epiboly

Haoyu Wan[1], Ashley Bruce[1]

[1]University of Toronto

During zebrafish gastrulation, epiboly, the thinning and vegetal spreading of the muti-layered blastoderm over the yolk cell, is essential for the correct formation of the adult body plan. My focus is on the yolk actin and microtubule cytoskeletal networks, which are both crucial for epiboly. Although yolk actomyosin contraction is the main driver of epiboly, various mutants with delayed epiboly exhibit both yolk actin and microtubule defects, pointing to a connection between the networks and suggesting that they might interact during epiboly. I hypothesize that yolk actin and microtubules interactions facilitate epiboly. My approach is to examine cytoskeletal dynamics in the yolk cell by live confocal imaging. I identified Camsap2, a microtubule minus-end stabilizing protein expressed in the yolk cell during epiboly, as a candidate to mediate yolk actin and microtubule interactions. I generated *camsap2a* CRISPR/Cas9 knockout mutants which show delayed epiboly and abnormally elongated embryos. Interestingly, yolk microtubules appear largely unaffected in *camsap2a*mutants. However, microtubules located vegetal to the yolk actomyosin ring are more disorganized in mutant embryos. The most severe defects in mutant embryos are in the yolk actin network, as a significant decrease in marginal accumulation is observed. Reduced actin ring formation likely leads to reduced contraction, and epiboly delay. Our current model is that Camsap2 is involved in the formation of the yolk actin ring by mediating actin retrograde flow or turnover. It is also possible that Camsap2 directly regulates the structure and dynamics of marginal microtubules. Alternatively, the microtubule defects might be secondary to reduced actomyosin contraction, as perturbing contraction in the yolk cell by expressing constitutively active myosin phosphatase leads to similar defects in marginal microtubules. Future experiments will focus on the mechanisms of Camsap2 function, which should provide new insights into the roles of yolk actin and microtubules during epiboly.

274 - The function of Bucky ball and the Balbiani body in oocyte polarity and germ cell development

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Oocyte polarity is important for formation of the embryonic body axis and germ cells. The Balbiani body (Bb) is a large, membrane-less, mitochondrial-rich, electron-dense structure observed in the primary oocyte, which is conserved from insects to mammals, including humans. However, its function and the mechanisms regulating it are not fully understood. In frogs and fish, the Bb is postulated to establish oocyte polarity. mRNAs essential for body axis formation and germ cell formation localize to the Bb. As the Bb disassembles at the cortex in later stage oocytes, the Bb-localized transcripts and proteins become docked at the oocyte cortex. These vegetally-localized factors delivered by the Bb are postulated to specify the animal-vegetal (AV) axis. The Bb localized protein, Bucky ball (Buc), is the only gene known to function in Bb formation. Here, I established *buc* CRISPR mutant lines that are *buc* hypomorphic alleles, which differ to null alleles. In *buc* null mutants, AV polarity fails to form and the animal pole is expanded radially in the oocyte and embryo, which dies before 1 dpf. In *buc* hypomorphic mutants, embryos displayed normal AV polarity and survived to 1 dpf; however, many were severely ventralized. Those embryos with a WT phenotype failed to generate primordial germ cells and developed into sterile males. In *buc* hypomorphic oocytes, the mitochondria did not aggregate into the Bb and the Bb failed to form in early stage oocytes. Interestingly, in later stage oocytes, Buc and transcripts localized to the vegetal cortex, although in a reduced size domain. In these later stage oocytes the animally-localized mRNA, *cyclin B1*, was localized in an expanded animal pole domain. These results demonstrate the importance of Buc in germ cell formation and dorsal axis formation and suggest a Bb-independent mechanism of polarizing the oocyte.

275 - A Maternally Provided Sunscreen Protects Early Development

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External development is the most common mode of embryogenesis amongst animals, yet it entails that an animal's most vulnerable life stages are exposed to the environment. Ultraviolet radiation (UVR) is highly damaging to cells, and all organisms exposed to sunlight have evolved UVR-protective mechanisms. Although embryos and larvae of most fish species are exposed to sunlight, how these early life stages are protected from UVR is largely unknown. Mycosporine-like amino acids (MAAs) are thought to act as sunscreens in numerous microbes and invertebrates. Intriguingly, gadusol, a chemical structurally related to MAAs, is found in large quantities in the eggs of many fish. However, the role of gadusol as a potential UV protectant *in vivo* is untested. We used CRISPR-Cas9 mutagenesis to KO *eevs,* the first gene in the biosynthetic pathway of gadusol and successfully created healthy gadusol-lacking mutants in zebrafish. When exposed to UVR we found that embryos and larvae maternally-depleted of gadusol are highly sensitive to UV: accruing elevated levels of DNA damage, and surviving at much lower rates. Thus, we demonstrate that gadusol, is a true sunscreen, the first time a *de novo* synthetic sunscreen other than melanin has been shown to provide UVR-protection in a vertebrate. Furthermore, we found that gadusol-lacking mutants survived at lower rates than mutants only lacking melanin when exposed to UVR. We surveyed 100+ genomes and found the gadusol biosynthetic pathway to be present in the genomes of the vast majority of fish but absent among many species not exposed to UVR. We conclude that gadusol is not only a powerful sunscreen, but that its presence is intricately linked to external development, providing protection during critical developmental stages. Our work emphasizes the importance of contextualizing development within ecological pressures to understand how animals have evolved to successfully thrive in their environments.

276 - From colors to kidney stones: The biological control of organic-crystal forming cells

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Chameleons, spiders, planktonic crustaceans, fish, and many other animals use organic crystals for an astonishing variety of optical functions, from white light scattering in spiders to tunable reflecting colors in the copepods. These crystals are formed by specialized cells called iridophores, in which remarkable control over crystal shape, size, and assembly is obtained using strategies that are beyond state of the art in materials science. While these cells were identified many years ago, almost nothing is known about the biological and biochemical processes which enable this tremendous control over bio-organic crystals. We used the zebrafish skin iridophores as a model system to investigate: 1. How cells with different architectures and different optical properties are obtained, and 2. What is the cellular machinery which drives structural color change? Using a combination of biological and physical tools, we found that distinct iridophore types with different crystal morphologies, architectures, and optical properties were obtained by differentiation based on their microenvironments. We further found that only one type of iridophores was capable of changing its colors and that this color change was facilitated by motor proteins actively pulling the membrane-bound crystals.

277 - Histological analyses of scleral cartilages in zebrafish and other teloests

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The cartilage element embedded within the sclera of vertebrate eyes has a long evolutionary history. The morphology of this element varies both within and amongst different teleosts as well as other vertebrates. With this knowledge, a deeper investigation into the nuanced developmental differences between the development of this cartilage within zebrafish, *Danio rerio*, and the Mexican tetra morphs of *Astyanax mexicanus* was conducted. Distinct differences in the growth trajectories were observed as well as significant differences in scleral chondrocyte morphology. This study provides the first quantitative characterization of the scleral cartilage and indicates which characteristics of the cartilage is highly conserved and which characteristics are more plastic. Furthermore, our studies show a unique mode of ossification of this cartilage later in development. This research was funded by the Natural Sciences and Engineering Research Council of Canada

278 - Discovering Natural Pathogens and Host-Associated Immune Responses Via Cohousing

Marlen Rice[1], Nels Elde[1], James Gagnon[1], Keir Balla[1, 2]

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An awareness of the natural pathogens of model organisms, in particular mouse, has greatly enhanced our understanding of infection biology. Zebrafish is emerging as a powerful immune model, but currently only two viruses have been documented to cause natural infections in zebrafish. In order to better understand the immune responses of zebrafish to natural pathogens we have devised a novel cohousing method to uncover naturally-transmissible pathogens. We cohoused zebrafish purchased from a pet store with lab-reared fish in a pilot study using two separate cohousing tanks. We hypothesized that pet store fish could serve as convenient reservoirs of diverse natural pathogens of zebrafish, while our lab-reared fish, similar to SPF mice, would likely be highly susceptible hosts having a relatively naïve immune system. After one month of cohousing we dissected, intestines, kidneys, and spleens from our lab-reared fish and performed bulk RNAseq. In one tank we noted several fish with hemorrhaging along their bodies and sequencing revealed a novel birnavirus. This novel virus, we name Rocky Mountain Birnavirus, is related to infectious pancreatic necrosis virus (infects salmonids), and elicited robust expression of antiviral genes in all tissues sampled. In the second cohousing tank our lab-reared fish became heavily infected with a common intestinal parasite, *Pseudocapillaria tomentosa*. Their pathological phenotype included weight loss and splenomegaly, but few changes in tissue wide gene expression. Our pilot study strongly supports the use of cohousing lab-reared fish with pet store fish as a viable means of uncovering novel pathogens and documenting zebrafish immune responses to these naturally-transmissible agents. Our future work aims to better understand how the relatively “naïve” immune status of lab-reared zebrafish may be impacting our understanding of zebrafish immunology.

279 - Low frequency vibration results in fused skeletal elements and shifted skeletogenic condensations

Tamara Franz-Odendaal[1], Shirine Jeradi[1]

[1]Mount Saint Vincent University

Teleosts are superb models to study development and growth because their entire life history can be studied in a laboratory setting. The objective of this research is to understand the impacts of the external environment, namely whole body low frequency vibrations on skeletal development. We exposed zebrafish larvae at different stages of development to low frequency vibrations by raising them under vibration conditions for several days. Our results show age-related and bone-type specific effects. Our data suggests that chondrogenic cell progenitors are capable of sensing and reacting to mechanical stimuli early during development. Furthermore, we show that this effect is sox9-independent. This data shows that there is a critical window during early development that is susceptible to environmental influence. The plasticity of the skeleton to respond to and adapt to external influence is remarkable. These responses often have lasting impacts on the resulting phenotype and provides a hint at how development influences evolution.
This research was funded by the Natural Sciences and Engineering Research Council of Canada and the Canadian Space Agency.

280 - Integrated single cell molecular analysis of pericytes reveals the cis-regulatory logic governing their identity

Feston Idrizi[1], Nathan Lawson[2]

[1]Self, [2]Principal Ivestigator

Single cell “omics” platforms allow molecular analysis of previously inaccessible cell types. Pericytes are vascular mural cells that control capillary function, but characterization of their lineage determinants is deficient due to lack of specific markers and transgenes. To address this issue, we employed single cell RNA (scRNA) and ATAC (scATAC) sequencing to identify pericyte-specific gene and enhancer signatures. We performed scRNA-seq and scATAC-seq on cells isolated by fluorescent activated cell sorting from TgBAC(abcc9:gal4;uas:egfp) larvae, which drive transgene expression in pericytes among other cell types. scRNAseq identified a conserved pericyte gene expression signature, including candidate lineage-specifying transcription factors (TFs), while scATAC-seq revealed enrichment of cognate TF binding sites in pericyte-specific enhancers. Notably, expression of the Notch receptor, *notch3*, was specific to pericytes, while binding sites for Rbpj, its DNA binding partner, were enriched in pericyte-specific enhancers. Previous studies have noted reduced pericyte numbers in notch3 mutants. To determine if Notch directly induces pericyte gene expression, we generated stable reporter lines using candidate pericyte enhancer elements containing Rbpj sites driving Egfp expression. In particular, an enhancer downstream of the pericyte-specific *ndufa4l2a* gene that contains two Rbpj sites drove pericyte-specific expression. Furthermore, CRISPR-mediated deletion of this enhancer element from a ndufa4l2a:sfGFP BAC reporter in transgenic zebrafish embryos eliminated sfGFP expression, suggesting this enhancer is required to drive pericyte-specific gene expression. Our current studies focus on deleting Rbpj binding sites in this reporter line. Taken together, our results demonstrate the powerful approach of applying integrated single cell omics platforms to reveal heretofore unknown mechanisms of specification in the pericyte lineage. Our analysis has identified several pericyte-specifying TF candidates, while supporting a role for the Notch signaling pathway in the direct induction of pericyte-specific gene expression.

281 - From the blood to the brain: diffusion of the human peripheral extracellular vesicles into the brain of zebrafish larvae

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Extracellular vesicles (EVs) are produced and released by all cell types in the body. These nanometric EVs play an important role in intercellular communications with the transfer of their functional cargo to recipient cells. Peripheral EVs (pEVs) could propagate proteins, lipids, and other factors to different organs through biofluids. However, the shuttle of pEVs between the periphery and the brain is not clearly demonstrated *in vivo*. Furthermore, within the brain, their uptake by neuronal and glial cells remains to be studied. We hypothesized that pEVs could rapidly cross the blood-brain barrier and are taken-up by neuronal and glial cells. For this, we isolated pEVs from the human serum by precipitation. pEVs were then characterized according to the ISEV recommendations. PKH26 fluorescent dye-labeled pEVs were injected into the blood circulation of transgenic *Danio rerio* of 2 days post-fertilization. The biodistribution of pEVs into the body of larvae was followed 1h and 24h post-injection by 3D-confocal microscopy. In Tg(*flk1:EGFP*) line, we demonstrated that the passage of the pEVs into the brain and their diffusion into different cerebral areas are time-dependent. The important colocalization of pEVs with endothelial cells suggest that these vesicles can diffuse into the brain notably thanks to their passage through the blood-brain-barrier. Moreover with the Tg(*huc:EGFP*) and Tg(*gfap:EGFP*) lines, we demonstrated that pEVs were engulfed, respectively, by neuronal and glial cells. These results support our hypothesis, that cerebral cells can rapidly take-up pEVs coming from the blood circulation. Therefore, this study brings up new information about the fate of pEVs after their passage through the brain barriers and raises new questions on the impact of the brain-body interaction. By providing pathogenic factors from altered organs in the periphery, pEVs may contribute to the pathogenesis of neurodegenerative diseases.

282 - The dual roles of interleukin11a (il11a) in promoting regeneration and inducing fibrosis in zebrafish hearts

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Limited regeneration capacity of mammals causes the permanent fibrotic tissue in the damaged heart, leading to cardiac dysfunction. In contrast, regenerative species, like zebrafish, exhibit the robust heart regeneration along with scar resolution. However, secreted factors influencing cardiac regeneration program and fibrosis remain elusive. Here, we demonstrate that *interleukin-11a* (*il11a*) regulates cardiac regeneration and fibrosis. Expression analysis of the *il11a* knock-in reporter line revealed that *il11a* is robustly induced upon cardiac injury and peaked at the early regenerative phase. Our gain-of-function study demonstrated that conditional *il11a* overexpression in myocardium of uninjured hearts can trigger cardiac regeneration programs, including cardiomyocyte proliferation, vasculogenesis, epicardial activation, and immune cell recruitment. Prolonged *il11a* overexpression eventually leads to cardiomegaly. Interestingly, we also found that the chronic *il11a* overexpression results in collagen-rich scarring via emergence of myofibroblast. Overall, our findings unveil the conflicting roles of *il11* signaling in hearts and provide insights into the importance of transient delivery of regenerative factors for heart repair.

283 - An Initial Characterization of Novel Contributors to Superior Coloboma and the Closure of the Superior Ocular Sulcus

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The superior ocular sulcus (SOS) is a transient developmental fissure that forms within the superior eye. Improper closure of this structure can result in a phenotype in zebrafish that resembles the human condition superior coloboma, an atypical form of ocular coloboma. Coloboma results from improper closure of the choroid fissure and is the cause of 3-11% of congenital blindness in children. Even though the choroid fissure and coloboma of the inferior eye have been widely studied, the mechanisms involved in the formation and closure of the SOS and the genetic causality of superior coloboma remain largely understudied. Our lab has previously demonstrated the potential importance of Bmp-dependent ocular dorsal eye patterning in SOS closure. Recently, we have uncovered variants from superior coloboma patients in the mTOR regulator, *TSC2*; the ventral eye patterner, *VAX2*; and the planar cell polarity (PCP) gene, *SCRIB*. Our current investigations show that loss of *tsc2*, *vax2*, and the PCP gene, *vangl2*, independently, result in delays in SOS closure in zebrafish. Through RNA sequencing, we have also identified multiple novel candidate genes whose roles in the formation and closure of the SOS have not yet been studied. Taken together, our results have extended our knowledge of the regulation of SOS closure by implicating the possible roles of the PCP pathway, mTOR signaling, and ventral eye patterning. However, these results also affirm that the causes of superior coloboma are likely combinatorial and that multiple pathways might be involved in its causality. Our eventual goal is to determine the gene(s) and/or pathway(s) responsible for the closure of the SOS and to create an ultimate zebrafish model of superior coloboma.

284 - Genetic background, embryo media, and testing arena size modulate neural development and behavior in zebrafish larvae.

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[1]NC State University

Genetic diversity in human populations is likely a major driver of variation in disease susceptibility and drug responsiveness. Laboratory strains of zebrafish have a similar degree of genetic variation to humans, making them an attractive model for translational and ecological applications. Tests of larval zebrafish behavior are commonly used to assay neural development and function, and although several reports have described behavioral differences within and between strains, a comprehensive behavioral characterization has been lacking. Here, we show significant differences both within and between two different sources of AB, TLF, TU, and WIK-strain larvae across a battery of standard behavioral assays: locomotor activity, thigmotaxis, light-dark response (VMR), dark-flash response (O-bends), acoustic startle sensitivity, pre-pulse inhibition (PPI), and short-term habituation. Intra-strain differences in PPI were not found to be heritable, but acoustic startle sensitivity was strongly heritable, indicating a genetic basis. Strain also modulates the penetrance and expressivity of startle hypersensitivity in *cyfip2* mutant larvae, along with the effect of the NMDA-receptor inhibitor MK-801 on startle sensitivity. Staining for auditory nerve club-ending synapses on the Mauthner cells shows that startle sensitivity in 4 strains of larvae directly correlates with the number of club-ending synapses, indicating that genetic differences between strains likely include pathways regulating synapse formation. We also analyzed how two extrinsic factors, embryo media and testing arena size, affect these same behaviors in TLF-strain larvae. We tested 11 commonly-used media and found that methylene blue, Ca^{2+} concentration, and sodium bicarbonate significantly alter startle sensitivity, PPI, STH, and general locomotion. Arena size affects both startle sensitivity and overall locomotion, with larger arenas decreasing startle frequency but increasing spontaneous swimming and turning movements and altering thigmotaxis. These data demonstrate how intrinsic and extrinsic factors influence multiple behavioral endpoints, and they provide critical information for the field to improve experimental design and replicability.

285 - Mechanism of translocation of bacterial elements from the gut to internal organs in zebrafish

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Translocation of microbiota related products outside the intestine has been suggested to occur in people affected by gut-related chronic diseases. However, the translocation of viable bacteria has not been clearly demonstrated and mechanisms inducing bacterial translocation are poorly understood. We are using transparent zebrafish larvae to investigate the possible translocation of bacteria from the gut to internal organs, upon metabolic stress, and to assess its impact on the host physiology. To monitor gut colonization of zebrafish larvae, we fed ZF larvae with ciliates engorged with transformed *E. coli* strain expressing a fluorescent protein (mCherry2), which we observed with confocal microscopy. To spatially locate the signal in the gut, we used a zebrafish line expressing GFP within intestinal cell membranes.

Our preliminary results show the presence of mCherry2-positive bacteria in the gut lumen of ZF larvae. We also observed the presence of mCherry2 signal outside of the intestinal lumen, suggesting a translocation of bacterial products (or intact bacteria) through the intestine. We observed that mCherry2 signal accumulates into lysosome rich enterocytes, in accordance with findings from other groups. We next aim to test the hypothesis that specific gut microbiota metabolites alter the intestinal barrier by modifying expression or function of tight junction proteins, enhancing gut permeability. We also aim to examine the role of iNOS and Occludine in bacterial translocation, since these proteins have been implicated in humans for playing a role in the selectivity of the intestinal barrier.

Our experiments using the zebrafish model should help elucidate the molecular mechanisms of bacterial translocation from the host gut to internal organs and to eventually develop compounds to reduce or prevent this process, as well as to assess the impact of these manipulations on host physiology.

286 - Mechano-molecular control of heart morphogenesis

Kevin Manuel Méndez Acevedo[1], Motahareh Moghtadaei[1], Anca Margineanu[1], Anne Merks[1], Daniela Panakova[1]

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Linear heart tube (LHT), a transient structure present in all vertebrates, undergoes major tissue deformations while forming the first functional organ. The polarized tissue tension, torsion and anisotropic expansion, all contribute to the LHT embryonic remodeling. How the mechanical forces contribute to the heart formation and what developmental pathways and cellular processes regulate them is not fully explored. Here, we use theoretical modelling to demonstrate the tissue-scale supracellular polarization of actomyosin within the myocardial epithelium is essential for heart formation. Examining the molecular mechanisms governing the actomyosin activity along the heart tube, we demonstrate that both, cardiac-specific Myosin Light Chain Kinase 3 (Mylk3) and Rho-associated Protein Kinase 2a (Rock2a) regulate the actomyosin-based tissue forces through the phosphorylation state of the Myosin Regulatory Light Chain (MRLC). We find the preferential basal activity of Mylk3 and apical activity of Rock2a mediate not only the proper levels of phosphorylated MRLC, but also its polarized distribution along the apicobasal axis within the myocardium. Moreover, both Mylk3 and Rock2a are under genetic control of Planar Cell Polarity signaling, identifying Mylk3 as a novel tissue-specific effector of this pathway. We propose the antagonistic force-generating activities of Mylk3 and Rock2a facilitate mechano-molecular control of heart tube morphogenesis.

287 - bmp3 is a novel regulator of convergence and extension movements in the zebrafish pharyngeal skeleton.

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The acquisition of neural crest-derived structures, such as the pharyngeal skeleton, are hypothesized to have allowed for the transition from a sedentary, filter-feeding lifestyle to active predation, allowing for the explosive diversity observed in vertebrates today. However, the factors that contribute to the development of the pharyngeal skeleton, and, more specifically, the morphogenetic movements that take place to shape the individual pharyngeal skeletal elements, remain poorly understood. We have identified the gene *bone morphogenetic protein 3* (*bmp3*) as a regulator of zebrafish jaw development. Adult *bmp3* mutants have altered cranial morphology compared to wild type siblings, and micro-computed tomography scans of adult zebrafish indicate that *bmp3* mutants display significantly shorter premaxillae, maxillae, and mandibles compared to wild type siblings. Additionally, Alcian blue staining of larval jaw cartilage indicates that the chondrocytes of several pharyngeal skeletal elements are disorganized in *bmp3* mutants when compared to wild type controls, further implicating *bmp3* as a regulator of jaw development. *In-situ* hybridization reveals that *bmp3* is expressed in the pharyngeal arches at times when significant cell rearrangements are occurring in the pharyngeal skeleton, and pharmacological inhibition of Smad3 (the intracellular protein activated by Bmp3) in 12-hour intervals from 24 to 84 hpf indicate that *bmp3* is likely regulating convergence and extension (C&E) movements of jaw cartilage precursors. Consistent with this, several components of the non-canonical Wnt/planar cell polarity pathway (*fzd3b, fzd7a, vangl2, ankrd6b,* and *prickle1a*) are down-regulated in *bmp3* mutants when compared to wild type controls, suggesting that *bmp3* may be regulating C&E via this pathway. Current investigations include live imaging of pharyngeal C&E movements and analysis of the spatial distribution of the planar cell polarity components during C&E in wild type and *bmp3* mutant embryos. Taken together, this research will contribute to our understanding of the shaping of pharyngeal cartilage elements during development.

288 - Modeling human genetic variants in the Undiagnosed Diseases Network using genome editing technology

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Known or newly identified human genetic variants of unknown function can be validated in model organisms by introducing the same mutations into their genomes by genome editing. The clinical sites of the Undiagnosed Diseases Network (UDN) identify candidate DNA sequence variants in undiagnosed patients, submit the information to the UDN gateway database, and the multi-institutional Model Organism Screening Center (MOSC) tests the human variants in relevant model organisms. The Washington University Zebrafish-MOSC is modeling 13 UDN cases, including *BCL6 COREPRESSOR* (*BCOR*) and *Down Syndrome Cell Adhesion Molecule Like 1* (*DSCAML1*), submitted by the UCLA Clinical Site. To assess pathogenicity of such human variants, we have been using a conventional CRISPR/Cas9 approach or prime editing to knock-in variants into the orthologous zebrafish genes. Here, we present modeling of two human variants. First, a novel 18 bp in-frame deletion p.G1464_R1469del of *BCOR* on X chromosome was associated with eye abnormalities. We generated two frame-shift indel mutants and an in-frame 7 amino acid deletion mutation, which is highly similar to the human mutation. Adult mutants homozygous for the frame-shift alleles show loss of dorsal, pelvic, and/or anal fins with craniofacial abnormalities. The in-frame deletion homozygotes have all the fins, but mutant larvae exhibit abnormal behavior, suggesting a neomorphic nature of the human mutation. Second, a *de novo* p.V1237L heterozygous autosomal mutation in *DSCAML1* was associated with idiopathic epilepsy. DSCAML1 protein, including p.V1237, is conserved in the zebrafish genome. Using prime editing, we generated two frame-shift alleles, which are lethal at 8-10 dpf. We also generated a p.V1237L knock-in allele, but homozygotes for this mutation develop into morphologically normal adults. However, RNA-seq and ongoing phenotypic studies suggest a pathogenic nature of the Dscaml1 p.V1237L variant. Taken together, our studies demonstrate that zebrafish is a useful platform to link human genetic variants and pathological symptoms.

289 - Analyzing 3D cell and nuclear shape in zebrafish lateral line neuromasts

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The lateral line is a sensory system that fish use to detect changes in water flow. Lateral line organs, called neuromasts, are composed of clusters of sensory hair cells surrounded by nonsensory supporting cells. Supporting cells can be further divided into subtypes with distinct locations, transcriptional profiles, and behaviors. However, it is not known whether neuromast cell subtypes have distinct shapes. Our goal is to understand the variation in three-dimensional (3D) cell and nuclear shape in neuromasts. We also wish to study how well cell and nuclear shape states correspond with markers of neuromast cell identity. Using confocal microscopy, we imaged live five day old transgenic zebrafish that express membrane-bound GFP in neuromast cells (*Tg:cldnb:lyn-eGFP).* Nuclei were stained using the far red dye DRAQ5. Cells and nuclei were segmented using a semi-automated, Python-based workflow. To analyze 3D cell and nuclear shape, we used a pipeline developed by the Allen Institute for Cell Science. Briefly, this pipeline uses spherical harmonics expansion (SHE) to represent the shapes of cells using several hundred coefficients. We found that SHE coefficients are sufficient to accurately reconstruct most neuromast cells and nuclei. Following SHE, we used Principal Component Analysis (PCA) and clustering to visualize the main modes of shape variation within the dataset. Hair cells, peripheral supporting cells, and interdigitating supporting cells formed distinct clusters from each other. There was also a cluster that appeared intermediate between peripheral supporting cells and hair cells. Additionally, a marker of dorsoventral supporting cell identity was expressed more strongly in certain shape clusters, suggesting dorsoventral cells have distinct shapes. We are currently studying how cell and nuclear shapes differ in mutants that cannot produce hair cells. Insights from this work could eventually be leveraged to understand cell shape states and transitions during dynamic processes such as hair cell regeneration.

290 - Plakin Cytolinkers: Coordinators of Microridge Morphogenesis

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Plakins are a diverse family of proteins that organize and coordinate cytoskeletal elements. We previously found that two members of this family, periplakin and envoplakin, are critical for the morphogenesis of microridges in the outer periderm layer of the zebrafish epidermis. Microridges are elongated protrusions arranged in maze-like patterns on the apical surface of many kinds of mucosal epithelial cells. Periplakin and envoplakin are two members of the plakin family that form heterodimers or oligomers with each other and bind both F-actin and keratin filaments. Periplakin deletion analyses revealed that its actin-binding N-terminal region is required for initiation of microridge morphogenesis, while its keratin-binding C-terminal region is required for microridge elongation. In addition to their F-actin- and keratin-binding activities, these plakins contain many protein-protein interaction domains that likely enable them to coordinate various cytoskeletal regulatory activities to promote microridge morphogenesis. For example, the plakin N-terminal domains contain an unmapped actin-binding domain, 4 spectrin repeats, and an SH3 domain. Envoplakin and periplakin both contain these domains and both are required for microridge morphogenesis, but the distinction between their roles is unknown. To better understand the relationship between plakin structure and function, we are using a combination of mutagenesis techniques and immunoprecipitation-mass spectrometry. First, to identify the function of specific regions within the N-terminal domain, we are making mutant versions of periplakin and will express them in periplakin knockout mutants to determine if they rescue microridge formation and/or elongation. Then, to elucidate functional differences between envoplakin and periplakin, we will use chimeras to determine unique regions required for microridge morphogenesis. Additionally, we will discover plakin interacting proteins by immunoprecipitating GFP-tagged periplakin from embryos and use mass spectroscopy to identify associated proteins. Together, these approaches will shed light on the ability of plakins to coordinate cytoskeletal activities resulting in the creation of microridges.

291 - Optogenetic investigation of microglia dynamics during zebrafish brain development

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Microglia are brain cells involved in different functions, such as immune response or brain development. Through their phagocytic activity, microglia can eliminate cells or prune synapses, thereby controlling the formation of neuronal networks. A potentially important influence in this process is the intestinal microbiota, which has been shown to modulate the microglia. To learn more on the impact of the gut microbiota on the phagocytic function of microglia during CNS development, we are establishing an optogenetic larval zebrafish model.

We are using a cross between two transgenic lines to visualize glutamatergic neurons in red (DsRed) and microglia in green (GFP). Larvae from 3-16dpf (day post-fertilization), either rendered germ-free or colonized with specific bacteria, are imaged live using confocal or 2-photon microscopy. We are developing deep-learning based tools to categorize microglia morphology and quantify phagocytosis.

Our preliminary results suggest that microglia change their morphology during the development of zebrafish brain circuits. In the optic tectum, they are minimally branched and very mobile in 3-5dpf larvae. At 5-6dpf, their protrusions are longer and more branched. At 7dpf, microglia are mostly immobile, while their branched protrusions are moving to scan the nearby environment.

Phagocytic function is detected by the inclusion of DsRed from the neurons inside the microglia, as phagosome-like structures. We find that the extent of phagocytosis differentially changes over the 3-7dpf period.

We are now comparing how this activity is affected by changes in gut microbiota. We aim to exploit this model to characterize the role of microglia in synaptic pruning.

292 - Macrophages and neutrophils are necessary for ER stress-induced β cell loss

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Persistent ER stress induces islet inflammation and loss of β cells. How islet inflammation contributes to β cell loss remains uncertain. We have previously reported that chronic overnutrition-induced ER stress in β cells causes Ripk3-mediated islet inflammation, macrophage recruitment, and β cell loss in a zebrafish model. We show here that β cell loss results from the intricate communications among β cells, macrophages and neutrophils. Macrophage-derived Tnfa induces cxcl8a in β cells. Cxcl8a in turn attracts neutrophils to macrophage contacted “hot spots” where β cell loss occurs. We also show potentiation of chemokine expression in stressed mammalian β cells by macrophage-derived TNFA. In Akita and db/db mice, there is an increase of CXCL15-positive β cells and intra-islet neutrophils. Blocking neutrophil recruitment in Akita mice preserves β cell mass and slows diabetes progression. These results reveal an important role for neutrophils in persistent ER stress-induced β cell loss.

293 - Intestinal immune response, one decision and two roads: oral tolerance or inflammation

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The intestines of vertebrates, including mammals and fish, is constantly exposed to a multitude of foreign material that may either be harmful or beneficial for the organism. Consequently, the intestinal immune system must discriminate between pathogens/toxins and commensal bacteria/food antigens to induce either an inflammatory or tolerogenic response, respectively. Oral tolerance is defined as the local (the small intestine in mammals) and systemic immune unresponsiveness to innocuous food antigens. On the contrary, inflammation is the process by which the immune system responds to a harmful stimulus, removing it and thus restoring homeostasis.

Combining cellular and molecular approaches we are analyzing *in vivo* how oral tolerance is established in the intestine of zebrafish larvae and how this differs from the induction of inflammation. We are focused in determine which immune cells are recruited to the intestinal epithelium to take up antigens, as well as when and where antigen-loaded cells interact with other key immune cells to establish and maintain oral tolerance or conversely to trigger an inflammatory process.

The results obtained showed that macrophage is the main immune cell type that take up antigen in the gut *in vivo*, both during tolerance and inflammation. In addition, we distinguished two macrophage populations taken up antigen, one small-rounded and the other big-stellate. During the establishing of oral tolerance, the percentage of each population that take up antigen is similar. In contrast, when oral tolerance is already established the percentage of rounded macrophage that take up antigen is significantly higher. During inflammation, mainly stellate macrophage takes up antigen. At the moment, we are analyzing the interaction of both macrophage populations with T lymphocytes in the gut.

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294 - Deciphering the cellular and molecular mechanisms of celsr3 dependent CNS regeneration

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Functional regeneration requires injured axons to traverse the injury site and sustain growth toward their targets. The mammalian Central Nervous System (CNS) has retained limited capacity for axonal regeneration. Unlike mammals, axons in the zebrafish CNS regenerate spontaneously. To decipher the underlying molecular mechanisms of this regenerative capacity, we previously established a laser mediated transection assay for Mauthner axon regeneration in live, 5-day-old zebrafish larvae. The Mauthner neurons, a pair of bilateral hindbrain neurons, each extend a single, myelinated axon to the tip of the spinal cord, providing a powerful model to study long distance CNS regeneration with single axon resolution. Using live-cell imaging we discovered that Mauthner axon regeneration is biphasic as axons initially cross the injury site at a slow rate before switching to sustained axonal regrowth at 3 times the rate. We identified the Cadherin EGF LAG Seven-Pass G-Type Receptor (*celsr3)* to be dispensable for the initial processes of axonal regeneration, including growth cone formation and rate of growth across the injury site. Instead, we find that axons in *celsr3* mutants achieve only half the growth rate of siblings after crossing the injury site. Moreover, axons in *celsr3* mutants regenerate to only 25% of their sibling length, revealing that the *celsr3* dependent switch in axonal regrowth rates is critical for CNS regeneration. Celsr3 is a core component of the planar cell polarity (PCP) pathway, extensively studied in development, yet previously unrecognized for its role in CNS regeneration. Here, we combine live-cell imaging and molecular genetics to determine the function of *celsr3* in CNS regeneration. Ongoing work will define the cell autonomy and signaling pathway by which *celsr3* promotes regeneration. Combined, this work reveals how a previously unrecognized regulator of axonal regeneration promotes CNS regeneration.

295 - Hyperaminoacidemia induces pancreatic α cell proliferation via synergism between mTORC1 and CaSR-Gq signaling pathways

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Glucagon has emerged as the main regulator of extracellular amino acid (AA) homeostasis. Insufficient glucagon signaling results in hyperaminoacidemia, which drives adaptive proliferation of glucagon-producing α cells. Aside from mammalian target of rapamycin complex 1 (mTORC1), the role of other AA sensors in α cell proliferation has not been described. Here, using *gcgr*-deficient zebrafish (*Danio rerio*) and primary mouse islets, we show α cell proliferation requires the AA-sensitive calcium sensing receptor (CaSR), and downstream extracellular signal- regulated protein kinase (ERK1/2). Inactivation of CaSR dampened α cell proliferation, which can be rescued by re-expression of CaSR or activation of the downstream Gq, but not Gi, signaling in α cells. CaSR was also unexpectedly necessary for mTORC1 activation in α cells. Furthermore, co-activation of Gq and mTORC1 induced α cell proliferation independent of hyperaminoacidemia. These results reveal another AA sensitive mediator and identify major pathways necessary and sufficient for hyperaminoacidemia-induced α cell proliferation.

296 - Zebrafish Huntingtin (HTT) promotes CNS axonal regeneration

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Following injury, axons in the Central Nervous System (CNS) initiate molecular pathways to respond and regrow. Unlike most mammals which exhibit a very limited capacity for axonal regrowth in the CNS, zebrafish exhibit robust and functional regeneration, providing a powerful system to decipher the molecular and cellular mechanisms of spontaneous CNS regeneration. To visualize and quantify axonal regeneration in the spinal cord we previously established a laser-mediated assay to transect Mauthner cell (M-cell) axons (Bremer et al.,2019). Following transection, M-cell axons reform a growth cone, cross the injury site and regrowth to their preinjury length within 96 hours. Using a candidate gene approach, we recently identified the Huntingtin (HTT) gene to be required for M-cell regeneration. Like wild type siblings, M- cell axons in HTT mutants form a regenerative growth cone, yet unlike wild type siblings, majority of M- cell axons in HTT mutants fail to recover to their pre-injury axon length and about 40% of mutant M-cell axons display a more severe phenotype, failing to cross the injury site, suggesting a role for HTT to promote injury site crossing. A functional role for HTT in spinal cord regeneration has previously not been reported. Using a transgenic rescue approach, I will determine whether HTT promotes regeneration via a neuron intrinsic or via an extrinsic mechanism. I will also determine whether HTT is selectively required for spinal cord regeneration by assaying axonal regeneration of RGC axons in the optic nerve as well as peripheral motor axons. Combined, the results from these experiments will provide the first insights into the molecular and cellular mechanisms by which an evolutionary conserved gene previously not implicated in this process promotes spontaneous CNS regeneration.

297 - Formation and epigenomic foundation of long range cis-regulatory landscape during zebrafish embryonic development

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The DANIO-CODE consortium created a central repository for zebrafish developmental functional genomic data. Its Data Coordination Center (https://danio-code.zfin.org) currently holds 1,802 unpublished and reanalysed published genomics datasets minablele in genome browsers. Here we report exploitation of this multi-omics database for comprehensive enhancer annotation and novel biological observations on gene regulatory principles during embryonic development. We identified and annotated >140,000 cis-regulatory elements, which we classified into functional categories (including novel classes) and assigned to cell-type specificity by whole embryo single-cell ATAC-seq. Our integrated genome-wide analyses revealed the developmental dynamic of cis-regulatory topology organisation. From zygotic genome activation, through gastrulation, to early somitogenesis the activation of developmental regulator genes is facilitated by promoter proximal regulatory elements. They overlap with large 'super-enhancer'-like H3K27ac domains, which we called H3K27ac ensembles. These ensembles are enriched in active gene promoters and facilitate chromatin interactions during topologically associating domain (TAD) formation. The ensembles are decommissioned during organogenesis and cis-regulatory elements of developmental regulator genes are scattered at long-range throughout the entire TAD. Our observations argue for a dynamic function of H3K27ac in topology organisation and regulatory grammar, different from previously described super-enhancers. To further explore the functional relevance of uncovered epigenomic features, we explored their evolutionary conservation. However, establishing conservation of epigenomic patterns (e.g. H3K27me3 or H3K27ac) across vertebrates is challenging due to the inability to establish their equivalence in the absence of direct sequence similarity. To meet this challenge, we developed a multispecies synteny-anchoring approach to detect conservation in epigenomic domains between zebrafish and mouse. We found remarkable similarities in H3K27me3 coverage of subTAD domains associated with orthologous Polycomb target genes, as well as similarities in architectures of epigenomic domain-associated regulatory elements. Our comparative epigenomic tool also demonstrated evolutionary conservation of H3K27ac ensembles suggesting their pan-vertebrate role in subTAD organisation.

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298 - Characterization of photoreceptor microvilli in zebrafish

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The photoreceptor outer segment (OS) is a modified cilium filled with photopigment-laden membranous disks. Surrounding the base of the OS are calyceal processes (CPs), microvilli-like protrusions with an actin core. While CP disruption has been associated with altered OS morphology and loss of vision, the role of the processes remains elusive. In this project, we use zebrafish as a model to investigate the structure and function of CPs. By analyzing immunostained eye sections of the adult zebrafish retina, I have determined CP number, absolute length, length relative to OS height, and organization. Although CP length is comparable between rod and UV cone photoreceptors (5.9±0.6 and 6.6±0.9 µm), there is a stark difference relative to the respective OS height (30% versus 80%). Despite the claims that CPs might participate in retinomotor movements in teleosts, we did not observe any difference in CP length between light- and dark-adapted retina. Remarkably, we also found a close association between cone CPs, villous extensions of adjacent retinal pigment epithelium, and rod inner segments, all arranged in a regular pattern around the UV cone OSs. Detailed transmission electron microscopy imaging of the larval zebrafish retina revealed that CPs are present when the OS is beginning to emerge. To access CP actin dynamics, we introduce heat shock inducible expression of tagged actin using the Tol2 transposase system and compare new actin localization to whole actin staining after induction in the larval and adult retina. Larval photoreceptors feature a fully dynamic CP actin core including in the actin roots extending into the inner segment. In summary, our data provide basic characteristics of CPs in zebrafish, suggest variable CP-OS interactions between photoreceptor subtypes, and indicate a close relationship between CPs and surrounding tissues.

299 - Developing a High-throughput Zebrafish Brain Activity Mapping Pipeline to Analyze Autism Risk Gene Function

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Autism spectrum disorder (ASD) is a clinically and genetically heterogeneous neurodevelopmental disorder characterized by deficits in social communication and restricted, repetitive behaviors. Recent whole exome sequencing studies of individuals with ASD have identified >100 high confidence ASD risk (hcASD) genes. However, our understanding of risk gene function in neurodevelopment is limited, yielding a need to develop high-throughput *in vivo* approaches. Using CRISPR/Cas9, we generated zebrafish mutants of 10 hcASD genes to investigate how loss of hcASD gene function affects brain activity and structure during early neurodevelopment. We developed a high-throughput analysis pipeline that measures regional changes in brain volume and activity from immunostaining of active (pERK) and total neuron (tERK) populations in whole-mount larval zebrafish brain. We quantify activity by mapping confocal images onto a standard zebrafish reference brain and calculating the ratio of pERK/tERK across 149 brain regions, using the Z-brain atlas. This allowed us to identify candidate regions from the Z-brain atlas based on altered size or neural activity in 10 mutant lines. We automated image registration and ran parallel analyses utilizing High Performance Computing (HPC) Systems across the 10 mutant lines. We visualized significant differences in baseline brain activity in mutants. We identified unique phenotypes involving differences in regional brain volume and activity across mutants. This novel pipeline will help us uncover the molecular mechanisms underlying altered neurodevelopment in ASD and expand our understanding of how mutations in unique hcASD genes might lead to differences in brain activity.

300 - Zebrafish Panx1 channels promote both anti- and pro-seizure-like activities via p2rx7 receptors and ATP signaling

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The molecular mechanisms of excitation/inhibition imbalances promoting seizure generation in epilepsy patients are not fully understood. Evidence suggests that Pannexin1 (*Panx1*), an ATP release channel, modulates the excitability of the brain. In the mouse others and we have demonstrated that targeting Panx1 channels is anti-convulsant. In this report, we exploited the evolutionary divergence of mammalian and fish and tested anti-convulsant activities of the zebrafish paralogs Panx1a and Panx1b. We performed electrophysiological, behavioral, and molecular phenotyping experiments on zebrafish larvae bearing genetic or pharmacological knockouts of Panx1a and Panx1b channels, each homologous to human PANX1. When Panx1a function is lost, or both channels are under pharmacological blockade, seizures with ictal-like events and seizure-like locomotion are reduced in the presence of pentylenetetrazol. Transcriptome profiling by RNA-seq demonstrates a spectrum of distinct metabolic and cell signaling states which correlate with the loss of Panx1a. Furthermore, the pro- and anticonvulsant activities of both Panx1 channels affect ATP release and involve the purinergic receptor P2rx7. Our findings suggest a subfunctionalization of Panx1 enabling dual roles in seizures, providing a unique and comprehensive perspective to understanding seizure mechanisms in the context of this channel.

301 - Selective targeting and mRNA delivery of zebrafish scavenging endothelial cells with lipid nanoparticles as an hepatic mammalian model system

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Scavenging of macromolecular waste from blood plasma is a common feature of sinusoidal endothelial cells in diverse vascular beds, most prominently in the liver, spleen and bone marrow. These 'scavenging endothelial cells' –or SECs– are specialized in receptor-mediated endocytosis and lysosomal degradation of a wide variety of ligands using a limited number of promiscuous transmembrane receptors. They also provide a niche for macrophages (*e.g.* the Kupffer cells of the liver) that remove colloidal material such as nanoparticles (important in drug delivery applications) and aging (red) blood cells.

Previously, we identified that the early venous endothelium of the zebrafish is primarily composed of SECs and acts as a model for mammalian sinusoidal endothelial cells.[1] Zebrafish SECs clear the blood plasma from macromolecular waste and colloids, including proteins, DNA, RNA, lipoproteins and viral particles, from the onset of circulation. We found the clearance of anionic nanoparticles is mediated by Stabilin-1 and Stabilin-2 receptors, where surface charge and size are key parameters determining their functionality.[2]

Recently, the SECs-nanoparticle interaction, mediated by Stabilin-1 and -2, was exploited to rationally design a lipid nanoparticle formulation able to preferentially target the hepatic reticuloendothelial system (RES) and deliver mRNA. Here, a single lipid was replaced within the lipid composition of the clinically approved Onpattro® to design srLNPs. This replacement changes the surface charge, from neutral to anionic, allowing the redirection of srLNPs to preferentially target hepatic RES. We showed the biodistribution of srLNPs in the zebrafish embryo and the subsequent preferential intracellular delivery of the carried mRNA.[3]

This research demonstrates the versatility and predictability of the zebrafish as a valuable model system to study mechanisms involved in nanoparticle-mediated drug delivery. In addition, it reveals that RNA can be preferentially delivered to zebrafish SECs with srLNPs, opening up opportunities in vascular biology studies where SECs regulation is needed.

302 - NMDA receptor mutation dysregulates neuroblast proliferation generating supernumerary neurons in the forebrain of zebrafish larvae

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Normal brain development depends upon precise spatiotemporal regulation of neural stem and progenitor cells (NSPC). N-Methyl-D-Aspartate receptors (NMDAR) are glutamate-gated cation channels known to play essential roles in dynamic neurodevelopmental events yet their role in the regulation of neurogenesis is not fully understood. Some studies indicate suppression of NMDAR-mediated signaling promotes neurogenesis, while other studies support the opposite view.

To probe the role of NMDARs in neurogenesis we developed a mutant zebrafish line that lacks all NMDAR-mediated signaling (*grin1* double mutants). These fish survive far beyond the comparable age of rodent knockout models, thereby providing a singular opportunity to examine the role of NMDARs in all stages of neurodevelopment.

We performed detailed quantification of 12 different forebrain cell populations in 3 days post fertilization (dpf) zebrafish. We found that relative to wild type fish, *grin1* double mutants show significantly increased cell densities in the anterior regions of the forebrain. At 5dpf, increased cell densities were observed in all forebrain cell populations. We performed Immunohistochemistry (IHC) against GFAP and PSA-NCAM to ensure that these increased cells were neurons and not glial cells. Morphometric analysis demonstrated that increased neuronal density occurs without any gross anatomical changes to the brain. Activated caspase-3 IHC indicated that the supernumerary neurons did not result from diminished programmed cell death. We then assayed the NSPCs using IHC to determine the origin of dysregulation. We find that *grin1* double mutants have a higher percentage of mitotic neuroblasts, as indicated by PCNA expression, which inappropriately amplifies the neuronal population.

These data suggest that NMDAR signaling is required for suppression of neurogenesis in the neuroblast transit amplifying cell populations, without which proliferation progressively increases unchecked. Furthermore, as NMDAR mutations are correlated with neurodevelopmental diseases (NDD), as are supernumerary neurons, this work also suggests an unexplored mechanism of NMDAR-mediated NDD etiology.

303 - Functional central vestibular topography emerges in developmental time in the larval zebrafish

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Neuronal birthdate predicts many anatomical and functional properties of individual neurons, but the complexity of most circuits has made the relationship between birthdate and circuit-level organization previously intractable. We leveraged a simple model – the larval zebrafish vertical gaze stabilization reflex – to determine how neuronal birthdate organizes a functional sensorimotor circuit. To transform vertical pitch-tilt rotations (e.g., nose-up) into corrective eye rotations (e.g., eyes-down), the vertical gaze stabilization circuit uses a precisely-organized three-neuron arc of vestibular sensory afferents, central brainstem projection neurons, and extraocular motor neurons. To determine how canonical nose-up and nose-down subtypes are specified and integrated into circuit architecture, we examined their development in space and time. We designed a vertical rotation stimulus, compatible with both two-photon and volumetric, single-objective light-sheet (SCAPE) microscopy, to differentiate cardinal subtypes of central vestibular neurons. To correlate subtype function with development, we presented this stimulus to anatomically-birthdated neurons. We discovered that neuronal birthdate predicts both the cardinal subtype identity and somatic localization of individual vestibular neurons, and that somatic localization anticipates subtype identity. Neuronal birthdate further predicted other functional properties, such as responses to non-preferred directional rotations and to impulse rotations. Lastly, neuronal birthdate predicted subtype integration with both upstream semicircular canal inputs and downstream extraocular motor neuron outputs. Our findings demonstrate that circuit organization emerges as early as when neurons become post-mitotic and suggest a key mechanism: spatiotemporally-available molecular cues. Currently, we are investigating the functional and transcriptional contributions of motor, sensory, and extrinsic signals to vestibular circuit organization. With a genetic loss-of-function tool, we demonstrated that motor-derived signals are dispensable for central vestibular development. Our work speaks to the developmental principles that organize functional sensorimotor circuits.

304 - Strength of interactions in the Notch gene regulatory network determines lateral inhibition patterning

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Gene regulatory networks (GRNs) determine the generation of diverse gene expression patterns, which in turn regulate cell fate during the development of multicellular organisms. However, how the topology and parameters of GRNs determine this patterning remain largely unknown due to the complexity of most experimental systems. To address this, we use the zebrafish notochord, an organ where coin-shaped precursor cells are arranged in a simple unidimensional geometry. These cells differentiate into vacuolated and sheath cells in a Notch-dependent manner. We identify *jag1a* and *her6*/*her9* as the main components of an unconventional GRN that generates a lateral inhibition pattern, a possibility previously thought to be restricted to the other Notch ligands. Using a mosaic gene overexpression approach, we identify that these genes are sufficient to determine sheath (*her6* and *her9)* and vacuolated (*jag1a*) fate in the cells where they are expressed. Making use of this experimental system and mathematical modeling we show that such lateral inhibition patterning requires that ligand-receptor interactions are stronger within the same cell than in neighboring cells. Altogether, we establish the zebrafish notochord as an experimental system to study Notch patterns, and identify and characterize a GRN that determines gene patterning and drives cell fate.

305 - Variants in the human MYLPF gene disrupt sarcomere organization in zebrafish

Tayo Adekeye[1], Sabrina Varga[1], Emily Tomak[1], Dmitrii Krivorotko[1], Jared Talbot[1]

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MYLPF is a myosin regulatory light chain protein that binds tightly to the head of the myosin heavy chain, stabilizing the lever arm structure as it connects with actin. Recently, specific MYLPF variants were identified exclusively in patients with Distal Arthrogryposis (DA), a skeletal disease thought to arise by immobility in utero, but the impact of these gene variants on muscle structure had not been determined. In that study, we presented a zebrafish mylpfa knockout that mirrors aspects of the human disease. We now show a fin skeletal defect in mylpfa that mirrors the contracture defects that characterize DA and is likely caused by the fin paralysis seen in the mutant. This fin paralysis is due to a failure of sarcomere assembly in the fast-twitch muscle of the mylpfa mutant. This defect in sarcomere structure also manifests in the truck muscle and contributes to reduced peak velocity of the larvae during swimming. To test whether Mylpf is functionally conserved we expressed human MYLPF in the zebrafish mylpfa mutant and find restored sarcomere structure. Two of the MYLPF gene variants found in DA patients (MYLPF-G163S and MYLPF-C157F) were then expressed in the wild type and in mylpfa mutant zebrafish. The recessive variant (C157F) could partially, but not fully, restore sarcomere formation. The dominant gene variant (G163S) could not rescue sarcomeres at all and in some cases even worsened the defect. Furthermore, the G163S allele disrupts sarcomere organization even in wild-type embryos. These findings demonstrate that these human gene variants may cause disease via a failure of sarcomere assembly and suggest that inheritance patterns may be governed by hypomorphic (C157F) versus antimorphic (G163S) activity.

306 - Investigating the Role of Complement System Activation in Spine Curvature Formation in Zebrafish Models of Idiopathic Scoliosis

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Idiopathic scoliosis (IS) is an irregular curvature of the spine, which occurs in 4% of humans in the absence of underlying muscular or congenital vertebral defects. Recent advances in scoliosis research have resulted in the development of multiple zebrafish models of IS such as the *protein tyrosine kinase 7a* (*ptk7a)* mutant and *SCO-spondin* (*sspo*) mutant. A major similarity between these two models is the presence of inflammatory signatures, including, but not limited to, a strong upregulation of complement immune factors.

The complement system is part of the innate immune response, a non-specific response classically defined as the first line of defense to damaged host cells. This response is controlled by soluble proteins which recognize said cells via pattern-recognition sites, thereby marking the cells for clearance and/or triggering acute local inflammation. The upregulation of complement genes in *ptk7a* and *sspo* models is intriguing because it offers an immune mechanism that could be responsible for the observed neuroinflammation in zebrafish. Here, we investigate the causal relationship between complement activation and IS in zebrafish.

To test the functional consequence of modulated complement activity in IS models, I utilized CRISPR/Cas9 mutagenesis to generate “transcriptless” loss-of-function alleles of *complement component 5* (*c5*), *regulator of complement activation group 2 gene 1* (*rca2.1*), and *regulator of complement activation group 2 gene 2* (*rca2.2*). *c5* mutants were generated to assess the effect of losing the terminal complement pathway in IS models, while *rca2.1* and *rca2.2* double mutants were generated to assess the effect of exacerbated complement activation. In addition to creating complement mutants, I generated fish with transgenic overexpression of *complement component 3* (*c3*) to assess the effect of increased complement activation on axial development. By modulating complement activity in both wildtype animals and IS models, we hope to further understand how the inflammatory response regulates spine development.

307 - A novel role for NMDA receptors in neural crest development

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The neural crest (NC) is a migratory, pluripotent cell type of neuroepithelial origin that gives rise to a host of neural and non-neural tissues including the autonomic, enteric, and peripheral nervous systems, as well as cartilage, cardiac and pigment cells. Given the ubiquity of NC-derived cells, and their implication in multiple disease states, a more thorough understanding of their regulation is essential.

N-Methyl-D-Aspartate receptors (NMDAR) are glutamate-gated cation channels that play a key role in excitatory calcium transmission. Though their function in dynamic neurodevelopmental events is well described, their part in NC development has been virtually unexplored. Surprisingly, we found that zebrafish mutants that lack the obligatory NMDAR subunit (*grin1* double mutants) show defects in multiple NC-derived tissues.

We assayed craniofacial cartilage and pigmentation, both NC-derived populations, in *grin1* double mutants and wild type controls, at 5 days post fertilization (dpf). Craniofacial cartilage was examined using two methods. First, zebrafish larvae were embedded in paraffin and serially sectioned in 5µm coronal sections; sections were Nissl stained for visualization and assessed for the presence of ectopic cartilage growth. Additionally, whole-mount embryos were stained with Alcian Blue dye for histological analysis of craniofacial cartilage morphology. We found that although craniofacial abnormalities are a rare occurrence in wild type fish – appearing in less than 15% of fish assayed and only in very mild forms – 100% of *grin1* double mutants assayed displayed severe craniofacial abnormalities. Live imaging of 6dpf zebrafish larvae revealed that *grin1* double mutants display hyperpigmentation over the entire anterior to posterior body axis.

Our work is the first to demonstrate a role for NMDAR-mediated signaling in the regulation of NC-derived cell types. Our future plans include examination of the autonomic and peripheral nervous systems of *grin1* double mutants.

308 - Osteoclast activity sculpts craniofacial form to permit sensorineural patterning in the zebrafish skull

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Efforts to understand the morphogenesis of craniofacial structures have previously focused on chondrocytes and osteoblasts. Bone–resorbing osteoclasts act in balance with osteoblasts to maintain adult skeletal structures, but there is limited information on their role in craniofacial development. Using a *cathepsinK* reporter, we documented osteoclasts daily in individual developing zebrafish skulls over several weeks, from 5.18 mm to 9.6 mm standard length (15 to 34 days post fertilization). While distribution of osteoclasts is consistent across individuals, they are sparse and their locations vary between fish and developmental time points. Interestingly, we observed osteoclasts concentrating at areas associated with neuromasts and nerves, in particular at the hyomandibular foramina and the supraorbital lateral line, areas of active remodeling. In contrast, other areas of rapid bone growth, like the osteogenic fronts of the frontal and parietal bones, do not show a concentration of osteoclasts, suggesting a special role in shaping bone surrounding neuromasts and nerves. In *csfr1a*^{mh5} mutants lacking functional osteoclasts, the morphology of the cranial bone was disrupted in both areas. The hyomandibular foramen is present in the initial cartilage template, but after the initiation of ossification, the area of the canal opening is decreased by 85.5% in the absence of osteoclasts. The diameter of the supraorbital lateral line canals was also reduced in the mutants, as was the number of pores which allow for the passage of efferent nerves from the neuromasts through the bone. We have found previously unappreciated critical roles for osteoclasts in shaping craniofacial skeletal structures. In particular, they act to model bone around peripheral cranial nerves, providing a scaffold for the sensioneural system during craniofacial development. This has important implications for the formation of the evolutionarily diverse lateral line system, as well understanding the mechanism of neurologic sequelae of congenital osteoclast dysfunction in human craniofacial development.

309 - Wnt, FGF, and Notch signaling modulate progenitor fate in the zebrafish lateral line

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Although mammals are unable to functionally regenerate sensory hair cells in their inner ear, hair cells in the zebrafish inner ear and lateral line are readily regenerated following injury or loss. This regeneration is mediated by proliferation of neighboring supporting cells that divide to produce both new hair cells and replacement supporting cells. In the lateral line neuromasts, the mantle cells that lie at the outside edge of the sensory epithelium rarely divide in response to normal levels of damage, but can re-enter the cell cycle following more significant damage. We have begun to explore differences in the fates of mantle cells and support cells in response to varied pharmacological manipulation. By activating Wnt signaling and simultaneously inhibiting FGF signaling, we are able to convert supporting cells back into migratory progenitors that mimic the primordium that deposits protoneuromasts during development. These migratory progenitor cells will, upon washout of the drugs, reform into neuromasts and produce innervated hair cells. The reformation of the neuromasts appears to be coordinated by Notch signaling much like the initial development of the neuromasts, as inhibition of Notch leads to significant overproduction of hair cells at the expense of supporting cells, though mantle cells are still produced. This suggests that Notch signaling may not be necessary for differentiation of mantle cells, but that the progenitors require Notch signaling in order to produce a balance between hair cells and supporting cells. In contrast, we find that Notch is required in order for regeneration of mantle cells from supporting cells following targeted ablation. This suggests that Notch may be a critical factor for supporting cell progenitors to make key cell fate decisions as they reproduce specific cell types, but that mantle and supporting cell progenitors may utilize different pathways for establishing their fates.

310 - Embryo-larval zebrafish, a multi-scale model to study the neurotoxicity of methylmercury and the protective effects of selenoneine.

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Methylmercury (MeHg) accumulates in marine predators regularly consumed by Inuit people. This neurotoxin is especially worrisome in the context of maternal transfer during pregnancy since MeHg can cross both placenta and blood-brain barriers. Early developmental stages are particularly vulnerable to MeHg toxicity that can cause *a posteriori* multi-scale effects. However, recent studies found selenoneine, an anti-oxidative molecule that reportedly forms a complex with MeHg, to be enriched in red blood cells of northern Canadian populations. This discovery led to the hypothesis that consumption of selenoneine-rich traditional food could afford protection against MeHg neurotoxicity.

To test this hypothesis, we rely on embryo-larval zebrafish (ZF) conducting MeHg waterborne exposures combined or not with a selenoneine pretreatment. We are developing means to introduce selenoneine in ZF larvae to test its putative protective effects. We are thus optimizing quantitative analyses to measure bioaccumulations of MeHg and selenoneine in ZF larvae by isotopic dilution gas chromatography with inductively coupled plasma mass spectrometry (ID-GC-ICP-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS), respectively. To characterize MeHg toxicity, we are carrying out assays at sublethal concentrations on embryo-larvae development, including heart rate measurement, growth profiles and apoptosis assays. We are also investigating the impact of MeHg on neuronal circuits development using fluorescent microscopy with immunostaining and transgenic ZF lines that carry optogenetic indicators. Preliminary results suggest that MeHg can induce a rise in brain cell apoptosis. We will then exploit a fluorescent sensor of Hg^{2+} to monitor its accumulation within different tissues, as a result of MeHg demethylation in live ZF larvae.

These experiments aim to further understand how MeHg exposure induces cognitive impairments. Our findings on the MeHg-selenoneine interaction may provide useful data to populations that rely on seafood consumption. Ultimately, this experimental model may generate new knowledge regarding human neuropathologies associated with MeHg neurodevelopmental toxicity.

311 - Excess glucose or fat differentially affects gene expression during zebrafish embryogenesis

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Several studies in vertebrates have shown that excess nutrients can change gene expression. The two main forms of energy fuel can be found in the form of sugar (glucose) or fat (triacylglycerides (TAGs)). During development, zebrafish embryos use their yolk sac reserve as the sole nutrient source and will only start to feed by themselves at around 132 hours post-fertilization (hpf) when their mouth will open. We have previously shown that the zebrafish yolk sac is full of different lipids with the majority of lipids deposited in the yolk sac by the mother being cholesterol, phosphatidylcholine, and TAGs and that the proportion of lipids in the yolk sac evolves as development progresses. Our laboratory and others have shown that we can modify the embryonic glucose and free fatty acid (FFA) and TAG content in zebrafish embryos. We exposed zebrafish embryos to 3% glucose (24-72 hpf) and injected 2 nL of BODIPY-FL C12:0 dissolved in canola oil directly into the yolk sac at 24 hpf. At 72 hpf embryos were collected for analysis. Quantitative PCR analysis of glucose exposed embryos demonstrated that glucose exposure activates the insulin pathway while FFA/TAG injections activates the lipolysis and beta-oxidation pathways. At 72 hpf embryos were collected and bulk RNAseq was performed on control, glucose exposed and FFA/TAGs injected embryos. More gene expression was affected by glucose exposure compared to FFA/TAGs injection. Our RNAseq and qPCR validation also reveal a different regulation of appetite regulation genes by excess glucose or excess fat, all these events happening in absence of a true appetite as the embryo does not yet feed by itself. Our project reveals differential gene expression by excess glucose or excess FFA/TAGs and sheds light on how the embryo regulates its nutrient intake prior to feeding.

312 - Laminin-111 mutant studies reveal a hierarchy within laminin-111 genes in their requirement for basal epithelial tissue folding

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The process of morphogenesis carefully crafts cells into complex organ structures which allows them to perform their unique functions. During brain development, the neuroepithelial must go through both apical and basal folding which is mediated through the instruction of both intrinsic and extrinsic factors. While much is known about apical folding, which is mediated by apical constriction, the mechanisms that regulate basal folding are less understood. Using the highly conserved zebrafish midbrain-hindbrain boundary (MHB) as an epithelial tissue model, we have identified the basement membrane protein laminin-111 as a key extrinsic factor in basal tissue folding. Laminin-111 is a highly conserved, heterotrimeric protein that lines the basal surface of the neuroepithelium. Laminin-111 is comprised of the alpha, beta and gamma chains encoded by *lama1*, *lamb1a*, and *lamc1* genes respectively. Human mutations in individual laminin-111 genes result in disparate disease phenotypes; therefore, we hypothesized that each laminin gene would have a unique role in tissue morphogenesis. Using zebrafish mutants for each laminin-111 gene, we found that each laminin chain has a unique impact on basal folding. We found that *lamc1* is the most critical for basal folding, followed by *lama1*, and finally *lamb1a*. This hierarchy was discovered when we analyzed single cell shape in the anterior-posterior dimension and the dorsal-ventral dimension. The hierarchy was also apparent when we examined localization of myosin regulatory light chain (MRLC), quantified cell shape outside of the MHB, and analyzed the MHB tissue fold at prim-15. These data indicate that lamc1 is the most critical laminin-111 chain for basal tissue folding. These findings are critical for novel techniques in tissue engineering and will help to elucidate differences in human diseases due to specific chain mutations.

313 - Dermal appendage-dependent development of zebrafish atoh1a+ Merkel cells

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Skin is our primary interface with the tactile environment and can distinguish a range of touch inputs with exquisite acuity. Touch system function requires precise interactions between specialized skin cells and somatosensory axons, as exemplified by the mechanosensory Merkel cell-neurite complex. Development and patterning of Merkel cells and associated neurites during skin organogenesis remains poorly understood, partly due to the in utero development of mammalian embryos. Here, we discover Merkel cells in the adult zebrafish epidermis and identify Atonal homolog 1a (Atoh1a) as a marker of zebrafish Merkel cells. Similar to their mammalian counterparts, zebrafish Merkel cells express neurosecretory and mechanosensory machinery, extend actin-rich microvilli, and complex with somatosensory axons. Merkel cells populate all major adult skin compartments, with region-specific densities and distribution patterns. in vivo photoconversion reveals that Merkel cells undergo steady loss and replenishment during skin homeostasis. Merkel cells develop concomitant with dermal appendages along the trunk. Preventing dermal appendage formation reduces Merkel cell density by affecting both cell differentiation and maintenance. Overall, our studies provide insights into touch system maturation during skin organogenesis and establish zebrafish as an experimentally accessible in vivo model for the study of Merkel cell biology.

314 - Development and function of stress-regulating neurons in the posterior hypothalamus

Priscilla Figueroa[1], Debora Brito De Andrade[1], Cade Kartchner[1], Jennifer Cheng[1], Richard Dorsky[1]

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Wnt/β-catenin signaling through the transcriptional effector Lef1 is fundamentally required for hypothalamic neurogenesis, and *lef1* mutant zebrafish exhibit increased stress-related behavior. However, it is unknown whether a specific population of *lef1*-dependent neurons in the hypothalamus inhibits the stress response. RNA-seq analysis of the *lef1* mutant hypothalamus revealed a significant decrease in expression of *crhbp*, which encodes a negative regulator of cortisol release through the hypothalamic-pituitary-interrenal axis. Furthermore, null mutants for the Lef1-dependent gene *otpb* also exhibit decreased hypothalamic *crhbp* expression and increased stress-related behavior. However, *otpb* mutants do not have a significant neurogenesis defect, consistent with a role for this gene in terminal differentiation of specific neuronal subtypes. To determine the fate and function of *crhbp*+ neurons, I developed a reporter transgene driven by a short upstream regulatory region of *crhbp* and generated a transgenic line in which GFP is expressed in hypothalamic neurons. Transgene expression is absent in the hypothalamus of *lef1* mutants, consistent with the role of Lef1 in neurogenesis. In contrast, GFP+ neurons are still present in the hypothalamus of *otpb* mutants, despite the absence of *crhbp* mRNA, suggesting that a different regulatory region may mediate this transcriptional interaction. I am now using this reporter line to label transplanted cells from wild-type donor embryos into *lef1* and *otpb* mutant hosts, in order to determine whether restoring either hypothalamic *crhbp*+ neurons or *crhbp* expression is sufficient to rescue normal behavior. To determine the necessity of *crhbp*+ neurons in regulating stress-related behavior, I have used the same regulatory region and the Gal4/UAS system to express Nitroreductase and perform ablation experiments with Metronidazole, followed by behavioral analysis. Together this work will test a novel pathway for regulating the stress response through hypothalamic neurogenesis.

315 - A Posture and Gait Perspective on Zebrafish Behavior after Spinal Cord Injury

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I measure zebrafish posture and gait, challenging common beliefs about functional recovery during spinal cord (SC) regeneration. After SC transection, a fish swims poorly according to objective measures such as activity and endurance. An injured fish also appears to swim more awkwardly, analogous to having an injury-induced limp. For approximately eight weeks after complete SC transection, an injured zebrafish experiences two aspects of recovery: structural and functional. Structural recovery refers to physical healing as the SC reconnects across the injury site. Functional recovery refers to improved locomotor ability, presumably because structural recovery restores neural communications caudal to the injury site. Most fish recover well enough to swim with high activity and endurance, but no objective standards exist to test whether gait awkwardness, or a limp, persists. By measuring comparable attributes of posture and gait including posture novelty, injury-induced lateral scoliosis, and sinusoidal locomotion, I address this understudied perspective on functional recovery.

316 - Mutations in the new disease-causing gene ARF3 have disruptive consequences on Golgi integrity and brain development

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Rare diseases affect more than 400 million people worldwide. Most of these conditions are characterized by highly disabling malformations of cortical development (MCD). Yet, despite the recent increase in disease-genes/variants discovery, heterogenous MCD remain without treatment due to poor knowledge of the underlying mechanisms. Here we employed an integrated functional genomics pipeline comprising human exome sequencing, *in silico*, *in vitro* and *in vivo* cell/developmental biology analysis in zebrafish to tackle a previously unidentified disease showing variable degrees of MCD, *i.e.* microcephaly, cortical atrophy associated with skeletal anomalies. We identified *de novo* missense variants affecting ARF3, a far neglected member of small GTPases of the RAS superfamily involved in Golgi-trafficking, as causative of the disease and provide first insights into ARF3 activity throughout vertebrate embryogenesis. In silico and biochemical investigations demonstrated that microcephaly-causing ARF3 mutations affect highly conserved residues regulating the catalytic activity of the protein participating in GTP binding. Experiments in fish embryos corroborated this finding and proved disruptive consequences of aberrant ARF3 on trans-Golgi integrity. Comparable in vitro results substantiated the pathophysiological role the newly discovered ARF3 mutations, leading to various patterns of Golgi dysfunction, as an underlying mechanism of this new form of Golgipathy. Our zebrafish models further validated the occurrence of a severe microcephalic trait caused by the severe mutations. The data showed a fundamental perturbation of precursor cells proliferation in the developing forebrain as well as planar cell polarity (PCP)-dependent cell processes establishing the body plan axes, resembling a known effect caused by dominant ARF1. In conclusion, utilizing an integrated multi-level analysis (genomics, *in silico*, *in vitro* and *in vivo*), our work 1.provides molecular classification for disease stratification, 2.offers a mechanistic knowledge of a previously unrecognized neurodevelopmental disorder and 3.document an obligate dependence on proper ARF3 function for Golgi homeostasis and early developmental processes.

317 - Optimization of transgenic strategies to study neuronal populations

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Transgenic approaches are especially powerful in zebrafish to characterize neural cell types and their connections, and monitor their activity. However, systematic efforts to combat transcriptional silencing and variegated expression of transgenes would be beneficial. In prior work, we adopted the QF/QUAS binary transcriptional regulatory system of *Neurospora crassa* to generate neural-specific driver lines and a variety of QUAS-driven reporter/effector lines. Here, we report on a CRISPR/Cas9 mediated strategy for targeted integration of QF2 (a modified version of the QF transcription factor) to drive its expression under control of endogenous cis-regulatory regions. The construct relies on short homology arms and includes a transiently expressed secondary reporter, which expedites screening for transgenic founders and establishment of stable lines. We have also identified regions of open chromatin that are prevalent in zebrafish brain tissue and have used CRISPR/Cas9 editing to integrate QUAS and UAS reporter constructs into precise genomic sites. By monitoring reporter labeling over multiple generations, we will determine whether enhanced chromatin accessibility maintains transcription of QUAS/UAS regulated transgenes with high fidelity. Through a combination of targeted driver and reporter constructs in the zebrafish genome, we aim to recover consistent and robust labeling of identified neuronal populations and new tools for functional manipulations.

318 - Deciphering the Role of islet-1a in Zebrafish Hair Cell Regeneration

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Mechanosensory hair cell loss leads to permanent hearing deficiencies in humans. However, our understanding of how to trigger hair cell regeneration is still limited. Zebrafish possess sensory hair cells and support cells within mechanosensory organs called neuromasts that share a striking resemblance to mammalian hair cells and support cells in the inner ear. Unlike mammals, zebrafish robustly regenerate hair cells throughout life. We previously identified central support cells as the direct hair cell progenitors in zebrafish. However, the molecular mechanisms that trigger central support cell proliferation and their differentiation into hair cells after injury are not well understood. *isl1a* is a transcription factor specifically expressed in central support cells with a dynamic response to hair cell death in zebrafish.

We generated an *isl1a* whole gene deletion mutant and discovered that homeostatic mutant larvae possess significantly bigger neuromast with more hair cells and support cells. Time lapse and EdU incorporation analyses revealed that this increase is driven by overproliferation. However, the increase in these cell populations is proportional to the neuromast size demonstrating that *isl1a* loss leads to an increase in proliferation but does not affect cell type specification in homeostasis. However, during regeneration *isl1a* mutants generate a disproportionately higher number of hair cells, showing that *isl1a* normally inhibits the hair cell fate. *isl1a* loss results in the upregulation of multiple central support cell genes, suggesting that it plays a repressive role in this population. *isl1a* downstream targets are currently under investigation.

Even though *isl1a* is necessary for proliferation in other tissues, our data suggest that *isl1a* negatively regulates central support cell proliferation and further differentiation into hair cells in the neuromast. Isl1 is a promising candidate for improving regeneration in mammals. Understanding how *isl1a* is dynamically regulated and identifying its downstream targets is essential to optimize its therapeutic effectiveness.

319 - A regulatory network of Sox and Six transcription factors initiate a cell fate transformation during hearing regeneration in adult zebrafish

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Using adult zebrafish inner ears as a model for sensorineural regeneration, we performed a targeted ablation of the mechanosensory receptors in the utricle and saccule and characterized the single-cell epigenome and transcriptome at consecutive time-points following hair cell ablation. Using deep learning on the regeneration-induced open chromatin sequences, we were able to identify unique, cell-specific transcription factor (TF) motif patterns enriched in the raw data. We correlated enhancer activity with gene expression to identify gene regulatory networks. A clear pattern of overlapping Sox- and Six-family transcription factor gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Pseudo-time analysis of single-cell transcriptomic data demonstrated that the support cells within the sensory epithelium changed cell identity to a multipotent “progenitor” cell population that could either differentiate into hair cells or return to a support cell identity. We showed that sox2 becomes enriched in the progenitor cells and is reduced again when the cells differentiate in either direction. Analysis of the scATAC-seq data identified a 2.6 kb DNA sequence element upstream of the sox2 promoter that dynamically changed in accessibility during hair cell regeneration. When deleted, the upstream regulator of sox2 showed a dominant phenotype that resulted in a hair cell regeneration-specific deficit in both the lateral line and adult inner ear.

320 - In vivo functional validation of new disease-genes and variants impairing trafficking and cytoskeleton dynamics as underlying cause of undiagnosed neurodevelopmental diseases

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Rare diseases represent a serious societal burden, with at least 70% of the cases manifested already during childhood in chronic forms often affecting the nervous system and resulting in extremely debilitating conditions, which include early onset neurodegeneration. The lack of a fundamental understanding of the underlying pathophysiological mechanisms, which might involve a variety of cell populations and developmental processes, make them difficult to diagnose and treat. New and potentially pathogenic gene variants are continuously identified in undiagnosed patients thanks to advanced genomic technologies, resulting in an increased need for effective in vivo disease models to obtain functional validation. In the framework of the "Undiagnosed Patients Program" at Ospedale Pediatrico Bambino Gesù (OPBG, Rome, Italy) more than 20 new rare diseases have been clinically and genetically classified since its launch in 2015, in collaboration with healthcare and research premises worldwide. Here, we show the most recent examples which benefited from functional validation through ad hoc modeling and analysis in zebrafish obtained at the newly established OPBG zebrafish laboratory. In this "in-house" in vivo workflow we utilize transient and stable protein knockdown, and overexpression approaches for loss and gain of function conditions, respectively, coupled to whole embryo imaging-based phenotype characterization at the cellular and subcellular levels. The in vivo pipeline is paralleled by functional investigation in simple and complex in vitro models and in silico analysis. We present zebrafish data that recently contributed to: 1.validate the pathogenicity of new disease genes and variants involved in endosomal trafficking and Golgi homeostasis affecting neurodevelopment (in this context we describe a new Golgipathy); 2.provide in-depth insights into the role and function of small GTPases and microtubules chaperons for CNS and motoneurons development; 3.characterize the impact of previously unidentified genetic variants and the efficacy of newly synthesized molecules inhibiting the altered RAS/MAPK signaling involved in RASopathies

321 - The role of Phoenixin, a neuroendocrine peptide, in the control of reproduction in zebrafish, Danio rerio

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In vertebrates, gonadotropin-releasing hormone (GnRH1) is the major hormone controlling fertility. Additionally, most vertebrates have additional isoforms (GnRH2, GnRH3) where fishes generally express all three (GnRH 1-3). We have shown that *gnrh1* is not present in the zebrafish genome (Whitlock et al., 2019). Furthermore, the loss of function of the *gnrh2* and *gnrh3* isoforms (Marvel et al., 2018) does not affect fertility. Because GnRHs are part of a highly conserved signalling pathway that controls reproduction, it is unusual for zebrafish to not use these hormones for reproduction.

We proposed that Phoenixin (Pnx) a novel neuropeptide acting on the brain-pituitary axis and potentiating the action of pituitary gonadotropins through the Gpr173 receptor (Yosten et al., 2013; Treen et al., 2016) is a potential candidate to replace GnRH in zebrafish reproductive axis (Whitlock et al., 2019). Our laboratory confirmed for the first time that Pnx is expressed in the zebrafish brain area (Ceriani et al., 2021). Using a zebrafish specific Pnx antibody, we found that PNX expression in the pituitary starts at the onset of puberty (~35dpf). Next, we confirmed that the Pnx receptor Gpr173/Sreb3, is expressed in the adult zebrafish pituitary. Finally, we investigated the potential physiological role of PNX in the reproductive axis by intraperitoneal injections of Pnx in adult male and female fish. Injection of zebrafish Pnx was associated with an increased expression of gonadotropins (*lhb*) in the adult fish. Currently we are analysing CRISPR/Cas9 based knockout of *pnx* to better understand the role of Pnx in zebrafish reproduction.

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Whitlock et al., 2019, Frontiers Neuroendocrinology.

Marvel et al., 2018, Biology of Reproduction.

Yosten et al., 2013, J Neuroendocrinol.

Treen et al., 2016, Mol Endocrinol.

Ceriani et al., 2021, GEP.

322 - Dixdc1b acts downstream of the Cdk5-Nckap1l axis to regulate biliary system morphogenesis.

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Impaired formation of the biliary system could lead to cholestatic liver diseases. Cdk5 orchestrates branching morphogenesis of the intrahepatic biliary network by regulating its downstream effectors including *nckap1l*. We combined a forward genetic approach in zebrafish with unbiased computational quantification to identify a new mutation that genetically interacts with *nckap1l*. The interacting mutation impacts the *DIX Domain Containing 1b* (*dixdc1b*) gene, the homolog of a known direct substrate for Cdk5. The genetic interaction is strong so that even trans-heterozygous larvae for *dixdc1b* and *nckap1l* show a similar phenotype to that in *dixdc1b* homozygous mutant larvae, indicating that these two genes work in the same pathway. The *dixdc1b* gene has three alternative splice isoforms, and CRISPR/Cas9-based isoform-specific knockout indicated that each isoform has a different role in the biliary system formation. We show that *nckap1l* mutant phenotype was rescued by overexpressing *dixdc1b* at least partially, while *dixdc1b* mutant phenotype was not rescued by overexpressing *nckap1l*, indicating that *dixdc1b* acts downstream of *nckap1l*. These data together indicate that *dixdc1b* acts downstream of the *cdk5-nckap1l* pathway to regulate the branching morphogenesis of the intrahepatic biliary network.

323 - Annotation of novel dynamic UTRs as functional targets of miRNAs during cardiac development

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A growing body of evidence suggests that congenital heart disease (CHD) can result from posttranscriptional or epigenetic regulation, including RNA regulatory events such microRNA (miRNA) directed mRNA degradation. Small noncoding microRNAs play essential roles in post-transcriptional gene regulation in development and disease, predominantly through interactions with mRNA untranslated regions (UTRs). However, the dynamic and tissue specific expression of miRNAs and target UTRs throughout development is poorly characterized. In order to understand the RNA regulatory events that drive cardiac morphogenesis, we captured a developmental time course series of simultaneous small RNA-seq and total RNA-seq from zebrafish hearts. Our algorithms provide *de novo* genome annotation of dynamically changing UTRs, reveal over ten thousand significantly changed transcripts, and use inverse correlation analysis to predict regulatory miRNA/UTR interactions during development. Additionally, to share this dataset as widely as possible with the scientific community, we built a Shiny web-based application in R that allows the on-line user to investigate their genes of interest rapidly and easily. In addition to miRNA and mRNA transcriptome resources for studies of RNA regulatory events in cardiac development and disease, this study provides bioinformatic approaches that should be applicable to discover posttranscriptional regulation in other developmental systems. In a collaboration between this Cardiovascular Development Consortium (CvDC) project and the Pediatric Cardiac Genomics Consortium (PCGC), we used human WGS and WES datasets to identify numerous 3'UTR's of these candidate miRNA targets that contain rare variants in CHD patients. This dataset provides an important resource for studies of RNA regulatory events implicated in cardiac development and CHD.

324 - The Adaptor Protein 2 (AP2) complex modulates habituation and behavioral selection across multiple pathways and time windows

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Animals constantly perceive and integrate information across sensory modalities, and their nervous systems must select behavioral responses appropriate to the current situation and prior experience. Genetic factors supporting this behavioral flexibility are often disrupted in neuropsychiatric conditions, and our previous work revealed the disease-associated *ap2s1* critically supports habituation learning during acoustically-evoked escape behavior of zebrafish. *ap2s1* encodes a subunit of the AP2 endocytosis adaptor complex and has been linked to autism spectrum disorder, though its mechanism and direct behavioral importance have not been established. Here, we show that multiple domains and subunits of the AP2 complex regulate acoustically-evoked behavior selection and habituation learning. Furthermore, *ap2s1* biases the choice between distinct escape behaviors in sensory modality-specific manners, and more broadly regulates action selection across diverse acoustic, visual, and mechanosensory contexts. Using tissue-specific and inducible transgenic rescue, we demonstrate that the AP2 complex functions acutely and in the nervous system to modulate acoustically-evoked habituation, identifying at least three spatially and temporally distinct mechanisms through which AP2 regulates different aspects of escape behavior selection and performance. Altogether, we demonstrate that the AP2 complex coordinates action selection across stimulus modalities and contexts, providing a new vertebrate model for the role of *ap2s1* in human conditions including autism spectrum disorder.

325 - Reporter gene knock-in strategy employing minicircle donor DNA and the hsp minimal promoter

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Transgenic reporters are a powerful tool to visualize gene expression. Among multiple approaches to generate transgenic fish, knock-in reporter animals provide a precise way to recapitulate the endogenous gene expression. Here, we employ minicircle technology and the heat shock minimal promoter (hsp) to improve the efficacy of the knock-in strategy in zebrafish. We utilize a minicircle, a circular vector absent of bacterial backbone sequences, as a donor template to reduce the number of linearization cutting sites. Lowly expressed genes often fail to generate knock-in reporter lines due to undetectable reporter gene expression. To address this obstacle, we compare the transcription ability between hsp and endogenous promoters of target genes. For several genes, the endogenous promoter cannot direct expression of the integrated reporter gene. Despite inducing non-specific expression in muscle cells of some larvae, hsp can induce notable reporter gene expression in the expected tissues. We will optimize our strategy to establish an efficient knock-in reporter method for zebrafish.

326 - Epigenetic regulation of postembryonic neurogenic plasticity by the histone methyltransferase Ehmt2

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During postembryonic development, the brain exhibits experience-dependent neuroplasticity, in which sensory experience modulates brain growth. We characterized the importance of motor experience on postembryonic neurogenesis in larval zebrafish and found that physical cues associated with movement appear critical to maintain forebrain neurogenesis. Reduced movement results in a decrease in the size of the proliferating precursor pool in the dorsal forebrain, and increased movement yields the opposite effect. Moreover, these physical cues are conveyed, in part by dorsal root ganglia. Epigenetic regulation plays an important role in neurogenesis, controlling both neural stem cell self-renewal and differentiation potential. The histone methyltransferase Ehmt2 (also known as G9a) has been implicated in neuronal development and plasticity, as well as in learning, memory, and responses to environmental stimuli in different organisms. Ehmt2 catalyses the addition of two methyl groups onto lysine 9 of histone 3 (H3K9me2), promoting repression of transcription. To examine the role of Ehmt2 in mediating neurogenic plasticity, we generated a zebrafish *ehmt2* mutant (*ehmt2*$^{D4/D4}$) through gene editing. Mutant fish are viable and fertile and, as expected, display reduced H3K9me2 levels in the brain. Interestingly, compared to wildtype larvae, the size of the proliferating precursor pool does not decrease in response to reduced movement, which may be correlated with an altered cell cycle in mutant neural stem cells. Our results demonstrate that movement-dependent plasticity in forebrain neurogenesis requires the epigenetic regulator Ehmt2.

327 - KLC4 shapes axon arbors during development and mediates behavior

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Precise delivery of axonal cargos by motor proteins like kinesin-1 is critical for neurons to establish their complex polarized morphologies. How kinesin-1 directs delivery of specific cargo to cellular compartments to facilitate neuronal morphogenesis is poorly understood. We identified the kinesin light chain subunit KLC4 as an important regulator of axonal branching and arborization pattern in sensory neurons. Mutation of Klc4 in humans causes the early-onset neurodegenerative disease hereditary spastic paraplegia, but specific cargos and functions of Klc4 are largely unknown. Loss of *klc4* in zebrafish results in reduced sensory axon branching and altered directionality of axon growth. Live imaging during axon outgrowth revealed that *klc4* mutants (*klc4-/-)* generated branches at the same rate as wild-type (WT) but nascent branches in *klc4*-/- failed to stabilize and were lost at an over 5x greater rate than in WT. To effectively tile a sensory field newly formed branches must spread apart to innervate a wider area. Branches in *klc4*-/- spread less than WT branches, and often fasciculated aberrantly indicating failure of normal contact repulsion. Since MTs are important in stabilization of axon branches, we next quantified MT dynamics by expressing the plus-end marker EB3-GFP in sensory axons. Frequency and velocity of MT comets was increased in *klc4*-/- axons, suggesting disruption of MT dynamics may contribute to changes in neuronal morphology observed in *klc4* mutants. Finally, we asked whether these cell biological changes could lead to behavioral phenotypes in larvae and adults. We found that *klc4* mutant larvae were overreactive to touch, swimming longer and performing more swim bouts after a touch stimulus. Adult *klc*4-/- showed an increase in anxiety-like behavior, including freezing and failure to explore the environment in a novel tank test. Overall, these data suggest that *klc4* plays a unique and important role in neuronal morphogenesis and function.

328 - Critical negative regulators of macrophage activation for tissue development and homeostasis

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Surprisingly little is known about the mechanisms that prevent inappropriate macrophage activation at steady state which can lead to detrimental conditions of autoinflammation and autoimmunity. Because macrophages are critical resident innate immune cells strategically integrated in virtually all the organs of the body, they can exert sustaining impact on the development and homeostasis of various tissues from brain to gut over a lifetime. Changes to macrophage activation in part rely on metabolic enzymes. One such enzyme is aconitate decarboxylase 1 (ACOD1; also known as immunoresponsive gene 1, IRG1) that is highly conserved and induced in activated macrophages from zebrafish to humans. We are investigating the roles of cytoplasmic nucleotide binding oligomerization domain (NOD)-like receptors (NLRs) and other genes that when disrupted cause spontaneous abnormal *acod1/irg1* induction using a forward genetic screen. We previously identified a deleterious mutation in *nlrc3l* that causes inappropriate macrophage activation (including *acod1/irg1* induction) at baseline that prevents microglia development. Furthermore, we generated a novel endogenous GFP knock-in reporter for *acod1/irg1* using CRISPR-Cas9 for precise and quantifiable single-cell resolution tracking and isolation of macrophages to define heterogeneous activation states in different contexts in vivo. We found lysosomal defects associated with inappropriate macrophage activation and possible dysregulation of lysosomal regulator TFEB. Overall, we will discuss new results from our genetic and functional genomic analyses combined with dynamic in vivo imaging that implicate previously unappreciated connection between endosomal-lysosomal processes and regulation of immune activation. This study provides critical insights into how the innate immune system keep itself in check.

329 - Characterizing Functional Effects of scn1lab Mutation in a Zebrafish Genetic Model of ASD

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Background: Previous exome-sequencing studies have identified over one hundred high confidence risk genes contributing to autism spectrum disorder (ASD). Variants in the gene *SCN1A* and *SCN2A*, which encode the voltage gated sodium channels Nav1.1 and Nav1.2, respectively, have been associated with Dravet Syndrome, infantile-onset epilepsy, intellectual disability, and ASD. Here we investigate functional effects of *SCN1A/2A* mutations in larval zebrafish mutants of *scn1lab*, an ortholog of the ASD risk genes *SCN1A* and *SCN2A.*

Methods: We evaluated *scn1lab* mutation effects on three levels: behavior, circuit activity, and transcriptional profiles. First, we characterized behavior of *scn1lab*-mutant larval zebrafish using two paradigms: rest-wake assay and visual-startle response assay. To assess pan-neuronal activity, we then conducted whole-brain activity mapping and measured differences in brain volume. To understand how *scn1lab* mutations affect global brain transcriptional profiles and biological pathways, we performed RNA-seq and Ingenuity Pathway Analysis.

Results: *scn1lab*-mutant larvae show reduced daytime activity, nighttime hyperactivity, and hyperactive startle response to light. *scn1lab*-mutants also show decreased brain activity, decreased brain volume, and a deficit in GABAergic neuronal populations. We found dysregulated synaptic, GABAergic, and glutamatergic pathways in *scn1lab*-mutants.

Conclusion and Discussion: These data show that *scn1lab* mutants exhibit disruptions in visuomotor, rest-wake, and excitatory-inhibitory circuitry, leading to alterations in visual-startle responses and rest-wake activity. In future studies, we aim to assess associations between zebrafish and human *SCN2A* RNA-seq datasets by directly mapping across species and examining correlations between global t-statistic and FDR of differentially expressed genes.

330 - Zebrafish as a model system to understand the role of the myh genes in development

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MYH genes encode highly conserved actin-based motor proteins, non-muscle myosins (NMIIs), which have essential roles in cell division, cell migration, and cell shape changes. Zebrafish have two *myh9* genes which encode for the NMIIA protein, *myh9a* and *myh9b*, likely due to the teleost genome duplication. Zebrafish also express *myh10* and *myh14*, which encode for NMIIB and NMIIC, respectively. Our homology studies indicate that human and zebrafish share >77% gene and protein homology for the *myh9a*, *myh9b,* and *myh10* genes, but not the *myh14* gene. To understand the role of *myh* genes in development, we obtained a null mutant for the *myh9a* gene and generated null mutants for the *myh9b* and *myh10* genes using CRISPR/Cas genome editing. We did not examine *myh14* due to the low sequence homology. Through our studies we identified *myh9b*, not *myh9a* or *myh10,* as the critical *myh* gene required for normal zebrafish development and morphogenesis. Consistent with this finding, *myh9a* and *myh10* homozygous mutants are viable through adulthood and do not develop visible phenotypes. However, *myh9b* homozygous mutants are semi lethal and develop pericardial edema, a phenotype consistent with kidney dysfunction, between 48 and 96 hours post fertilization. This phenotype reverses shortly after onset, leading us to hypothesize that there is compensation by other NMII proteins in these mutants. In the human population there are five clinical disorders resulting from mutations in the *MYH9* gene that are classified as *MYH9*-related disease (*MYH9*-RD), with many patients developing kidney dysfunction. Our results suggest that *myh9b* mutants can be used as a model for *MYH9*-RD. Current experiments are investigating NMIIA and NMIIB protein levels during the time of pericardial edema presentation and future experiments will examine kidney patterning, glomerular function, and glomerular structure in our mutant models.

331 - Mesendodermal enhancers predicted by Eomes, FoxH1, and Mixl1 binding

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The development of distinct cell types requires precise spatio-temporal control of gene expression. Enhancers act as gene-regulatory elements spread throughout the genome, controlling cell-type specific gene expression through the binding of tissue- or signaling-specific transcription factors. In zebrafish, maternally contributed transcription factors, including EomesA and FoxH1, cooperate with zygotic co-factors and BMP/Nodal-controlled Smads to achieve spatio-temporal target gene control in mesendoderm patterning, such as towards lateral plate mesoderm (LPM). However, the complement of mesendodermal and in particular ventro-lateral gene-regulatory elements remains incompletely understood.

Here, building on previous work in the field, we build a computational pipeline to identify early mesendodermal and LPM enhancers. We previously established that the combination of EomesA, FoxH1, and Mixl1 acts on the early LPM enhancer in the zebrafish draculin (*drl*) gene in cooperation with Smads as basic input for LPM-primed mesendodermal gene regulation. We now mapped the regions concomitantly occupied by EomesA, FoxH1, and Mixl1 (EFM) using available ChIP-seq data and annotated likely target genes. We used our regulatory element-testing workflow with Tol2-based, minimal-promoter-coupled *mCerulean* reporter transgenes to validate the individual activity patterns of enhancer candidates in transient and stable zebrafish transgenics.

Our analysis revealed EFM-responsive gene-regulatory elements in several well-characterized mesendoderm genes at the base of classic ventro-lateral expression patterns in zebrafish. Among these, we identified EFM-responsive elements the *vent*/*vox* and *sox32* loci that recapitulated their early gene expression patterns as reporters and tracked descendant lineages as creERT2 drivers. *In toto* timelapse imaging of *sox32*-based reporters documented endoderm and yolk syncytial layer dynamics from early epiboly stages, underlining the capabilities for early developmental transgene expression of minimal promoter-coupled mCerulean reporters. We further identified EFM-bound elements in the *eomesa*, *foxh1*, and *mixl1* loci, indicating positive feedback regulation. Our data define a series of mesendodermal gene-regulatory elements as developmental cell fate sensors in zebrafish and beyond.

332 - Position-independent development of functional connectivity in vagus motor neurons

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The vagus nerve (10th cranial nerve) mediates brain-body communication by innervating and controlling various internal body parts, including the pharynx, heart, and stomach. The sensory component sends peripheral information to the brain, and the motor component reflexively regulates essential body functions, such as swallowing, heartbeat, and digestion. Diverse motor neurons underlying these body functions topographically organize their cell bodies in the brainstem. However, a group of motor neurons innervating a common body target, a “target group”, are partly intermingled with other target groups without clear boundaries. It remains unanswered how this intermingled diverse population of vagus motor neurons regulate each distinct body function. Through calcium imaging of vagus motor neurons in larval zebrafish, we identified that local sensory stimulation (noxious or mechanosensory) along the gastrointestinal tract reproducibly induces stereotyped motor responses by activating specific target groups appropriate for stimulation locations. Thus, individual target groups develop specific upstream connectivity despite the coarse spatial organization. We provide three lines of evidence supporting that this fine-scale connectivity is not a simple outcome of topographic mapping during embryogenesis, but rather a product of subsequent activity-dependent connection specification in larvae. First, upstream connectivity becomes progressively more fine-tuned between 4 and 10 days post fertilization. Second, abnormal connectivity develops in motor neurons with impaired neurotransmitter release from the axon terminals, suggesting that activity-dependent axon-target communication retrogradely instructs upstream connectivity. Third, fine-scale connectivity is largely resilient to disrupted topographic organization. Local stimulation can successfully activate specific target groups even when motor neurons within the groups are topographically mispositioned. Therefore, independent of topographic organization, vagus motor neurons that are wired together to the same body targets manage to fire together to cooperatively regulate distinct body functions. This study establishes a tractable model for investigating how brain connectivity for effective brain-body communication might be guided by peripheral motor performance.

333 - Flipping the Script on Hearing Loss: A Zebrafish Model of atp11a Flippase-Mediated Sensorineural Hearing Loss

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Phospholipid transferases (flippases) are integral membrane proteins required to maintain the asymmetrical distribution of phospholipids within the plasma membrane (PM) of all cell types. This asymmetry is crucial in signal transductions that maintain cellular homeostasis. *ATP11A,* a P4-type, class-VI flippase, actively transports phospholipids across the PM using ATP-hydrolysis. Recently a novel splice site variant of *ATP11A* was determined to be the causative gene of autosomal dominant neurosensory hearing loss (NSHL) in a Newfoundland family. As knowledge about *ATP11A* is lacking, an animal model is needed to understand its underlying mechanisms and how it contributes to disease.

Herein we utilize a novel CRISPR-Cas9 knockout zebrafish model to determine the cellular mechanisms, function, and disease pathogenesis of *atp11a*. Given similar expression profiles, sequence similarity, and gene synteny to its human orthologue, our model is an excellent candidate for future genetic disease research. Zebrafish expression patterns demonstrate that *atp11a* participates in the development of the ear, neural crest cells, and outer photoreceptor/RPE layers. We have observed a significant decrease in overall hair cell numbers in *atp11a*$^{-/-}$ and *atp11a*$^{+/-}$ mutants in contrast to WT counterparts, specifically within the medial crista and posterior macula. These structures are responsible for orienting head position relative to angular/linear acceleration respectively, and gravity. Preliminary data from ongoing swimming behaviour studies suggest mutant larvae have difficulties maintaining proper orientation in the water column by 6 and 10 dpf when swimming and resting. These findings are consistent with "deaf" zebrafish phenotypes. Furthermore, we aim to assess how our *atp11a* mutant allele affects these cells by examining apoptosis and vesicle formation through staining and TEM techniques. Though further research is ongoing, our model shows great promise in elucidating NSHL pathogenesis resulting from flippase knockout.

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334 - The olfactory organ is a unique site for neutrophils in the brain

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It is widely believed that neurogenesis contributes to learning and memory and can be regulated by immune signaling molecules as exemplified by the localization of immune system proteins in the developing and adult nervous system. Within the vertebrate nervous system the olfactory system is unique in that new neurons are continuously produced throughout life. Previously, we have shown that olfactory imprinting, a specific type of long-term memory, is correlated with a transcriptional response in the olfactory organs that includes up-regulation of genes associated with the immune system. We made use of dissected, intact adult brains from cell-specific fluorescent reporter lines to examine the association of the olfactory sensory neurons with neutrophils and blood-lymphatic vasculature. Surprisingly, neutrophils were found only in the olfactory epithelia and associated blood-lymphatic vasculature of the olfactory organs. Damage to the olfactory epithelia resulted in a rapid increase of neutrophils both within the olfactory organs as well as the central nervous system. Cell tracking using a photo-convertible fluorescent reporter line suggests that neutrophils move from the olfactory epithelia into the brain. Our results reveal an intimate relationship between the peripheral olfactory epithelia and neutrophils suggesting a dual olfactory-immune function for this unique sensory system.

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335 - Characterizing hair cell regeneration in the larval zebrafish inner ear

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A common cause of deafness and vestibular impairment is death of the sensory hair cells of the inner ear. Adult mammals are unable to regenerate auditory hair cells and have only a limited ability to regenerate hair cells in the vestibular organs. In contrast, robust hair cell regeneration throughout life is common in non-mammalian vertebrates such as fish and birds. Understanding the mechanisms of hair cell regeneration in these highly regenerative vertebrates will inform preventative measures and therapeutic approaches for hair cell loss in humans. The zebrafish inner ear has been historically understudied in the context of hair cell regeneration, despite its high level of conservation with the mammalian inner ear and potential for *in vivo* imaging. This work examines hair cell regeneration in the zebrafish inner ear during the larval stage of development, at which point the inner ear sensory organs become fully functional. To distinguish hair cell addition during organ growth from addition during regeneration, we have established the baseline rate of homeostatic hair cell addition during the larval stage. Our preliminary data suggest that little hair cell turnover occurs naturally during this time. To study hair cell regeneration, we are employing a novel, genetically-encoded method for inner ear hair cell ablation. This method results in complete ablation of crista hair cells after just one hour of treatment, and the fish exhibit a robust regeneration response in the 48 hours post-ablation. In an effort to identify the hair cell progenitors involved in this process, we have analyzed a single-cell RNA-seq dataset of zebrafish hair and support cells. Our analysis revealed multiple transcriptionally distinct clusters of hair and support cells. Future work will include fate mapping support cells during regeneration to identify hair cell precursors and determine the underlying mechanisms of regeneration.

336 - Listening to the scale-beat: signalling waves and tissue morphogenesis in zebrafish regeneration.

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Regeneration is the spectacular process in which a lost body part regrows to its original form. Regeneration poses fundamental questions regarding how chemical and mechanical signals act to recover a tissue of the right size, shape and pattern, starting from variable injuries. Several chemical factors important for regeneration have been discovered in the past, but it is unclear how they are organized in time and space to coordinate morphogenesis. We address these questions using a quantitative approach that combines live imaging with measurements and theory, using zebrafish scales as a model. Scales are millimeter-sized bone disks forming a skeletal armor on the body of the fish. The scale bone matrix is deposited by a monolayer of osteoblasts whose regeneration is amenable to live imaging. We discovered that the regenerative growth of the osteoblast tissue is organized by a train of concentric waves of Erk activity, broadcasted from a central source. Erk activity propagates as an excitable wave and traverses the entire millimeter-sized scale in approximately two days. Erk activity waves induce patterned tissue expansion, leading to overall scale growth. The final scale size is determined by the number of waves that traverse the tissue. We are now investigating how Erk activity wave generation is tuned to control final scale size. Does a mechanism inform the wave source on tissue size and stops wave generation once scales get to their appropriate final form? How does signaling integrate mechanical inputs from the neighboring bone to coordinate tissue growth and bone matrix deposition? Overall, our findings are revealing trigger waves as a regulatory strategy to coordinate regeneration.

337 - Elovl4b Ablation in Zebrafish Leads to Loss of Ocular C30 to C36 Very-Long-Chain Polyunsaturated Fatty Acids

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Purpose: Very-long-chain polyunsaturated fatty acids (VLC-PUFAs) are a distinct class of lipids with chain lengths > 24 carbons found in the retina and a few other tissues in vertebrates. ELOVL4 is a member of the ELOVL elongase family responsible for the rate-limiting step of VLC-PUFA biosynthesis. Patients with autosomal dominant Stargardt-3 disease, a juvenile form of macular degeneration, have genetic mutations in ELOVL4 leading to the formation of a non-catalytically active protein and loss of retinal VLC-PUFAs. Interestingly, low retinal VLC-PUFAs are also characteristic of age-related macular degeneration. Moreover, retinas isolated from patients with age-related macular degeneration have low omega-3 (n-3) to omega-6 (n-6) fatty acid ratios compared to healthy controls.The retinal pathology caused by VLC-PUFA depletion has historically been difficult to study because homozygous *Elovl4* mutations cause skin and neurological disorders in humans and neonatal lethality in mice. Mice with homozygous *Elovl4* mutations are susceptible to catastrophic drying from losing their protective skin barrier. Thus, we created and examined a zebrafish model of Elovl4 deficiency and haploinsufficiency.

Methods: We created a deletion mutation in exon 2 of the *Elovl4b* gene using CRISPR-Cas9. F0 mosaic mutant zebrafish were screened and outcrossed with wild-type fish over two generations to generate a stable line. At ~4 months post-fertilization, their eyes were isolated, total fatty acids extracted, and the quantity of n-3 and n-6 VLC-PUFAs was determined through gas chromatography and mass spectrometry.**Results:** We found that homozygous *Elovl4b* mutant zebrafish eyes had no detectable C30-C34 VLC-PUFAs in their eyes, in contrast to age-matched wild-type controls. Heterozygous fish with one functional copy of *Elovl4b* had intermediate lipid profiles.**Conclusions:** Our data indicate that the loss of Elovl4b in zebrafish alters ocular biochemistry in a way that is comparable to macular degeneration. We plan to correct such abnormalities by feeding synthetic VLC-PUFAs.

338 - Beyond cancer – how oncogenic clones modify long bone size

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Somatic mutations can occur very early in life. The result is the formation of a patchwork of mutant cell clones in the embryo. Such genetic mosaics have been implicated in a broad range of birth defects, however little is understood about how mutant clones interfere with normal development. A striking example are cases where a somatic oncogenic mutation in either PIK3CA or AKT1 leads to overgrowth of an entire limb or digit. Underlying long bones are both longer and wider, yet overall retain their normal shape and proportional relationships. How oncogenic clones can cause such controlled and coordinated overgrowth of entire entities is puzzling. Here, we develop a zebrafish model that allows live imaging of somatic clones with oncogenic PI3K/AKT signaling. These fish develop oversized long bones of the fin endoskeleton, thereby mimicking limb long bone overgrowth in patients. We unravel the cellular and developmental mechanisms by which mutant clones cause long bone overgrowth and pinpoint the cell type and timing of mutation event in the embryo. Intriguingly, our results reveal that mutant clones induce a heterochronic shift in skeletal condensation leading to the observed change in long bone size. This study exemplifies the power of zebrafish in identifying the mechanisms by which somatic mutations cause congenital disorders.

339 - Rigorous evaluation of putative antiseizure drugs in scn1lab mutant zebrafish

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Dravet syndrome (DS) is a severe pediatric epilepsy primarily caused by *de novo* mutation in a voltage-activated sodium channel gene (SCN1A). Patients face life-threatening seizures that are largely resistant to available antiseizure medications (ASM). Existing preclinical animal models of DS are used to identify novel ASMs for these patients. Among these, *scn1lab* mutant zebrafish that exhibit spontaneous seizure-like activity were developed in our laboratory and successfully identified “standard-of-care” ASMs used in this patient population (e.g., valproate, benzodiazepines, stiripentol), as well as novel serotonin-modulating drugs (e.g., fenfluoramine, clemizole, and lorcaserin). Our phenotypic screening platform consists of two stages: (i) a locomotion-based assay measuring high-velocity convulsive behavior and (ii) an electrophysiology-based assay using *in vivo* local field potential recordings to quantify seizure-like events. Unfortunately, more widespread use of our zebrafish *scn1lab* model has led to modifications in these well-established assays and in turn, confused drug discovery claims. Here, we curated a list of 9 antiseizure drug candidates identified in the preclinical DS model literature: donepezil, soticlestat, lisuride, vorinostat, mifepristone, pargyline, 1-EBIO, chlorzoxazone and AA43279. To rigorously evaluate drug efficacy, we tested these drug candidates in *scn1lab* mutants and age-matched wild-type (WT) controls. We first tested each drug at 3 different concentrations on 3 independent trials in the locomotion assay. Only lisuride, pargyline and 1-EBIO were positive hits from this screen and proceeded to electrophysiological testing to validate network-level seizure-like event suppression. Electrophysiology also included soticlestat, a cholesterol 24-hydroxylase inhibitor in Phase 3 clinical trials, as no preclinical data was available. Interestingly, we found exposing WT larvae to soticlestat induced seizure-like discharges. As our results failed to replicate clear antiseizure efficacy for any of the drugs tested, and yielded a potential adverse side-effect for at least one, it highlights the necessity for strict scientific standards in preclinical identification of effective patient treatment options.

340 - Charting the Epigenetic Dynamics during Zebrafish Fin Regeneration at Single-Cell Resolution

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In zebrafish, after the caudal fin is amputated, mature somatic cells at the site of incision are believed to re-enter cell cycle and de-differentiate, self-proliferate, and re-differentiate in a lineage-restricted fashion. Although landmark studies have elucidated intricate biochemical and genetic pathways that direct fin regeneration, the picture of epigenetic dynamics across regeneration stages is not complete yet. Our previous study has shown that cells in bulk level undergo dynamic but coordinated changes of gene expression and chromatin accessibility during zebrafish caudal fin regeneration. However, this bulk-level observation cannot represent cell type/state-specific changes. As single-cell/nucleus sequencing technology develops, here we took advantage of single nucleus ATAC (snATAC-seq) and single nucleus RNA (snRNA-seq) sequencing to generate chromatin accessibility and transcriptional profiles from zebrafish caudal fin tissue across regeneration stages. We demonstrate that the assignments of cell identity in both modalities are highly consistent. In each cell type, gene expression undergoes dynamic changes across stages, whereas chromatin accessibility shows dynamics first then goes into a more static mode, suggesting chromatin opening/closing occurs prior to gene expression change. Our single-nucleus multi-omics approach improves the ability to detect corresponding transcriptomic and chromatin accessibility changes in a cell type/state-specific manner. Moreover, we predicted cell type/state-specific transcription factors and enhancers that will be properly validated in our future work.

341 - Fish and water in drier world: The future is knowledge

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Almost a decade ago our scientific outreach program “Ciencia Al Tiro (Science Immediately https://cienciaaltiro.cl/) remodeled building to create a permanent home called the "Green Building”. In this project we installed innovative technology including a thermal mass heating system for the fish facility and an Acuaponics System, the first in Chile. The Ciencia al Tiro/ Aquaponics project was selected to be part of the "Climate Change, Science and Technology" program (Cabala Producciones) that was broadcast on National Television. Since then we have developed a stand alone Acuaponics System to be used in schools to teach science workshops related to themes essential for the future as we grapple with the effects of climate change: water quality (water pH, nitrogen cycle), photosynthesis, fish development, and sustainable agriculture. These workshops are an elaboration of our original Acuaponics Chapter: http://cienciaaltiro.cl/acuaponia-2/. Thus we have developed a base for the incorporation of fish, water, and sustainability into our science outreach program for people of all ages as we emphasize the importance of water https://cienciaaltiro.cl/en/proyects/water-the-root-of-life/.

As researchers using fish as a model system we are absolutely dependent on the amount and quality of the water we use, yet the world is facing a water crisis characterized by both water scarcity and pollution. We have analyzed our water use and are stunned by the amount of water that we, as zebrafish researchers, “throw away” down the drain. Only by highlighting the magnitude of inefficient water use and discussing potential solutions can we move forward. Currently we are initiating a project in our fish facility to recover water.

342 - Tracing differences in susceptibility to a naturally occurring picornavirus infection

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Viruses are an incredibly diverse group of genetic elements that can profoundly influence the evolution of host organisms. Viral infection of the same virus strain can result in dramatically different clinical severity due to the ability of some viruses to target more than one host tissue in some but not all patients. The zebrafish, with unrivaled optical accessibility and a suite of well-developed genetic and molecular tools, is a powerful but currently underappreciated model system to study host-virus interactions. Our recent discovery of a naturally occurring picornavirus - Zebrafish picornavirus (ZfPV) - that infects zebrafish provides a new opportunity to study enteric virus-host interactions of an endemic virus. ZfPV infections of the Tübingen strain appear asymptomatic but elicit strong host immune responses in the intestine. In contrast, infection in the CG2 strain results in significantly higher viral load and infection of the CNS. Using single-cell RNA sequencing, we identify specific cell populations infected by ZfPV in the intestines of TU and CG2. Preliminary results suggest that while infection did not lead to increased cell death, genes involved in mitophagy and autophagy activities are upregulated. Additionally, differences in basal interferon activity may underlie differences in susceptibility to infection. Using comparative transcriptomic to investigate host-picornavirus interactions locally and systemically, we will gain a better understanding of cellular mechanisms underlying the observed strain-specific differences in host susceptibility to enteric virus infection.

343 - Optimizing conditions for long term colonization of the gut of axenic and conventional zebrafish larvae

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The zebrafish is an excellent model for the observation of the impact of the gut microbiota on the health because of its small size, its optical transparency, and the established protocol to make them axenic. With axenic larvae it is possible to colonize the gut with different bacteria to see the impact that those specific microorganisms have on biological system. Then, with the transparency of the larvae and fluorescence, it is possible to observe the bacteria in the gut. But, to do so, a protocol for the colonization is needed. Different protocols have been used in different laboratory and are different one another, reducing the reproducibility between the different experiments. Unoptimized protocols can reduce the number of bacteria in the gut and prevent colonization. Therefore, in this project, different conditions were tested to develop a clear and reproducible protocols allowing the colonization of the zebrafish's gut from 2 to 9 days post fertilization. Multiple settings, like the addition of live food, the number of larvae colonized simultaneously and more, were tested. Every fish was imaged using fluorescent microscopy to observe the level of fluorescence and gut content were plated to statistically prove that the number of bacteria were increased with optimal conditions. This experiment allowed to see that an addition of live food simultaneously with bacteria is helping for colonization and that a population of 1 larva per mL allows a more constant colonization. By using fluorescent bacteria, it was possible to monitor the colonization over a week and showed that with the optimal protocol, fluorescent was observed from 5 to 9 days post fertilization. With those results, a protocol has been developed, allowing a more reproducible colonization from one fish to another and between the different labs working with zebrafish.

344 - Embryonic and Post-Embryonic Notch-Receiving Secretory Cells Have Different Roles During Intestinal Development in Zebrafish

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Vertebrate intestines across species share many similarities in structure and function. The intestinal epithelium is organized into column structures called villi (mammal, bird) or folds (zebrafish, xenopus) wherein stem cells (SCs) at the base of these columns are responsible for renewing epithelial cells lost to apoptosis at column tips. These SC compartments are also home to secretory cells which aid in regulating proliferation and asymmetric divisions of SCs. Less is known about the structure and cell-cell signaling within the immature intestinal SC niche. We have identified a subset of secretory cells that receive Notch signaling as they differentiate (termed Notch receiving secretory cells- NRSCs). NRSCs associate with proliferative centers during embryogenesis and the immature post-embryonic intestine, suggesting that they may play a role in regulation of epithelial proliferation. Here we disrupt development of NRSCs to determine their role in epithelial proliferation during last half of embryogenesis and the first week of post embryogenesis. Since NRSCs appear to use Notch to differentiate, disruption of Notch signaling should disrupt development of NRSCs and interrupt their ability to function. We find that inhibition of Notch signaling leads to increased proliferation along with increases in proliferation promoting signaling pathways such as EGF, and IGF. We propose that NRSCs play a role in downregulating EGF and IGF pathways resulting in downregulation of epithelial proliferation. NRSCs may then be part of a system to prevent intestinal epithelial cells from over proliferating.

345 - Alagille Syndrome: from genetic mechanism to FDA-approved drug

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Alagille Syndrome (ALGS), which occurs in approximately every 35,000 births, is associated with heterozygous loss of the Notch ligand gene, *JAGGED1*, leading to a postnatal paucity of intrahepatic ducts (IHDs). Cholestasis due to this IHD loss resolves in certain ALGS cases, but fails in most, with no clear mechanisms or therapeutic interventions. Failure to regenerate these lost hepatic ducts consequently leads to progressive liver dysfunction and 76% lethality by late adolescence. Our *in vivo* studies revealed that increasing Jagged/Notch signaling enhances liver duct regeneration in zebrafish models of Alagille Syndrome, implicating Notch signaling increase as a viable therapeutic strategy. This discovery led us to identify and rigorously validate the small molecule, NoRA1, and its more potent analog NoRA2, as direct Notch receptor agonists and potential therapeutics for Alagille Syndrome. NoRA2 is an FDA approved, standard-of-care drug with unknown molecular target and therapeutic mechanism – until now. We find that these Notch receptor agonists robustly augments Notch signaling in ALGS patient primary cells and in livers of orally treated mice and stimulates regeneration of liver duct cells in *jagged* mutant zebrafish that normally fail to regenerate.

346 - Regenerative failure of intrahepatic biliary cells in Alagille Syndrome rescued by elevated Jagged/Notch/Sox9 signaling

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Despite the robust healing capacity of the liver, regenerative failure underly numerous hepatic diseases, including the*JAGGED1* (*JAG1*) haploinsufficient disorder, Alagille Syndrome (ALGS). Cholestasis due to intrahepatic duct (IHD) paucity resolves in certain ALGS cases, but fails in most, with no clear mechanisms or therapeutic interventions. We find that modulating *jag1b* and *jag2b* allele dosage is sufficient to stratify these distinct outcomes, which can be either exacerbated or rescued with genetic manipulation of Notch signaling, demonstrating that perturbations of Jag/Notch signaling may be causal for the spectrum of ALGS liver severities. Although regenerating IHD cells proliferate, they remain clustered in mutants that fail to recover, due to a blunted elevation of Notch signaling in the distal-most IHD cells. Increased Notch signaling is required for regenerating IHD cells to branch and segregate into the peripheral region of the growing liver, where biliary paucity is commonly observed in ALGS. Mosaic loss-and-gain-of-function analysis reveals Sox9b to be the primary Notch transcriptional effector, required cell-autonomously to regulate these cellular dynamics during IHD regeneration. Treatment with a Notch agonist small molecule stimulates Sox9 expression in ALGS patient fibroblasts and enhances hepatic *sox9b* expression, rescues IHD paucity and cholestasis, and increases survival in zebrafish mutants, thereby providing a proof-of-concept therapeutic avenue for this disorder.

347 - Shedding light on the dark yolk phenotype: Identifying novel regulators of lipoprotein metabolism

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Prior large-scale forward genetic screens in zebrafish expanded our understanding of complex biological processes governing development and organogenesis, but yolk biology was not of interest and the presence of abnormal yolk tissue was used as an exclusion criterion. Since these foundational screens, the biology of the yolk and surrounding yolk syncytial layer (YSL) have been further explored, revealing genetic and functional overlap with the liver and intestine. In the YSL, lipid is packaged into ApoB-containing lipoproteins (ApoB-lps) and secreted for distribution to other tissues. When ApoB-lp production is disrupted, lipid is abnormally stored in the YSL. Abnormal lipid storage reduces light transmission which gives the yolk an opaque appearance that is easily identified by low-magnification light microscopy. We have leveraged this recently appreciated, unique, and readily screenable phenotype to identify additional genes involved in ApoB-lp processing using an unbiased forward genetic screening approach. While screening is ongoing, we have already identified 20 dark-yolk mutant families, the majority of which represent previously unknown dark-yolk loci. Of the collection, many produce abnormal numbers and sizes of ApoB-lp particles and exhibit YSL lipid accumulation. A whole-genome sequencing approach has been developed to efficiently map and identify gene candidates for follow-up. Ongoing characterization of novel dark-yolk loci is shedding light on previously unappreciated genetic factors controlling ApoB-lp metabolism, for example, mutant family 14 has been identified as an allele of mia2/ctage5 which we find plays a critical role in the production of large ApoB-lps. In addition, hypomorphic alleles of known dark-yolk loci are expanding our understanding of known factors. By taking advantage of the unique features of the zebrafish model, we have designed a screen optimized for the identification of genes regulating ApoB-lp metabolism which will expand our understanding of ApoB-lp related disease which includes atherosclerosis and fatty liver disease.

348 - Coordinated regulation of the actomyosin cytoskeleton, cell shape, and chamber morphology in the zebrafish heart

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During cardiac morphogenesis, the linear heart tube expands to create cardiac chambers, each with a convex outer curvature (OC) and a concave inner curvature (IC). This stereotypical chamber shape facilitates the function of the embryonic heart, and errors in chamber morphogenesis are frequently associated with congenital heart disease. Using zebrafish as a model organism, our studies show that regional changes in cardiomyocyte morphologies underlie curvature formation: as the ventricle emerges, OC cells enlarge and elongate along their lateral axis, whereas IC cells remain relatively small and round while extending along their apicobasal axis. These divergent growth behaviors result in more squamous cells in the OC and more cuboidal cells in the IC. Coupled with these changes in cell morphology, we find distinct organization of the actomyosin cytoskeleton in each curvature: whereas F-actin and phospho-Myosin are distributed primarily along the basal and lateral cortices of developing OC cells, IC cells exhibit relative enrichment of this network along their apical cortex. These data suggest a link between actomyosin dynamics and patterns of cell shape change; indeed, we find that modulation of actin polymerization or myosin activity disrupts curvature-specific cell shapes. We therefore hypothesize that ventricular curvature formation involves the coordination of curvature-specific reorganization of the actomyosin network with acquisition of squamous and cuboidal cell morphologies in the OC and IC. Intriguingly, we find that the T-box transcription factor Tbx5a is required for both of these processes. Ongoing studies aim to connect the effector genes downstream of Tbx5a with the regulation of actomyosin organization and the attainment of OC and IC cell morphologies. Altogether, our work provides a new model for the control of chamber morphogenesis by localized cytoskeletal dynamics that create patterns of cardiomyocyte cell shapes.

349 - The Role of Autism Risk Gene CHD8 in Neural Development

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There is an urgent need to understand the neurobiological mechanisms underlying abnormal brain development in autism spectrum disorder (ASD), a neurodevelopmental disorder affecting communication and behavior. *De novo* mutations in *Chromodomain Helicase DNA Binding Protein 8* (*CHD8*) are strongly associated with ASD, and individuals with *CHD8* mutation may represent a subtype of ASD. *CHD8* encodes a chromatin modifier that affects cell cycle progression through its role in gene expression regulation. Cell cycle control affects the timing of neural progenitor cell (NPC) proliferation and differentiation into neurons, which lays the foundation for proper neurodevelopment. Although numerous studies to date have improved our insight into CHD8 function, there are still **critical gaps in our understanding of how *CHD8* mutation alters cell cycle progression and neural proliferation, when these alterations take place, and the molecular mechanisms underlying these changes.**

To study the effects of *CHD8* mutation on early neurodevelopment, we generated a zebrafish line with a frameshift deletion in an early exon of the zebrafish ortholog, *chd8,* and performed behavioral, molecular, and gene expression analyses. Heterozygous and homozygous mutants survive to adulthood. Behavioral analyses show homozygous *chd8* mutants have decreased daytime activity and decreased total sleep at night compared to wild-type siblings. Brain activity mapping using phosphorylated extracellular signal-related kinase (pERK) and total ERK staining shows differences in baseline neural activity and brain volume. RNA sequencing suggests chd8 mutants have alterations in expression of genes involved in cell cycle regulation. Experiments to further explore the effects of *chd8* mutation on neuronal proliferation and differentiation at earlier timepoints are ongoing. This work suggests disrupting mutations in a master regulator gene have significant effects on zebrafish behavior and neurodevelopment. The *chd8* mutant zebrafish line will be useful for dissecting the molecular mechanisms that underlie these changes and provide insight into the altered neurodevelopment of autism.

350 - Mios is required to establish female fates in Zebrafish

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It has been consistently observed across several species, including mammals, that nutrition availability during and after development can induce sex biases in a population. In laboratory strains of zebrafish, alterations in nutritional availability due to factors such as rearing density and food competition can bias sexual development. A female bias is observed in high nutrient environments whereas the opposite conditions introduce a male bias. The factors regulating this process are not understood, but several pathways involved in nutrient sensing provide strong candidates for investigation. The mammalian Target of Rapamycin (mTOR) pathway has been well characterized and its role in nutrient availability sensing has been widely accepted. To determine if activity of the mTOR pathway is involved in zebrafish sex determination, we investigated the role of *missing oocyte* (*mios*), a protein required for mTORC2 activation. Mios, a component of the GATOR2 subcomplex, was identified in *Drosophila* (*mio*) where its loss causes oocyte maturation defects and sterility in females. We have characterized the role of *missing oocyte* (*mios*) in zebrafish sex determination and found that, as in *Drosophila*, loss of *mios* results in loss of oocyte fate, which in zebrafish results in male only development. In zebrafish, Mios acts downstream or parallel to the vertebrate specific RNA binding protein Rbpms2 to promote ovary fate. To determine if the *mios* phenotype was due to insufficient activation of mTORC2, we genetically reduced mTORC2 negative regulators, *SPO11 initiator of meiotic double stranded breaks* (*spo11*) and *TSC complex subunit 2* (*tsc2*) in *mios* mutants. We found that loss of *spo11*, unlike in *Drosophila* (*mei-W68*), did not restore female fates in the *mios* mutant gonad. Similarly, reducing Tsc2 was not sufficient to prevent male differentiation. Further studies will investigate alternative regulators and targets of *mios* to better understand the role of *mios* in zebrafish sex determination.

351 - Conserved Myosin light chain function is essential to sarcomere assembly in fast-twitch skeletal muscle

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Sarcomeres are the basic contractile unit of muscle, in which myosin-rich thick filaments interact with actin-rich thin filaments to generate force. Tightly bound to the head of myosin heavy chain in fast-twitch muscle is a protein, 'Myosin Light Chain Phosphorylatable Fast' (Mylpf), that stabilizes the myosin lever arm in the skeletal muscle of vertebrates. We previously showed that zebrafish *mylpfa* is needed for muscle fiber integrity, and that *MYLPF* allelic variants are found in human patients with Distal Arthrogryposis (DA1), but the effect of these variants on muscle development remained unclear. We hypothesized that Mylpf is essential to sarcomere assembly and began testing this hypothesis using knockouts for the two zebrafish *MYLPF* homologs, *mylpfa* and *mylpfb*. We find severe fast-twitch sarcomere defects in the *mylpfa* single mutant and a complete lack of fast-twitch sarcomeres in *mylpfa*$^{-/-}$*;mylpfb*$^{-/-}$ double mutants. The *mylpfa;mylpfb* double mutant fails to link sarcomeric components; instead, actin localizes to the periphery of fast-twitch cells while skeletal myosin is found centrally within the cytoplasm, a defect that begins by the first day post fertilization and persists through larval development. The *mylpfb*$^{-/-}$ single mutant fails to swim at high speed, but appears to have normal sarcomeres, consistent with lower *mylpfb* transcript abundance than *mylpfa* in the wild type. Transgenic expression of *mylpfb, mylpfa*, or human *MYLPF* under the highly expressed *mylpfa* promoter efficiently restores sarcomeres in *mylpfa*$^{-/-}$ mutants, indicating conserved function. However, two allelic variants found in DA1 patients did not rescue *mylpfa-/-* mutant sarcomeres, suggesting that myofibril formation defects may also contribute to this disease. Furthermore, expressing the dominantly-inherited variant MYLPF-G163S in wild-type animals induces sarcomere disorder, indicating antimorphic activity. Collectively this study reveals a vital and conserved role for Mylpf in sarcomere assembly and demonstrates that DA1-causing human *MYLPF* variants can negatively influence sarcomere organization in zebrafish.

352 - Evaluating a New Allele of MTP through Forward Genetic Screen

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[1]Research Assistant, [2]Post Doctorate, [3]Principle Investigator

Lipids are required as sources of fuel, as building blocks for cellular membrane components, and act as signaling molecules. To become available for usage, these lipids must be transported from tissues where they are absorbed or synthesized (intestine, liver) to peripheral tissues; this is achieved through ApoB-containing lipoproteins (ApoB-lps). Lipids, including triglyceride and phospholipid, are packaged onto ApoB through the lipid transfer activity of microsomal triglyceride transfer protein (MTP). Previous characterization of a zebrafish allele of MTP, *stalactite* (*stl*), showed that both phospholipid and triglyceride transfer activities are lost and suggests that MTP(*stl*) is essentially non-functional. However, in a forward genetic screen for genes regulating ApoB-lp biogenesis, we identified a new allele of MTP, named *Mut7*, which exhibits a more extreme phenotype. To establish *Mut7* as an MTP allele, we performed complementation and sequencing analysis. *Mut7* fails to complement MTP(*stl*) as well as a partial function allele of MTP, MTP(*c655*). Bulk segregant analysis using whole genome sequencing identified a non-synonymous point mutation in exon 2 which has been validated by sequencing individual larvae. In contrast to $MTP^{stl/stl}$ fish which survive to adulthood and are fecund, $MTP^{mut7/mut7}$ larvae do not survive past 4 days post fertilization. By utilizing an ApoB-nanoluciferase reporter line, we have found that Mut7 larvae produce no detectable ApoB-lps, while $MTP^{stl/stl}$ fish produce very few, small, ApoB-lps. These data provide evidence to suggest that Mut7 is more severe than stl suggesting there may be additional functions or features to MTP that are, as of yet, not characterized. By further evaluating *Mut7*, we may discover characteristics of this protein that were previously unknown, or discover molecular interactions that further shed light on ApoB-lp assembly.

353 - Visualizing cell cycle dynamics in the developing midbrain

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Embryonic cells are both proliferative and pluripotent, gradually progressing to more restricted fates while also dividing rapidly. Enormous progress has been made in understanding pluripotency genes as well as the genetic machinery that controls the mitotic cell cycle to maintain pluripotency. Importantly, cell cycle control, pluripotency, and cell fate decisions are interconnected. Members of the Zic (zinc finger in the cerebellum) gene family of transcription factors are among key pluripotency controls in mammals and function in a number of embryonic lineages including tectal, cerebellar and neural crest lineages. Zic functions are deeply conserved since the fly homolog of Zics, odd-paired (opa), has been shown to control progenitor pluripotency. We have previously shown that zebrafish *zic2a* controls neuronal progenitor proliferation in the developing midbrain, and hypothesize that it plays a key role in transcriptional regulating the G2/M checkpoint. To test this hypothesis, we will examine *zic2* control of cell cycle progression through live in vivo imaging. Since dynamics of cell cycle regulation are best understood through long-term tracking of single cells in a living embryo, we have set out to engineer mitotic cell cycle reporter transgenics for interrogating *zic2* function during neuronal proliferation. We are using the recently developed GeneWeld CRISPR/Cas9 method to integrate FUCCI, a live cell-cycle reporter, into the *zic2*a locus to place it under the control of endogenous *zic2*a regulatory elements. In a parallel approach, we are using GeneWeld to integrate KalTA4 into the zic2a locus to be used with a UAS:FUCCI transgene. To simplify analysis of cell cycle dynamics in *zic2* mutants, we are using Tol2 to generate zicD5:FUCCI transgenics. These transgenic lines, now under construction, will be used to analyze cell cycle dynamics in the developing tectum of living embryos through high-resolution 4D imaging to ask how *zic2* regulates embryonic neurogenesis.

354 - Zebrafishology: Creating guidelines for rigorous and reproducible data using zebrafish

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The zebrafish (*Danio rerio*) is one of the most widely used research model organisms, second only to the mouse. However, for researchers new to the zebrafish, no guidelines have been created to aid in study design and analysis. Here, we want to open a discussion about key experimental requirements when using the zebrafish as an animal model. We are attempting to highlight both the advantages of the zebrafish while helping those new to the organism avoid experimental design pitfalls often seen. Finally, we are creating a list of critical methods and statistical components of a study that should be detailed within the method section. Additionally, this will help both editors and manuscript reviewers during the peer review process with the ultimate goal of improving reproducibility.

355 - Understanding the effect of mitochondrial localization on anesthetic response

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Though millions of surgical procedures are performed with the use of general anesthesia, we have yet to understand the mechanism by which anesthetic compounds induce states of unconsciousness. It has been shown that mitochondrial proteins are a major target for many of the general anesthetics, particularly within the electron transport chain. Interestingly, we recently demonstrated that propofol and etomidate inhibit kinesin, the motor protein responsible for anterograde movement, thereby preventing the movement of cargo, such as mitochondria, down axons. To investigate the *in vivo* significance of alteration in mitochondrial localization, we obtained two previously identified mutants within the kinesin pathway; a neuron-specific kinesin, *kif5Aa*, and kinesin binding protein (*kbp*), a regulatory protein important in removing kinesin from microtubules. Using IV anesthetics, propofol and etomidate, we are creating hill curves to define the concentration at which 50 percent of the animals respond to the drug (EC50) to both a sedation and general anesthetic behavioral endpoints (loss of spontaneous movement and tap respectively). Additionally, we are assessing movement to tap over time for alteration in behavior during induction and maintenance of anesthesia. Interestingly, within the sedation phenotype, we saw a resistance in *kif5Aa*$^{-/-}$ to both anesthetics. Unexpectedly, over time the larvae equilibrate when exposed to propofol and go back to wild type sensitivity. However, with etomidate we see an increased sensitivity to the drug over time. With *kbp*$^{-/-}$, we see a resistance to both anesthetics over time. These data demonstrate that movement of mitochondria through neurons are a novel mechanism by which anesthetic drugs maintain unconsciousness. Using these key mutant larvae, we will continue to explore the effect of mitochondrial localization on anesthetic response as well as the effect on synapse energy homeostasis.

356 - Whole-brain dynamics and state transitions underlying spontaneous behavior in larval zebrafish

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Simultaneous whole-brain imaging and behavioral experiments in larval zebrafish have started to reveal how distributed neuronal networks interact to generate a wide array of behaviors. While many studies have focused on neural mechanisms behind stimulus-evoked behavior, we ask how spontaneous motor events can be predicted from internal brain states and neuromodulation. Using volumetric resonant two-photon microscopy, we measure whole-brain neuronal activity in head-restrained transgenic zebrafish larvae expressing pan-neuronal nucleic calcium indicators, while monitoring tail movements using a high-speed camera. Unsupervised clustering algorithms reveal multiple spontaneous swim types with different kinematic properties and temporal organization. Using regression methods, these swim clusters are mapped onto spatially distributed neuronal populations which are correlated with movement at various time scales, suggesting distinct roles in generating and encoding behavior. To investigate the large-scale functional background wherein motor activity is generated, we use clustering to decompose brain dynamics into a sequence of recurrent states of activity across brain regions. These brain states are spatially organized into modules of structurally connected regions, while transition probabilities between states are similar across individuals and driven by structural connectivity. As pan-neuronal calcium imaging reveals global patterns of activity, the neurochemical identity of neuronal subpopulations remains mostly concealed. To reveal dopaminergic and noradrenergic cells, we use immunolabeling along with volumetric image registration to project labeled cells onto *in vivo* imaging data. With this information, we are currently studying how neuromodulators drive brain state transitions and motor circuits, leading to multiple observed behaviors of the larval zebrafish.

357 - Fin-specific differential contributions of hox13 paralogs to fin ray patterning

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The exoskeletal part of the zebrafish fins typically contains only soft rays or lepidotrichia. These soft rays are composed of two biconcave hemirays made of bone segments separated by fibrous joints, and contain actinotrichia fibers at their distal tip. Hox transcription factors that provide positional identity during development, and specifically, Hoxa13 and Hoxd13 have been shown to be essential for proper limb/fin development. To examine their potential role during ray development, we induced deletion mutations within the hoxa13a, hoxa13b, and hoxd13a genes in zebrafish using CRISPR-Cas9. No defects are visible in the rays of the single homozygous mutants and the only visible defects in the (*hoxa13a* -/-, *hoxa13b* -/+, *hoxd13a* -/-) and (*hoxa13a* -/+, *hoxa13b* -/-, *hoxd13a* -/-) mutants are slight reductions in ray length. The triple homozygous mutants, and the (*hoxa13a* -/-, *hoxa13b* -/-, *hoxd13a* -/+) mutants however, present more severe defects in the dorsal, anal, pectoral, and pelvic fins, including a loss of joints, severe shortening of the rays, and a severe reduction or absence of actinotrichia. These data suggest that the *hoxa13a/b* genes are more essential for proper lepidotrichia patterning compared to *hoxd13a* in zebrafish. Interestingly, the severe triple mutant phenotype, and especially the loss of joints is reminiscent of the structure of the spiny-rays present in acanthomorph fish. The dorsal and anal fins of acanthomorph fish contain both soft and spiny rays. During development, the spiny-ray domain, in contrast to the soft-ray domain, is characterized by the absence of *hoxa13a/b* expression (Woltering et al (2021) PNAS). Quantitative gene expression and morphological analyses is being conducted to further examine the resemblance *hox13* mutant rays to the spiny rays, and possibly provide evidence of altered *hox13* expression contributing to the formation of spine-like structures in teleost fish.

358 - hapln1 defines an epicardial cell subpopulation required for cardiomyocyte expansion during heart morphogenesis and regeneration

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Certain non-mammalian species like zebrafish have an elevated capacity for innate heart regeneration. Understanding how heart regeneration occurs in these contexts can help illuminate cellular and molecular events that can be targets for heart failure prevention or treatment. The epicardium, a mesothelial tissue layer that encompasses the heart, is a dynamic structure that is essential for cardiac regeneration in zebrafish. The extent to which different cell subpopulations or states facilitate heart regeneration requires research attention.

To dissect epicardial cell states and associated pro-regenerative functions, we performed single-cell RNA-sequencing and identified a particular cluster of epicardial cells had the strongest association with regeneration and was marked by expression of *hapln1a* and *hapln1b*. *hapln1* paralogs are expressed in epicardial cells that accumulate and enclose dedifferentiated and proliferating cardiomyocytes during regeneration. Genetic inactivation of *hapln1b* or induced genetic depletion of *hapln1*-expressing cells altered deposition of the key extracellular matrix (ECM) component hyaluronic acid (HA), disrupted cardiomyocyte proliferation, and inhibited heart regeneration. We also found that *hapln1*-expressing epicardial cells first emerge at the juvenile stage, when they associate with and are required for focused cardiomyocyte expansion events that direct maturation of the ventricular wall.

Our findings identify a subset of epicardial cells that emerges in post-embryonic zebrafish and sponsors regions of active cardiomyogenesis during cardiac growth and regeneration. We provide evidence that, as the heart achieves its mature structure, these cells facilitate HA deposition to support formation of the compact muscle layer of the ventricle. They are also required, along with the function of the *hapln1b* paralog, in production and organization of HA-containing matrix in cardiac injury sites, enabling normal cardiomyocyte proliferation and muscle regeneration.

359 - Exploring early atrial myocardium phenotypes of Atrial Fibrillation using myl4 mutant zebrafish

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Atrial Fibrillation (AF) is the most common longstanding cardiac arrhythmia that remains a global health burden. Increasing age, hypertension, heart failure, valve disease and ischemic disease are well known traditional risk factors of AF, however, the rising role of genetic variants and its interplay with environmental stressors present an exciting opportunity to decipher its molecular mechanism and advance towards therapeutic strategies. We have established a zebrafish AF model by knocking out atrial *myosin light chain gene (myl4).* The *myl4* knock-out fish recapitulated the molecular and electrophysiological phenotypes of AF, including increased connexin hemichannels density, lower heart rate and decreased nppb::luciferase levels. Using this model, we investigated whether dysfunction in *myl4* disrupts the metastable equilibrium in the atrial myocardium and whether this alters cardiomyocyte functionality to favor disease development. We tested if *myl4* knock-out zebrafish exhibited disruption in cell polarity of the embryonic atrial cardiomyocytes by examining a key regulator N-cadherin(*cdh2*). We monitored the dynamic expression and turnover of *cdh2* utilizing the transgenic zebrafish with a tandem fluorescent timer [tFT;TgBAC(cdh2:cdh2-sfGFP-TagRFP)]. We showed that loss of *myl4* altered the expression and localization of *cdh2*, specifically in atrial cardiomyocytes. Such molecular complex abnormalities at individual cardiomyocyte level disrupts adjacent cell polarity and alignment. We have further shown that these early structural and functional phenotypes lead to lateralization of N-cadherin, microtubulins, loss of nuclear lamina and electrophysiological changes in the adult zebrafish heart analogous to human AF. Furthermore, we are investigating the aggravation or inhibition of planar cell polarity to provide deeper insights into complex mechanistic pathways affected in AF, which could lead to new pharmacological targets. In conclusion, defect in *myl4* causes early molecular phenotypic implications in cell polarization and communication that can compound, further pushing destabilizing atrial cardiomyocytes to more pronounced AF phenotypes as the disease progresses.

360 - The glycosyltransferase Lh3 is critical for directing RGC axons toward the midline during optic nerve regeneration.

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The optic nerve conveys visual information from the retina to the brain through Retinal Ganglion Cell (RGC) axons. Injury to RGC axons can cause irreversible vision loss due to the poor capacity of the mammalian Central Nervous System to regenerate. Several RGC intrinsic signaling pathways have been identified that increase axonal growth after axonal injury. In contrast, the extrinsic mechanisms critical for guiding RGC axons toward and across the optic chiasm during regeneration are not well understood. To identify molecular mechanisms critical for optic nerve regeneration, we performed a genetic shelf screen using a robust optic nerve transection assay we developed in larval zebrafish. From this screen, we identified several genes that promote directed RGC axonal growth, including the gene encoding the glycosyltransferase Lh3, which modifies collagen proteins. In *lh3* mutants, regenerating RGC axons fail to cross the optic chiasm and instead project along aberrant trajectories. During initial RGC axonal regrowth as axons approach the optic chiasm, we observed that some RGC axons in wild-type animals initiate growth toward the midline at 24 hours post transection. In contrast, RGC axons in *lh3* mutants at 24 hours post transection project away from the midline. From our screen, we also found that mutants of *collagen18a1* display similar RGC axonal misguidance phenotypes to *lh3* mutants, in that RGC axons frequently fail to grow across the optic nerve chiasm. Ongoing experiments will further characterize the cellular and molecular mechanisms through which Lh3 and Collagen18a1 promote optic nerve regeneration. Results from this work will further define extrinsic guidance mechanisms required for robust RGC axonal regeneration.

361 - Primary cilia as mechanosensing regulators of vascular stability during embryonic development

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The process of vascular stabilization during embryogenesis—which includes mural cell recruitment to the blood vessels—is critical for organism development and survival. Intriguingly, the hemodynamic forces from blood flow itself contribute to vessel stabilization; however, there are still many gaps in our understanding of how this mechanosensitive signaling occurs and how it influences blood vessel integrity. Primary cilia on endothelial cells (ECs) have been proposed to be mechanosensitive structures that facilitate signaling in response to hemodynamic forces, though most of our current understanding of this process stems from *in vitro*work. We aim to determine if EC primary cilia are linked to mechanosensing and vascular stability during embryonic development using the zebrafish animal model. Our preliminary data shows EC primary cilia are present—and unexpectedly, abluminal—during axial vasculature stabilization events at 3 dpf, and that embryos with mutant cilia have phenotypic abnormalities associated with compromised vascular stability—including paracardial edema and cranial hemorrhage. Additional preliminary data suggests that both luminal and abluminal cilia can assemble or disassemble in response to changes in blood flow. Finally, embryos with mutant cilia display mis-targeted mural cell recruitment to veins. Together these data support the central hypothesis that EC cilia respond to hemodynamic forces to influence mural cell recruitment and regulation of gene profiles associated with vascular stability.

362 - Reduced enteroendocrine cell activation in response to nutrient stimulation in a syngap1 autism model

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Research into autism spectrum disorder (ASD), a collection of neurodevelopmental disorders that affects 1-2% of the population, has typically focused on addressing the diagnostic symptoms of socialization/communication impairment and behavior alteration. However, individuals on the autism spectrum and their caregivers frequently report gastrointestinal comorbidities accompanying various forms of autism, including those caused by mutations in *SYNGAP1*; these individuals commonly experience feeding issues and/or severe constipation. Using a CRISPR-generated zebrafish *syngap1* model, we sought to determine whether there is evidence of altered gastrointestinal function by assaying gastrointestinal motility and the enteroendocrine cell-enteric nervous system-smooth muscle circuit underlying motility. Upon confirming our hypothesis that *syngap1* mutants have decreased gastrointestinal motility, we further examined a key component of the motility circuit – enteroendocrine cell (EEC) activity. As EECs are the primary chemosensory cells in the gastrointestinal tract and are responsible for conveying information regarding gut contents to the enteric and central nervous systems, we hypothesized that the *syngap1* mutants would display reduced or absent EEC activity in response to nutrient stimulation. By utilizing calcium imaging techniques, we were able to demonstrate that *syngap1* mutant zebrafish exhibit a reduced EEC response to nutrient stimulation when compared to wild-type larvae. These results indicate that impaired EEC activation could underlie reduced gastrointestinal motility in the *syngap1* autism model. Future experiments will seek to address the mechanism by which EEC response is altered, as EECs do not express *syngap1,* indicating a non-cell autonomous cause.

363 - Using human single-nuclei RNA sequencing to model ascending aortic aneurysms in zebrafish

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Ascending aortic aneurysm (AscAA) is the most common thoracic aortic disease that affects approximately 1% of populations. The progress of an aneurysm eventually leads to dissections and ruptures, which are both fatal once they occur. To date, there are no treatments to prevent or slow down aneurysm progress. Tens of causative genes and variants are known to contribute to the disease, however, less than a quarter of cases can be explained by genetic predisposition, and the mechanism underlying AscAA remains largely unknown. To model human AscAA, we used CRISPR/Cas9 technology to knock-down Lysyl oxidase (*lox*) in zebrafish. *Lox* catalyzes the cross-linking of collagen and elastin stabilizing the extracellular matrix (ECM), and has been previously shown to cause familial thoracic aortic aneurysms. We hypothesized that mutated *lox* would alter the morphology of the embryonic zebrafish outflow tract (OFT), which is rich in elastin and mimics the human aortic root – the most common location for AscAA. CRISPR/Cas9-edited *lox* zebrafish showed more elongated OFTs at 5- and 8-days post fertilization (dpf). Furthermore, these embryos display more tapered, and tortoise ventral aorta (VA) compared to their control-injected counterparts. Moreover, immunofluorescence staining of vascular smooth muscle cells showed reduced coverage around the OFT, VA and proximal trunk area at 3 dpf compared to controls. To unravel genetic mechanisms and novel targets for AscAA, we also performed single-nuclei RNA sequencing (Sn-RNA seq) of human aortic aneurysm samples. The differentially expressed genes from this dataset will be further investigated using zebrafish models. In conclusion, we have developed zebrafish models that represent human AscAA. With transgenic reporter lines and acute CRISPR/Cas9 editing, we are screening a large number of gene targets that were derived from our Sn-RNA seq analysis. Ultimately, we will perform drug screening to rescue the aforementioned AscAA phenotypes and better inform therapeutical interventions.

364 - Lack of proteoglycans has no significant effect on dermal bone formation

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While proteoglycans (PGs) are known regulators of growth factor signalling during endochondral ossification (i.e., bone formation around a cartilage template), the role of PGs in BMP-mediated intramembranous ossification is not fully understood. Recently, unpublished data from our lab suggested that the accelerated endochondral ossification observed in PG-deficient zebrafish (*fam20b* mutants) was BMP-dependent. Therefore, we also hypothesized that PGs normally inhibit BMP signalling during intramembranous ossification. While no defects in intramembranous ossification were seen during embryonic development of *fam20b*$^{-/-}$ fish, we used fin regeneration as another intramembranous ossification system to test the hypothesis. Confirming that BMP signalling plays a role in caudal fin regeneration, DMH1, an inhibitor of the kinase domain of BMP type I receptors, decreased average outgrowth size in almost all the fin rays (except for ray # 10) in *Tg(5xBMPRE-Xla.Id3:GFP)*^{ir1189} fish line. Furthermore, significant differences were detected in the GFP signal and second to fourth rays on both dorsal and ventral edges in this fish line. According to these results, we concluded that BMP signaling was successfully inhibited by DMH1 which inhibited fin elongation. However, *fam20b*$^{-/-}$ showed normal caudal fin regeneration indicating that PGs had no significant effect on the regeneration. EGFP signals during the fin regeneration of *Tg(BmpRE:EGFP)*^{pt510} could be linked to vascularization which marks the artery but not vein in each fin ray in this fish line. Regenerated patterning as well as the EGFP signals was not affected by DMH1, which revealed that the artery reconstruction of this fish line was not influenced by the BMP signalling.

365 - zebrafish gut

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zebrafish gut

366 - Unraveling the developmental biology of atherosclerosis using ldlr mutant zebrafish

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Atherosclerosis is the leading cause of mortality globally and is characterized by low-density lipoprotein (LDL) carrying cholesterol accumulating in the arterial wall. Although late-stage atherosclerosis is well understood, the developmental mechanisms underlying the disease prior to cholesterol elevation are unexplored. Familial hypercholesterolemia (FH) is a Mendelian, accelerated form of atherosclerosis, often caused by mutations in *LDL* receptor (*LDLR*). Similar to FH patients and murine models, *ldlr* mutant zebrafish develop vascular hypercholesterolemia and hyperlipidemia. To unravel the earliest molecular and cellular changes underlying atherosclerosis, we interrogated *ldlr* homozygous mutant and wild-type zebrafish from 2 dpf using single-cell RNA sequencing (scRNA-seq) and whole-mount staining. ScRNA-seq analysis of flk:GFP+ endothelial cells (ECs) and flk:GFP- cells at 2 dpf demonstrated the presence of 10 major cell types that formed 15 distinct clusters. Intriguingly, the *ldlr* mutant group displayed one additional population of ECs that was significantly enriched for heat shock proteins (particularly *hsp70*) and other stress response genes. Further analyses confirmed this perturbed EC population was present in the trunk vasculature. Additionally, pro-inflammatory regulators *il1b* and *nfkb* were markedly upregulated in the immune cell population of *ldlr* mutants. Whole-mount staining for macrophage and neutrophil lineages revealed an excess of both cell types in the caudal hematopoietic tissue of *ldlr* mutants at 2 dpf and 3 dpf. Global reactive oxygen species (ROS) levels were also concomitantly enhanced at these timepoints. Following tail wounding, immune cell recruitment and ROS were augmented in *ldlr* mutants, corroborating our findings during steady state. Taken together, our data demonstrate that EC and innate immune biology are dysregulated during early development in *ldlr* mutants. Further work is required to unravel endothelial-immune-hematopoietic crosstalk in this model. Understanding the developmental biology of atherosclerosis prior to the emergence of dyslipidemia could ultimately improve primary prevention strategies for FH patients and other atheroprone individuals.

367 - Examining the genetic basis of behavioral diversity and evolution in the cavefish Astyanax mexicanus

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Animals evolve morphological, physiological and behavioral traits following colonization of a novel environment. However, identifying the genes contributing to the evolution of these traits remains challenging. We are investigating the genetic basis of behavioral evolution in the blind Mexican cavefish, *Astyanax mexicanus*. *A. mexicanus* exist in two interfertile forms, a river-dwelling surface form and a blind cave form. Cavefish from multiple, independently-evolved populations of *A. mexicanus* have repeatedly evolved a suite of traits including regression of eyes and reductions in pigmentation, reduced sleep and alterations to foraging behaviors. We have established the use of CRISPR-Cas9 gene editing in *A. mexicanus* to investigate the genes underlying behavioral evolution in this species. Using these approaches, we have evaluated the role of multiple genes implicated in the evolution of sleep in cavefish. One of the genes, *oculocutaneous albinism 2* (*oca2*) gene, is responsible for albinism in multiple cave populations. Utilizing surface fish with engineered mutations in *oca2*, we have found that mutations in *oca2* do indeed cause albinism, as well as behavioral changes in cavefish. Albino *oca2*-mutant surface fish sleep less than wild-type pigmented siblings, suggesting that *oca2* plays a role in the evolution of reduced sleep in cavefish. Further, albino *oca2*-mutant surface fish have defects in visually-mediated larval feeding behavior. We are currently investigating the neuronal bases of these changes. Together, these studies provide insight into the genetic basis of behavioral diversity in natural populations, and demonstrate that there is a link between morphological and behavioral traits.

368 - Deciphering the cellular and molecular mechanisms of angiogenesis by fluorescence-based bioimaging in zebrafish

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Angiogenesis refers to physiological and pathological processes through which new blood vessels form from pre-existing vessels. Under physiological conditions, angiogenesis is induced during embryonic development, wound healing and ischemic diseases to maintain homeostasis. It is also implicated in the pathogenesis of various diseases, such as cancer, arthritis, diabetic retinopathy, and macular degeneration. However, the cellular and molecular mechanisms underlying regulation of angiogenesis remain still largely unknown, because methods of analyzing this highly dynamic process in vivo have been lacking. To address these questions, we have established zebrafish lines in which endothelial cells express fluorescent biosensors that allow visualization of various cellular and molecular functions (Dev. Biol. 2014; Development 2015; Dev. Cell 2015; Development 2016; Dev. Cell 2019; Kidney 360, In press). By performing fluorescence-based bioimaging of these fish lines, we successfully visualized dynamic behavior of endothelial cells and delineated the signaling pathways underlying migration of endothelial cells during developmental angiogenesis. Recently, we have also established a novel live-imaging technique for adult zebrafish and investigated how endothelial cells and pericytes establish neovascular networks during cutaneous wound angiogenesis (Angiogenesis 2019). Although it has been believed that induction of angiogenesis induces detachment of pericytes from the vessel wall to facilitate sprouting of endothelial cells, we found that pericytes proliferate and migrate to cover the blood vessels upon induction of angiogenesis, revealing an unexpected role of pericytes in cutaneous wound angiogenesis. Furthermore, we recently demonstrated a novel role of blood flow-driven intraluminal pressure in wound angiogenesis by indicating that injured blood vessels mainly elongate from the downstream side, but not the upstream side, of blood flow (Nat. Commun. In press). In this symposium, we will present our recent progress on the mechanisms of angiogenesis, and introduce how fluorescence-based bio-imaging technique is useful for studying medical and life sciences.

369 - Generation of novel skeletal muscle fibres in the adult zebrafish

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Vertebrate skeletal muscles consist of morphologically and physiologically distinct types of fibre that express different myosin isoforms. The myosin regulatory light chain 10, encoded by the *myl10* gene, is expressed specifically in primary slow-twitch fibres in zebrafish embryos, juveniles and adults. Mutation of *myl10* disrupts the sarcomeric organisation of slow-twitch fibres, reducing locomotor activity of larvae and juveniles. Remarkably, however, locomotor activity recovers in young mutant adults, a recovery that coincides with the emergence of a novel population of slow-twitch fibres at the interface between the intermediate and slow-twitch muscle fibres. In wild-type fish, this interface is occupied by a poorly characterised population of small diameter fibres, the so-called Red Muscle Rim (RMR) fibres. We find that RMR fibres express distinct myosin regulatory light chains encoded by the *myl2a* and *2b* genes: strikingly, the newly arising slow-twitch fibres in myl10 mutants also express the myl2 genes, suggesting that they may derive from the RMR fibres or their progenitors. We are currently using transcriptome analysis to investigate this possibility.

370 - Two MARCKS in zebrafish neural tube morphogenesis

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In vertebrates, the neural tube is formed from the dorsal ectoderm during neurulation, through morphogenetic movements and cell polarity transitions in great part driven by actin and its modulating proteins. Among these are the gnathostome-exclusive MARCKS family, composed by two proteins encoded by respective genes in most taxa, duplicated into four in teleosts. Our group previously reported that the four zebrafish genes are expressed and their knock-down leads to a particular phenotype, with two of them, MARCKSb and MARCKS-Like1a (MARCKSL1a) appearing more relevant in central nervous system morphogenesis. Here, we further characterized the function of these proteins by generating specific antibodies and disrupting their expression through morpholinos and CRISPR-Cas9. Confocal imaging of embryos ranging from 10-somites stage to 24 hpf showed that MARCKSb is localized to the plasma membrane, as expected, while MARCKSL1a is surprisingly found on small cytoplasmic particles. Interestingly, we found an enrichment of immunoreactivity for both proteins towards the apical side of neural keel cells, with differential temporal patterns for each, before and around the onset of cavitation. We had previously found that morpholinos to *marcksl1a* generated an N-cadherin (*cdh2*)-like phenotype (a T-shaped neural tube cross section), which we now confirmed and quantitatively compared by using CRISPR-Cas9. We sought for a potential functional interaction between both genes by using sub-dose morpholino treatments, finding that doses that did not result in a visible phenotype on their own, generated a very penetrant T-shape neural tube phenotype upon co-injection. On the other hand, a similar experiment using low doses of both *marcksb* and *marcksl1a* morpholinos showed a more severe phenotype than each alone, indicating an important degree of functional interaction between them as well. In conclusion, both MARCKS proteins cooperate among them and MARCKSL1a cooperates with the adhesion molecule N-cadherin in the correct formation of the zebrafish neural tube.

371 - Modelling spinal muscular atrophy in zebrafish model.

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Spinal Muscular Atrophy (SMA) is a rare autosomal recessive neurodegenerative disease primarily caused by mutations in *SMN1*. It is the leading genetic cause of infant mortality. Despite recent advances in the SMA therapeutics, there remains a need to identify potential therapeutic compounds that can exert their effects via non-SMN dependent mechanisms. Morpholino-based knockdown is commonly used in zebrafish to study SMA pathogenesis. However, this *smn* knockdown model is transient and not appropriate for efficient drug discovery purposes. Thus, development of a stable model for screening candidate drugs would be valuable for identifying of lead compounds as potential therapeutics for SMA. Here we generated and characterized a *smn* KO zebrafish mutant using the CRISPR/Cas9 genome targeting. The *smn* mutants displayed impaired locomotor activity, axon branching defects and abnormal NMJ structures. We demonstrated that loss-of-function of *smn* specifically in motoneuron is principally the cause of motor deficits, motoneuron loss and muscle atrophy in zebrafish. As a proof-of-principal, we showed that treatment of the *smn* mutant larvae with salubrinal significantly improved locomotor activity. These findings show the potential value of using this zebrafish SMA model for efficient *in vivo* screening of potential therapeutic compounds.

372 - Anatomy and Development of the Pectoral Fin Vascular Network in the Zebrafish

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In this work, we describe the assembly of the vascular network supplying blood to the zebrafish pectoral fins, analogous structures to the forelimb of tetrapods. The superficial location of the pectoral fin and its developing vessels make it ideal for observing and studying a broad timespan of vascular development and vascular network assembly.

Our analysis of this process spans from initial sprout into the limb bud through the first adult-like vascular network forms at approximately 3-4 weeks post fertilization. The blood vessels that feed the pectoral fins begin via a highly stereotyped process that starts with invasion of dorsal and ventral sprouts from the common cardinal vein into the fin bud. These sprouts grow around the endoskeletal disk of the pectoral fin and converge to become the primary arc of the pectoral fin vasculature. Completion of the flowpath and perfusion of the primary arc with blood only occurs after a sprout from the base of the ventral arm of the primary arc migrates to and attaches to the dorsal aorta; this attachment follows a reproducible path in the trunk to supply an arterial feed near the base of the second trunk intersegmental vessel.

After the initial embryonic flowpath through the fin is assembled, we then show additional, and fascinating, reorganization events occur to bring about an adult-like vascular structure. The primary arc of the pectoral vessel remodels to form a distal vascular plexus in the fin fold that subsequently remodels a second time and sprouts separate arterial and venous networks that give rise to the vasculature that feeds the fin rays. We also show early data showing that notch signaling contributes to the patterning of specific branches of the initial circulatory circuit.

373 - Interrogation of GPR68 and acid signaling in Zebrafish development

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Our lab has identified Ogremorphin (OGM), a highly specific small molecule inhibitor of GPR68, in a Zebrafish based chemical genetic screen. GPR68 is a G-protein–coupled receptor (GPCR) originally described as sensing extracellular proton concentration (pH). Dysregulation of GPR68 has been implicated in several pathophysiological processes such as inflammation, bone absorption, and cancer. We have used OGM to show that GPR68 promotes glioblastoma (U87 and U138), human lung adenocarcinoma (A549), and murine pancreatic adenocarcinoma (Panc02) cell survival, migration, and proliferation. We demonstrated that in Zebrafish inhibition of GPR68 with OGM or Morpholinos resulted in a wavy notochord similar to the catastrophe mutant phenotype. Additionally, GPR68 knockdowns exhibited craniofacial malformations and loss of pigment consistent with neural crest defects. However, recent findings show GPR68 is a critical sensor of fluid flow and a sensor of mechanical stretch. OGM is the first small molecule to inhibit all three functions of GPR68. To interrogate how GPR68 is sensing changes in extracellular pH in vivo, we generated a transgenic zebrafish expressing a GPI-anchored form of the ratiometric pH-sensitive green florescent protein, pHluorin2, under a ubiquitin promoter. We found that while globally Zebrafish are at a neutral pH, they exhibit dynamic microdomains of low extracellular pH. High incidences of these domains occurred from 10-12 hpf, corresponding to periods of widespread cell migration. Currently, we are utilizing light sheet microscopy and transgenic RFP lines to identify specific cell populations and build 3D models of pH microdomains in vivo.

374 - Link between cerebellar pathology and loss of C9orf72 in zebrafish ALS model

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that specifically targets the motor neurons. A hexanucleotide repeat expansion in C9ORF72 is the major genetic cause of ALS. Loss of motor coordination and fine balance are the clinical hallmarks of patients suffering from ALS, but the brain region controlling those bodily functions, i.e. cerebellum, has been widely overlooked in ALS studies. Patients carrying the HRE in C9orf72 display widespread cerebellar atrophy in posterior lobe and vermis. Additionally, dipeptide repeats from translation of HRE in C9orf72 have been found in motor neurons and/or glia in the spinal cord/brainstem and motor cortex/ cerebellum. As cerebellum harbors most neurons responsible for the reciprocal connection between the brain and spinal cord, the study of cerebellar pathology could be valuable in ALS. To investigate the cerebellar pathology in ALS, we used synthetic micro-RNAs to specifically target the zebrafish C9orf72 gene (C9-miRNA) to develop an ALS model with reduced expression of C9orf72, as observed in patients. Loss of function of C9orf72 in zebrafish results in severe motor behavioural deficits.We then assessed the overall cerebellar morphology in our zebrafish C9orf72 ALS model by immunohistochemistry. We observed anomalies in cerebellar morphology in zebrafish upon loss of function (LOF) of C9orf72. We are gearing up to perform a detailed analysis of the cerebellum by (i) first assessing numbers of cerebellar inhibitory and excitatory synaptic puncta in C9orf72 LOF and wild-type zebrafish on larval and adult brain sections. As Purkinje cells (PC) play a general role in encoding, controlling, and/or adjusting parameters of larval zebrafish swimming, we will analyze the link between disturbances in GABAergic synaptic transmission in Purkinje cells and aberrant behaviour in C9orf72 mutants. Our study will ultimately shed light on the alterations in the cerebellum in a zebrafish ALS model and may help us understand better ALS pathogenesis.

375 - High-throughput automated behavioral analysis of novel calcineurin signaling pathway-modulating compounds using zebrafish larvae

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Calcineurin is a serine/threonine phosphatase known to act in various organ systems. In the brain, it is involved in modulating synaptic plasticity, learning, and memory. Increased calcineurin signaling in the brain is associated with the onset of neurological disorders and calcineurin inhibitors are considered potential therapeutics for treating these disorders. Even though the importance of calcineurin signaling in neurological disorders is evident, the exact mechanism in which calcineurin dysregulation results in the pathophysiology of neurological disease is elusive. Using zebrafish larvae, we screened a small-molecule library with FDA-approved drugs for effects on behavior, imaged in a 384-well format. We developed automated behavioral analysis to quantify behaviors and found that various drugs affect activity, habituation, startle responses, excitability, and optomotor responses. The changes in behavior were organized in behavioral profiles, which were examined by hierarchical cluster analysis. The calcineurin inhibitors Cyclosporine A (CsA) and Tacrolimus (FK506) produced a distinct behavioral profile that includes increased hyperactivity, acoustic hyperexcitability, reduced visually guided behaviors, and reduced habituation to acoustic stimuli. The calcineurin inhibitors form a functional cluster with unique drugs without any known direct implication in the calcineurin signaling pathway. We propose that drugs with ‘CsA-type’ behavioral profiles are promising candidates for the prevention and treatment of neurological disorders.

376 - Defining cell identity by simultaneously examining transcription and its epigenetic regulation.

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The identity of a cell can be defined by the expression of certain genes as well as its regulation at a DNA level. Lineage restriction can be viewed as a set of steps that allows an undefined cell to become committed, specified and finally differentiated into a certain cell type. How a noncommitted cell acquires the cell identity of a particular cell type is not well understood however it is implied that regulation of the DNA must be involved. In this study, we attempted to track the progression of a cell from a committed to differentiated state by examining the heterochromatin as well as the repression and activation of large genomic regions via nuclear lamina associated domains (LADs). 15 somite zebrafish embryos were subjected to single cell Dam&T-seq. In this next-generation sequencing technique, we are able to simultaneously generate transcriptomic and epigenetic regulatory information within the same cell at a single cell level. We applied this technique to the developing 15 somite stage zebrafish embryos and examined H3K9me3 and LaminB1 profiles according to the cell identity. As an example, we found that the notochord exhibited high levels and broad enrichment of H3K9me3 that was not seen in other cell types which may suggest that other than having specific markers for notochord cell identity, the maintenance of its cell fate may in part be due to the active repression of other lineages.

377 - Simulating Neurodegeneration in Zebrafish: From Lost Organelle Contact to Disrupted Swimming

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The rare, inheritable neurodegenerative disorder Hereditary Spastic Paraplegia (HSP) is mainly characterized by progressive spasticity and weakness of the lower limbs. The cause of these symptoms is the retrograde degeneration of the longest axons in our body also known as the corticospinal tracts. HSP is caused by mutation of about 80 different genes that represent only 50% of the total, which indicates that there are many more candidate genes for HSP. HSP genes are involved in a number of cellular processes such as membrane and axonal transport, endoplasmic reticulum modeling and shaping, mitochondrial functions, abnormalities in lipid metabolism, myelination etc. Here, we are investigating the role of a mitochondrial structural protein in HSP using zebrafish as a model. A single point mutation in *tomm70* changes a conserved amino acid from isoleucine to threonine. This mutation leads to the partial disruption of the organelle contact between Tomm70 and Lam6, an ER protein and the interaction partner of Tomm70, mainly known for the transport of sterols. Disrupted organelle contact leads to reduced cholesterol levels in the brain of *tomm70* mutants. An overall effect of this loss is seen on the locomotion of *tomm70* mutant zebrafish. They are found to be slow in their swimming movement as compared to their wildtype siblings. Overall, we want to define *tomm70* as a novel HSP gene.

378 - Understanding the embryonic zebrafish heart at single-cell RNA-seq resolution

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The zebrafish heart is composed of four morphologically and functionally distinct segments, comprising sinus venosus, atrium, ventricle, and bulbus arteriosus. Numerous studies aimed at molecular characterization of the heart have revealed that each of its segments is made up of heterogeneous cell types which work in synchrony to maintain proper heart function. With the growing use of the zebrafish to model human heart biology, a deeper insight into its complex cellular composition is critical for a better understanding of heart function, development, and associated malformations. Here, we delineated major cell lineages and sublineages of the developing heart by single-cell RNA sequencing. We utilized the transgenic lines *Tg2(myl7:mRFP)*, *sqet33mi59BEt* and *sqet31Et* to mark the cells of the myocardium and cardiac conduction system. We provide transcriptome profiles of over 50 000 cells representing building blocks of the zebrafish heart at 48 and 72 hpf and define at least 18 discrete cell populations. Taking advantage of the endogenous *egfp* expression in the transgenic lines and well-established gene signatures, we pinpointed a population of cells likely to be the primary pacemaker and identified the transcriptome profile defining this critical cell type. Our study established a cellular atlas of the zebrafish heart which constitutes a valuable resource for further investigations into cellular and molecular mechanisms of this organ.

379 - A novel cardiac enhancer drives trabeculae-specific expression

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Enhancers are *cis*-regulatory elements in the genome which increase the likelihood of transcription of one or more distally located genes. Due to their function in regulating gene expression they play an equally important role in developmental processes as protein-coding genes. Trabeculae formation is a key morphologic event during cardiogenesis and contributes to the formation of a competent ventricular wall. Disruption to this process could lead to abnormal morphology and improper functioning of the heart. The zebrafish (*Danio rerio*) harbours the potential for rapid discovery and functional analysis of genetic regulatory elements implicated in heart development. A screen based on transcriptome and chromatin accessibility analyses on zebrafish cardiomyocytes identified putative cardiac enhancers, among which, a specific enhancer located on chromosome 7 (Chr7_51.4M) was identified based on its ability to drive transient GFP reporter expression in the heart. Further analyses of stable transgenic lines carrying this enhancer by light-sheet microscopy revealed striated *gfp* reporter expression domain in the myocardial wall of the ventricle, marking the developing trabecular structures. In order to identify the target genes of this enhancer, we determined the expression patterns of its flanking genes. A mitochondrial gene *Glutamic-oxaloacetic transaminase 2b* (*got2b*) located ~6kb downstream showed the same expression pattern in ventricle, making it a potential target of the Chr7_51.4M enhancer and a possible player in trabeculae development. I will present our ongoing functional analyses of this enhancer and discuss the possible insights our study can contribute into understanding the role of regulatory elements in heart development and disease.

380 - Mmp13 plays expected and unexpected roles during zebrafish morphogenesis.

Matthew Mabey[1], Christopher Small[2], Bryan Crawford[1]

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The matrix metalloproteinases (MMPs) are large and ancient family of zinc-dependent endopeptidases widely known for their roles in degrading extracellular matrix (ECM) proteins, and are generally considered as primary effectors of tissue remodelling during development, wound healing and regeneration. In mammals, three MMPs are categorized as 'collagenases' capable of degrading fibrillar collagens (MMP-1, -8 and -13), but the zebrafish genome encodes paralogous copies of *mmp13a* and *mmp13b*, and no other collagenases. RNAseq data suggests that only *mmp13a* is expressed significantly during embryonic development. Using immunofluorescence we detect Mmp13 protein in the hatching gland, epidermis, myotome boundaries, notochord sheath, and associated with specific neurons in the ventral neural tube at 24 hpf. Starting at around 48 hpf, we see strong Mmp13 signals in the posterior notochord, and *intra*cellularly within the sarcomeres of maturing myofibrils. At 72 hpf and later stages, we see persistent accumulation of Mmp13 in the epidermis, in mesenchymal cells associated with collagenous actinotrichia in the fin folds, and in migrating macrophages. Using a fluorescent collagen-hybridizing peptide (fCHP) we can detect total and denatured collagen *in situ*, and find that Mmp13 localization correlates with some, but not all sites of collagen denaturation, suggesting that non-canonical collagenases (possibly Mmp14, which we also detect at many loci of collagen degradation) and possibly non-MMP proteases play a significant role in collagen remodelling during zebrafish development. We find that pharmacological inhibition of Mmp13 activity results in notochord and epidermal defects, consistent with essential roles of Mmp13 in remodelling fibrillar collagens during tissue morphogenesis in the zebrafish embryo. Finally, using CRISPR/Cas9 mutagenesis, we find that *mmp13a* crispants exhibit an unexpected pigmentation defect, suggesting that epidermally-mediated collagen remodelling may be important in neural crest cell migration/invasion/differentiation.

381 - A computational approach to identify cardiac disease-causing enhancers

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Congenital heart disease (CHD) is the most common cause of mortality among infants leading to premature death and stillbirths. Genome-wide association studies (GWASs) have identified a large number of single-nucleotide polymorphisms (SNPs) associated with various forms of CHD. These studies have highlighted the high prevalence of disease-associated variants within the non-coding regions of the genome, which could affect enhancers, a class of regulatory elements responsible for regulating gene expression. Here, we aim to elucidate the contribution of non-coding genomic SNPs towards the pathological mechanism of CHD through a network analysis approach. Understanding complex diseases with the perspective of organizing principles of the biological networks architecture offer a great potential to address fundamental properties of genes implicated in diseases. To this end, we developed a bioinformatics pipeline integrating multiple layers of publicly available data encompassing genome-wide genotype-phenotype associations, *cis*-regulatory elements (enhancers) database and epigenomic information from major genomic studies to systematically identify CHD-associated genetic variants. We applied network-based approach to establish a mechanistic link between coding and non-coding elements contributing to CHD and model their regulatory interactions represented in a comprehensive genetic interaction map. Combining the power of network analyses and *in vivo* targeted biological validations in the zebrafish (*Danio rerio*) model organism, results from our study is envisaged to reveal novel CHD-causing genetic factors and mechanisms which will facilitate basic and clinical science research in this field.

382 - Direct functional imaging of Piezo1 dynamics with the designed fluorescent reporter GenEPi

Periklis (Laki) Pantazis[1]

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Mechanosensing is a ubiquitous process to translate external mechanical stimuli into biological responses. However, non-invasive investigation of cellular mechanosensing remains challenging. Here, we developed GenEPi, a genetically-encoded fluorescent reporter for mechanical stimuli based on the essential mechanosensitive ion channel Piezo1. We demonstrate that GenEPi has high spatiotemporal resolution for Piezo1-dependent mechanical stimuli from the single-cell level to that of the entire organism. GenEPi revealed mobile and functionally dynamic Piezo1 clusters in the plasma membrane of single cells, resolved repetitive mechanical stimuli of contracting cardiomyocytes within microtissues, and allowed for robust and reliable in vivo optical analysis of Piezo1 function in zebrafish. GenEPi will enable non-invasive optical monitoring of Piezo1 activity in mechanochemical feedback loops during development, homeostatic regulation, and disease.

383 - Characterizing the role of RHOA signaling in regulating vascular development and integrity

Laura Pillay[1], Megan Ezeude[1], Joseph Yano[1], Andrew Davis[1], Jong Park[1], Matthew Butler[1], Madeleine Kenton[1], Daniel Castranova[1], Gennady Margolin[1], Joshua Forgy[2], George Davis[2], Brant Weinstein[1]

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RHOA is a small, monomeric GTPase that transduces stimuli from hormones, growth factors, cytokines, and transmembrane signaling proteins to downstream effectors of cellular signaling via direct activation of target protein effectors, including ROCK1/2. RHOA signaling is dysregulated in hemorrhagic stroke-prone human patients with congenital vascular malformations, suggesting that RHOA signaling may regulate vascular integrity. Previous *in vitro* cell culture studies suggest that RHOA can regulate many critical aspects of vascular endothelial cell (EC) biology, including junctional adhesion, focal adhesion, and actin stress fiber formation. However, the *in vivo* functions of RHOA in regulating blood vessel development and integrity are almost completely uncharacterized and, from a molecular perspective, it is unclear precisely how RHOA and its effectors regulate different EC biological processes.

To study *in vivo* developmental consequences of RHOA gain- and loss-of-function, we generated an allelic series of zebrafish RHOA-ortholog (*rhoaa*) mutants and transgenic embryos expressing wild type or mutant forms of *rhoaa* specifically in ECs. Our combined data suggest that vascular development and integrity in zebrafish is exquisitely sensitive to Rhoa and Rock1/2 gene dosage and/or activity, with too much or too little activity producing cranial hemorrhage and distinct blood vessel patterning defects.

Activated RHOA targets frequently exhibit distinct phosphorylation states. To further elucidate the downstream molecular mechanisms by which RHOA regulates vascular integrity and development, we conducted quantitative phospho- and total proteomic profiling of cultured human ECs with increased or decreased RHOA activity. We identified previously established as well as several new members of the vascular RHOA signaling network. Mutating some of these RHOA pathway targets in zebrafish embryos produces cranial hemorrhage and/or angiogenesis defects, suggesting that our proteomics dataset includes novel regulators of vascular development and integrity that we are now characterizing in-depth.

385 - A Reactive Response to Prolonged Glucose Exposure: Assessing the post-translational activation of Mmp13a in a zebrafish model of diabetic neuropathy.

Matthew Mabey[1], Bryan Crawford[1]

[1]University of New Brunswick

Essential for development and tissue homeostasis, the extracellular matrix (ECM) is a diverse landscape of proteins that are continuously secreted and remodeled by specific proteases. ECM homeostasis is disrupted in many diseases including but not limited to, fibrosis, cancer, rheumatoid arthritis, and diabetes. Matrix Metalloproteinases (MMPs) are zinc-dependant endopeptidases capable of degrading many ECM components and are essential for normal and pathological tissue remodeling. Collagen, an abundant ECM protein, is degraded by collagen specific MMPs (collagenases), whose upregulation is fundamental to numerous diseases. While mammals have several types of collagenases, zebrafish have only one - Mmp13 - which facilitates investigation of collagenase activity in this model. Recently, zebrafish have become established as an excellent model for investigating diabetic neuropathy, a painful and debilitating complication of diabetes. In this context hyperglycemic conditions cause an increase in production of reactive oxygen species (ROS) as well as Mmp13 activity, both of which are necessary for neurodegeneration. MMPs undergo post-translational activation, an often-overlooked checkpoint regulating MMP activity. We suggest that ROS-mediated post-translational activation of latent proMmp13 zymogens may be causative in diabetic neuropathy. Using a novel, doubly epitope tagged Mmp13 construct that reports on the post-translational activation of this collagenase *in vivo*, and improved image analysis methods for quantifying glucose-mediated neurodegeneration in the larval zebrafish caudal fin, we provide evidence for this model.

387 - Piezo1-mediated detection of mechanical force regulates post-translational activation of matrix metalloproteinase-2 (Mmp2) in growing zebrafish embryos

Jillian Hickey[1], Bryan Crawford[1]

[1]University of New Brunswick

To grow and change shape during development, wound healing and regeneration, multicellular tissues must remodel their extracellular matrix (ECM). The matrix metalloproteinases (MMPs) are the primary effectors of ECM remodeling, making their proper regulation central to normal development, and their mis-regulation central to many diseases. All MMPs are synthesized as inactive pro-enzymes that must be post-translationally activated by the proteolytic removal of an auto-inhibitory N-terminal domain. Using a novel transgenic zebrafish, we can visualize the proteolytic activation of matrix metalloproteinase 2 (Mmp2) in intact embryos. Interestingly, Mmp2 is activated in a patchwork-like pattern in the epidermis of growing embryos, suggesting that the mechanical stretching of this tissue as the embryo grows may stimulate ECM remodeling. Piezo1 is an integral membrane stretch-sensitive calcium permeable channel expressed in the epidermis; we hypothesized that mechanical activation of the Piezo1 channel regulates activation of Mmp2, resulting in this patchwork pattern. Consistent with Piezo1 regulating Mmp2 activation, we see dramatic expansion of the patches of activated Mmp2 following treatment with an agonist of Piezo1 (Yoda-1), and statistically significant reduction of Mmp2 activation in the presence of Gd^{3+} ions (which are known to inhibit Piezo1). Moreover, immunofluorescent localization of Piezo1 in zebrafish embryos reveals it is not only abundant in the epidermis, but also localizes to other structures that exhibit strong Mmp2 activation during development, suggesting that Piezo1-mediated detection of mechanical deformation may be a general mechanism for activation of ECM remodeling *in vivo*. These findings are the first to link mechanical stretching of epithelial tissues to the mechanisms of ECM remodeling in an intact tissue and shed light on the feedback mechanisms regulating tissue morphogenesis in vertebrates.

388 - Assessing anti-inflammatory effects of mushroom extracts using live imaging of immune effector cell motility and analysis of matrix metalloproteinase 2 activation in vivo.

Kate Gallant[1], Bryan Crawford[1]

[1]University of New Brunswick

Inflammation plays fundamental roles in both normal physiological responses to injury and pathogens, and can itself be part of a pathogenic feedback loop in diseases such as arthritis and cancer. This facet of the innate immune response is rapidly triggered by tissue damage, which releases cellular components into the extracellular milieu (damage associated molecular patterns - DAMPs), or by the presence of pathogens that are recognized by conserved molecular signatures (pathogen-associated molecular patterns - PAMPs). Zebrafish are emerging as a powerful model system for biomedical research into inflammation, and for testing novel compounds for therapeutic potential. I use transgenic zebrafish, challenged either with tissue damage (DAMPs), or bacterial lipopolysaccharides (PAMPs), to visualize and quantify inflammation-associated activation macrophages or neutrophils, as well as post-translational activation of matrix metalloproteinase 2 (Mmp2) *in vivo*. Using these assays I compare the effects of a known anti-inflammatory glucocorticoid with extracts of mushrooms used in traditional medicine because of their purported anti-inflammatory properties. This data will be compared with complementary *in vitro* studies of the effects of the same mushroom extracts on macrophage activation in response to DAMPs and PAMPs underway in our collaborators' lab at the University of Guelph. Taken together these data will improve our understanding of the efficacy and mechanistic underpinnings of these ancient natural remedies for inflammatory disorders.

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389 - Characterization of a novel zebrafish model of SPEG-related centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of congenital neuromuscular disorders characterized by hypotonia and diffuse muscle weakness, with no approved treatments. The pathobiology of these disorders has been linked to the triads, a skeletal muscle substructure that acts as the primary site for excitation-contraction coupling (ECC), and mutations in genes encoding proteins essential for triad biogenesis and ECC. *Striated muscle Preferentially Expressed Kinase (SPEG)* is a novel gene recently identified to cause recessive CNM. To better understand SPEG's role in skeletal muscle and develop therapy for *SPEG*-CNM, our lab has generated the first *speg* knockout (*speg*KO) zebrafish using CRISPR/Cas9 editing. Using immunofluorescence on isolated myofibres, we observed Speg expression at the sarcolemma and perinucleus at 2 dpf (day-post-fertilization) and predominantly at the triads from 3dpf in wild-type, while Speg expression is depleted in *speg*KO. The *speg*KO fish faithfully recapitulate phenotypes observed in patients with SPEG mutations and *Speg*KO mouse models, including reduced life expectancy, impaired muscle performance, aberrant calcium dynamics, and abnormalities in triad proteins (e.g. RyR1, Dhpr, and Serca-1a) and ultrastructure. Taking advantage of zebrafish models of multiple CNM genetic subtypes, we compared novel and known disease markers in *speg*KO with *mtm1*KO and DNM2-S619L transgenic zebrafish. We observed Desmin accumulation is common to all CNM subtypes, and Dynamin-2 up-regulation in both the *speg*KO and *mtm1*KO. In, all we have established a zebrafish model for *SPEG*-CNM that will enable us to explore SPEG's role in muscle development and CNM; ultimately allowing us to inch closer to developing and translating therapies for this devastating disease.

390 - Paralogues of Stromelysin-3 and Timp4 play Jekyll and Hyde during embryonic development

Nhu Trieu[1], Bryan Crawford[1]

[1]University of New Brunswick

Matrix metalloproteinase 11 (a.k.a. Stromelysin-3 or Mmp11 in zebrafish) plays paradoxical roles in mammalian tumours - both promoting primary tumour growth and inhibiting tumour metastasis - making it a molecule of great interest in cancer biology. Unfortunately little is known about its roles in normal physiology or development. There are two paralogues of Stromelysin-3 in zebrafish - *mmp11a* and *mmp11b* - both of which encode metalloproteinases with the typical zymogen structure: a pre-pro-protease with an N-terminal secretory signal, an auto-inhibitory pro-peptide with a cysteine switch motif, a zinc-binding catalytic domain, and a C-terminal hemopexin-like domain. In both paralogues the auto-inhibitory pro-peptide is linked to the catalytic domain by motifs expected to be cleaved by furin, and we have previously demonstrated this is the case for Mmp11b. Furthermore, we have previously shown that both paralogues of Mmp11 localize to the maturing myotome boundary, where they co-localize with tissue inhibitor of matrix metalloproteinase 4 (Timp4 - also encoded by duplicated paralogues in zebrafish), and that Mmp11 is both necessary and sufficient for the degradation of fibronectin during myotome boundary maturation. Finally, we know that the N- and C- terminal fragments of Timp4 paralogues interact differentially with catalytic and hemopexin domains of Mmp11 paralogues, suggesting that these TIMPs may function in both inhibitory and modulatory roles for these MMPs. Here we show that over-expression of N-terminal domains of Timp4a and Timp4b have dramatically different effects on embryonic development, and similarly that over-expression of Mmp11a and Mmp11b also have different effects. We suggest that the duplication of these homologues of Stromelysin-3 and Timp4 provide an opportunity to dissect the molecular functions of this idiosyncratic protease and its inhibitors *in vivo*, and possibly shed light on its paradoxical functions in tumour biology.

391 - Analyzing the roles of Kdm4ab as a novel epigenetic regulator of arterial specification and development in zebrafish

Miranda Marvel[1], Aniket V. Gore[1], Kiyohito Taimatsu[1], Andrew Davis[1], Daniel Castranova[1], Brant M. Weinstein[1]

[1]NICHD/NIH

Epigenetic mechanisms such as DNA and histone methylation play crucial roles in gene regulation and in directing cell and tissue differentiation during development, but most vertebrate tissue-specific epigenetic regulators are still unknown. Our lab recently developed a novel “EpiTag” transgenic epigenetic reporter zebrafish line that reliably reports tissue-specific epigenetic silencing and activation via the expression of a destabilized green fluorescent protein (GFPd2). Using this line, we performed the first ever large-scale vertebrate forward genetic screen for epigenetic-related mutants and identified a mutant in an uncharacterized histone demethylase, Kdm4ab, with defects in vascular sprouting and arterial vessel development. Transcriptomic analysis of mutants identified a number of dysregulated angiogenic and arterial-specific genes, with many arterial-related genes exhibiting downregulation and venous-related genes showing an unexpected upregulation in mutant tissues. Further *in situ* experiments revealed that these upregulated venous genes are ectopically expressed in arterial vessels of *kdm4ab* mutants. Additional analysis of histone methylation abundance on genetic loci of *kdm4ab* mutants via a novel CUT&RUN (ChIP-Seq) approach revealed many vascular-related genes also have aberrantly increased H3K9me3 abundance, signifying they may be targets of Kdm4ab demethylase activity during development. Our ongoing experiments are currently elucidating the endothelial cell-autonomous roles of Kdm4ab in epigenetically regulating arterial differentiation and vascular development. Our preliminary findings suggest that Kdm4ab is a novel critical epigenetic regulator of arterial specification and development, and we plan to further characterize the mechanisms of action of this histone demethylase.

392 - Dissecting Vascular Reperfusion and Remodeling After Injury in Zebrafish

Leah Greenspan[1], Daniel Castranova[1], Brant Weinstein[1]

[1]National Institutes of Health

The circulatory system is essential for life, and failed vessel reperfusion after tissue injury can cause life threatening complications such as heart attack and stroke, or for cutaneous injuries, defects in wound closure. Although the mechanisms regulating angiogenesis during development have been well studied, the molecular pathways driving vessel regrowth after injury are not well understood. Zebrafish provide an ideal model organism to visualize and study endothelial repair *in vivo* because of their optical clarity, experimental accessibility, conserved vertebrate vascular anatomy, and regenerative capacity. Using adult transgenic fish, I can monitor vessel reperfusion after cutaneous wounding through high-resolution confocal microscopy. I find that immediately after injury blood and lymphatic vessels are absent from the wound area but initiate sprouting by 2 days post wounding (dpw) and fully reperfuse the wound area by 9dpw. While some remodeling occurs, vessel patterning does not return to pre-injury conditions suggesting that signaling mechanisms that are required to set up vessel patterning initially may be absent during wound triggered angiogenesis. To better understand endothelial cell behavior in response to damage, I use the nitroreductase/metronidazole system in zebrafish larvae to mosaically ablate a small proportion of vessels throughout the fish. Most vessels reconnect and relumenize normally due to migrating endothelial cells that replace dying cells, but some vessels disconnect and regrow as the opposite vessel type. This change in arterial/venous identity suggests that vessels are plastic and can adapt to changes in flow after injury. I am now using TRAP RNAseq to probe endothelial gene expression changes upon damage and recovery. Together my studies will uncover the mechanisms that restore vascular networks in injured tissue, providing potential new targets for therapeutic approaches.

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394 - Stem cell mediated kidney regeneration: frizzled9b antagonizes canonical wnt signaling and drives convergent extension and invasion of newly formed kidney tubules after injury

Caramai Kamei[1], Will Sampson[1], Iain Drummond[1]

[1]MDI Biological Laboratory

Adult zebrafish kidneys harbor kidney stem cells that are stimulated by injury to aggregate and differentiate into new, functional nephrons. Nephron function requires engraftment of epithelial tubules onto an existing branched duct architecture and interconnection of tubule lumens. New nephrons show an invasive mesenchymal phenotype at their basal distal surface with pronounced invadopodia penetrating the duct architecture. Regeneration-linked new nephron addition is associated with expression of multiple Wnt ligands, receptors and target gene expression. Injured ducts express *wnt9a* and *wnt9b*, a canonical wnt reporter shows activity in adjacent new nephron aggregates that also express canonical wnt targets *lef1, lhx1a,* and *wnt4* as well as feedback wnt inhibitors *notum1* and *wif1*, and new nephrons express the wnt receptor *frizzled9b.* Treatment of injured fish with an inhibitor of wnt secretion, IWP2, or a Tankyrase inhibitor IWR1 blocked tubule interconnection suggesting a key role for Wnt signaling in new nephron addition. Adult homozygous mutations in *wnt9b, wnt4a,* and *frizzled9b* inhibited new nephron formation. Further analysis of two indel *frizzled9b* mutations revealed that mutant new nephrons exhibited ectopic cell proliferation, failed to undergo convergent extension, lacked properly positioned invadopodia, and showed expanded expression of canonical wnt target genes. We conclude that both canoncial and non-canonical Wnt signaling play key roles in regeneration-linked new nephron formation with non-canonical Wnt signaling being a driver of nephron morphogenesis and interconnection.

395 - SLALOM: A simple and rapid method for enzymatic synthesis of CRISPR-Cas9 sgRNA libraries

Joshua Yates[1], Robert Russell[1], Nathaniel Barton[1], Joseph Yost[1], Jonathon Hill[1]

[1]Brigham Young University

CRISPR-Cas9 sgRNA libraries have transformed functional genetic screening and have enabled several innovative methods that rely on simultaneously targeting numerous genetic loci. Such libraries could be used in a vast number of biological systems and in the development of new technologies, but library generation is hindered by the cost, time, and sequence data required for sgRNA library synthesis. Here, we describe a rapid enzymatic method for generating robust, variant-matched libraries from any source of cDNA in under 3 h. This method, which we have named SLALOM, utilizes a custom sgRNA scaffold sequence and a novel method for detaching oligonucleotides from solid supports by a strand displacing polymerase. With this method, we constructed libraries targeting the *E. coli* genome and the transcriptome of developing zebrafish hearts, demonstrating its ability to expand the reach of CRISPR technology and facilitate methods requiring custom libraries. We are now using this method to conduct a CRISPR forward-genetic screen in zebrafish.

396 - Studying pharynx development using the zebrafish

Kiyohito Taimatsu[1], Daniel Castranova[1], Madeleine Kenton[1], Gennady Margolin[1], Brant M. Weinstein[1]

[1]Division of Developmental Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, 20892

The pharynx and associated organs have important roles in breathing, swallowing, digesting, speaking and protecting the body from infection. Pharyngeal dysfunction can manifest as dysphagia, persistent palatal displacement, or exercise intolerance. Secondary complications are serious and life threatening, and can include aspiration pneumonia, weight loss, and death. However, pharynx development is not well understood, in large part because of the inaccessibility of the developing pharynx to observation and experimental manipulation in most model organisms. I am establishing the optically clear zebrafish as a powerful model for observation and genetic and experimental analysis of pharynx development. I have used histology and confocal imaging to describe adult and developing pharynx anatomy, and scRNAseq to identify resident cell types of the pharynx and their gene expression signatures. My anatomical and molecular findings show the zebrafish pharynx is a complex structure with numerous specialized cell types, many of which correspond well to cell types identified in the mammalian pharynx. I have also used genetic screens that to identify pharynx-specific mutants that I am currently characterizing in detail. My ongoing work also includes careful staged analysis of the early development of the zebrafish pharynx, generation of cell type-specific transgenic lines to characterize the development and function of specific pharyngeal cell populations, and identification and functional work-up of genes required for the proper development of some of these cell types. My results thus far have established the fish as a valuable new experimental model for experimental and genetic analysis of the pharynx and a useful platform for functional and molecular characterization of the molecular basis for pharynx disorders.

397 - Evaluation of the colors blue, green and red as an alternative to the standard black color used in the evaluation of the optomotor response (OMR), for immediate and adaptive responses.

Paula Marroquín-Morales[1], Daniela Suarez[1], Mayeli Abúndez-Olvera[1], Alfonso Rios-Perez[1], Cecilia Zampedri[1], Samantha Carrillo-Rosas[1]

[1]Tecnologico de Monterrey

The OptoMotor Response (OMR) is a visually mediated behavioral test in which zebrafish larvae start swimming prompted by visual motion clues given. These clues have been usually high-contrast images i.e. alternating black and white lines, in motion. However, zebrafish are highly visual tetrachromat animals with red (R, 570 nm), green (G, 480 nm), blue (B, 415 nm), and UV (U, 362 nm) cones, allowing them to have a full color vision. Additionally, there have been reports showing a color preference of zebrafish; the color red has been reported as the most strongly preferred color associated with learning and blue has been preferred in color maze tests. To assess and determine if there is a color preference driving the larvae behavior in the OMR assay, we tested in a free-moving environment zebrafish larvae at different stages (4-7 days post fertilization (dpf)) for the absolute colors that zebrafish can perceive.

To determine if any of the evaluated colors could evoke a greater response, we evaluated the immediate response, i.e., the movement achieved on average by the larvae in a fixed area after the first 10 seconds of the test; and the adaptive response, i.e., the movement achieved on average after 30 seconds of adaptation. Red and black colors did not show a difference in immediate response between them, while blue and green colors showed a significant difference compared to them. For the adaptive response, a significant difference was present only with green compared to the other colors, which suggests that the green color evokes a poorer response.

Our results show that the red color could represent a great alternative to the standard black color presenting a good OMR response and the best larvae-line image contrast for better quantification without the need for additional processing and adjustment to the image.

398 - From Wrappers to Drains: The diverse cellular identities of the Zebrafish Meninges

Marina Venero Galanternik[1], Ryan D. Gober[1], Daniel Castranova[1], Bakary Samasa[1], Tuyet Nguyen[1], Aniket Gore[1], Andrew E. Davis[1], Louis E. Dye 3rd[1], Gennady Margolin[1], Madeleine Kenton[1], Allison Goldstein[1], Avery Seaver[1], Van Pham[1], James Iben[1], Timothy Mulligan[2], Steven L. Coon[1], Jeffrey Mumm[2], Brant M. Weinstein[1]

[1]NICHD, NIH, [2]Neuroscience and Ophtalmology Departments, Johns Hopkins School of Medicine

The vertebrate brain is an essential but surprisingly delicate vital organ protected by several anatomical components, including supportive and frequently disregarded protective tissues called the meninges. Located between the brain and the skull, the cephalic meningeal tissue layers surround the brain shielding it from mechanical shock, providing blood supply, supporting brain buoyancy and maintaining homeostasis by producing, homing and filtering cerebrospinal fluid. Often thought of as “inert tissues,” the meninges remain poorly characterized, in part due to their thin morphology, their location immediately below the skull, and a lack of defined meningeal markers. To overcome these limitations, we are utilizing the transparent and imaging amenable zebrafish as a new research organism for studying the meninges. Our comprehensive teleost meningeal anatomical characterization using histology, electron microscopy and super-resolution confocal microscopy shows that like mammals, zebrafish have well-defined meningeal layers, arranged into the leptomeninges (pia and arachnoid mater) and the dura mater, all populated by a myriad of cellular components. Using scRNA-seq to comprehensively profile all of the resident zebrafish meningeal cell populations, we have identified approximately fifty unique cellular clusters, which include pericytes, fibroblasts, blood and lymphatic endothelial, pigment and immune cells, as well as a variety of uncharacterized cell populations. Among them, Ependymin-expressing cells (EPDs) represent a unique meningeal epithelial cell population that ensheaths other leptomeningeal cells and delimits the boundaries of the leptomeninges. Ependymin, a glycoprotein of the cerebrospinal fluid, represents a novel leptomeningeal specific marker, and our generated ependymin-dependent transgenic lines are allowing us to perform global or partial ablations of the leptomeninges to better understand the importance of this tissue during development, adulthood and post injury regeneration. Our zebrafish studies are leading to a new understanding of the meninges, their developmental origins, their roles in protecting the brain and the effects of meningeal damage.

399 - Cardiovascular Development Data Resource Center (CDDRC)

H Joseph Yost[1]

[1]University of Utah

The CDDRC provides an innovative cloud-based platform to facilitate the analysis, visualization and sharing of genomic data from research in cardiovascular development and regeneration in several species. Leveraging institutional investments in genomics and personalized medicine, the CDDRC will facilitate dynamic and investigator-interactive cloud-based interfaces with multiple -omics datasets from humans and multiple model organisms, resulting in collaborative discoveries of the genetic and epigenetic causes of congenital heart disease (CHD). In addition, our program will have educational components in Ethical, Legal and Social Implications (ELSI) of CHD genetics and genomics, and an outreach program in CHD computational biology for students from underrepresented groups. The CDDRC will bring together researchers with a diverse range of expertise, with the goal of understanding the causes of CHD in children.

The CDDRC is the newest component of the NHLBI Bench-to-Bassinet (B2B) program (https://benchtobassinet.com), along with the Pediatric Cardiac Genomics Consortium (PCGC) and the Pediatric Heart Network (PHN). The goals of the CDDRC are 1) to build and populate the CDDRC platform with multi-omics data (single cell-omics, RNA-seq, ATAC-seq, ChIP-seq, Hi-C, etc.) from multiple organisms from the previous basic science component of the B2B, the Cardiovascular Development Consortium (CvDC), 2) to recruit new datasets from the cardiovascular community to the platform, and 3) to develop, recruit and incorporate new bioinformatics analytic and visualization tools to the platform, and 4) intersect this cloud-based platform with human CHD genetics datasets and the NHLBI BioData Catalyst (BDC) initiative.

At the zebrafish meeting we will be announcing **Challenge Prize competitions** that will fund new projects in the CDDRC from outside investigators, and a new **CDDRC Fellowship program** that will provide training opportunities for outside investigators in cloud-based genomics computation.

400 - Developing a zebrafish gill model of mammalian lung endothelium

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Endothelial cells (ECs) are a major cellular component of the lungs and perform a central role in gas exchange. Recent reports in mice suggest that a subset of lung ECs are highly specialized, with unique anatomical and functional properties facilitating gas exchange. However, the development and specific properties of these unusual cells are still not well understood. I am using the zebrafish as an experimentally and genetically accessible vertebrate model to study the development and function of specialized gas exchange endothelium. Although zebrafish gills function in an aqueous environment, like mammalian lungs, they facilitate gas exchange and share functionally equivalent cell types, including an extensive specialized vascular endothelial network dedicated to oxygen transfer to the circulatory system. To establish the foundations for a zebrafish gas-exchange organ development and function model, confocal imaging was performed on blood and lymphatic transgenic reporter lines coupled with SEM/TEM to characterize the detailed gill vascular anatomy of the adult and compile a comprehensive description of blood and lymphatic vascular growth and patterning in the developing zebrafish gill. We also performed scRNAseq on dissected adult gills to characterize molecular signatures of its resident cells, identifying novel cell populations likely critical for gas exchange physiology. We are also carrying out in-depth targeted molecular profiling of adult and developing blood and lymphatic vessels in the gills, using “RiboTag” lines that permit specific *in vivo* visualization and transcriptional profiling of these EC types. We plan to fully characterize the early development of the gills, and in particular their specialized gas exchange endothelial cell populations, and then use the fish as a genetically and experimentally accessible comparative vertebrate model to study and better understand the roles of endothelial molecular pathways and genes implicated in human lung disease. This work was supported by the Intramural Program of the NICHD (to BMW).

401 - Lysosomal signaling in microglia and Alzheimer's disease

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Genome wide association studies of Alzheimer’s disease (AD) patient mutations strongly implicate immune pathways in disease onset or progression. In the healthy brain, microglia, primary immune cells of the brain, ensure nervous system well-being and function by eliminating dying cells, pruning synapses, and orchestrating appropriate immune responses. Multiple lines of evidence indicate that two essential microglial processes – lysosomal activity and inflammatory response – are aberrantly activated in AD. How these microglial processes, critical for brain development and function, become dysfunctional in the context of aging or disease remains largely unexamined. I propose to study lysosomal and immune responses of microglia in vivo using zebrafish in light of numerous observations showing that microglia cultured in vitro rapidly lose their identity and display aberrant expression of disease-associated genes. During my early postdoctoral training, I defined a lysosomal network centered around the key lysosomal transcription factors Tfeb and Tfe3. Using RNA-Sequencing of macrophages overexpressing Tfeb and Tfe3, I have uncovered the lysosomal targets of these transcription factors in the macrophage lineage. I find that many lysosomal targets of Tfeb and Tfe3 are dysregulated in AD and my ongoing experiments will reveal mechanisms through which aberrant Tfeb and Tfe3 activity contributes to the pathology in AD. As an independent researcher, I will leverage my training in microglial and lysosomal biology to study genes associated with AD risk. I have identified zebrafish homologs of genes mutated in AD, prioritized them based on microglial expression or lysosomal function, and performed a CRISPR mutagenesis screen. My preliminary data confirm that indeed many of these AD risk-associated genes function in microglia. Collectively, my research renders microglial biology accessible to live imaging and functional characterization in vivo, and will bridge the gap between genomic resources available for AD and molecular mechanisms underlying the pathology of this devastating disease.

402 - A new zebrafish model highlights the hallmarks of human Methylenetetrahydrofolate Reductase (MTHFR) deficiency and the role of folate metabolism in energy balance regulation during embryonic development

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Methylenetetrahydrofolate reductase (MTHFR) deficiency is the most common inborn error of folate metabolism associated with various metabolic consequences. In North America, synthetic folic acid (FA) is consumed in excess and may interact with *MTHFR* genotype. While pre-clinical murine models have been valuable to inform on diet-gene interactions, a recent folate expert panel has encouraged the validation of new animal models. The zebrafish (*Danio rerio*) is a proven model for embryonic and nutrient studies with a conserved folate pathway to humans. Using CRISPR-Cas9 mutagenesis, we developed a new model of MTHFR deficiency in zebrafish to explore how the interaction of *mthfr* genotype and FA supplementation affects metabolic dysregulation during embryonic development. Wild-type (WT) and *mthfr*$^{-/-}$ embryos were exposed to either no FA (control) or high FA (100µM, 100FA) throughout embryonic development from 0-5 days post-fertilization (dpf). Food intake (FI by DiD fluorescent dye), energy expenditure (EE by alamar blue), aortic and liver lipid deposition (by Oil-Red-O), and 1-carbon metabolites (by LC-MS/MS) were assessed at the end of the endogenous feeding phase of the zebrafish at 5dpf. *mthfr*$^{-/-}$ had perturbed 1-carbon metabolism as indicated by lower folates, methionine, s-adenosylmethione:s-adenosylhomocysteine (methylation index) and betaine as well as higher homocysteine compared to WT zebrafish independent of FA exposure. *mthfr*$^{-/-}$ zebrafish also had greater lipid deposition (~75%, P<0.0001) and lower energy expenditure (~38%, P<0.001) than WT, which was exacerbated by 100FA exposure. 100FA-mthfr$^{-/-}$ also had 120% higher FI compared to all other groups (P<0.001). Loss of *mthfr* function in zebrafish mimics clinical biochemical hallmarks of severe MTHFR deficiency and is a genetic modifier of energy balance regulation during embryonic development. High dose FA supplementation does not rescue and may even exacerbate these effects. Alternate folate forms may be better suited for patients with underlying genetic conditions. Funding: CIHR-INMD, NSERC-CGS D (EP), NSERC-CGSM (RS)

403 - Elucidating the gene regulatory network of the zebrafish pacemakers

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Rhythmical contractions of the heart are regulated by the electrical impulses generated by the pacemakers, namely, the sinoatrial node and atrioventricular node. The transcription factors Tbx3, Tbx2a/b, and Isl1, have been shown to be responsible for suppression of the cardiomyocyte genetic program and promoting development of the pacemaker cells. However, their downstream molecular mechanism in driving pacemaker development and function is still insufficiently determined. Here, we utilized DamID-seq to identify the genome-wide binding sites of these transcription factors in vivo and decipher their putative binding sequences in zebrafish. Taking advantage of the EGFP expression in transgenic lines sqet33-mi59BEt, we isolated cells of the pacemaking region and profiled their transcriptome and chromatin landscape. By combining the information on their specific binding sites with cellular transcriptome and epigenome profiles, we aim to identify target genes regulated by these transcription factors and determine the mechanism for pacemaker development and function. This in turn will facilitate the generation of zebrafish models for cardiac arrhythmia.

404 - A brain-wide analysis maps structural evolution to distinct anatomical modules

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Brain anatomy is highly variable and it is widely accepted that anatomical variation impacts brain function and ultimately behavior. The structural complexity of the brain, including differences in volume and shape, presents an enormous barrier to define how variability underlies differences in function. In this study, we sought to investigate the evolution of brain anatomy in relation to brain region volume and shape across the brain of a single species with variable genetic and anatomical morphs. We generated a high-resolution brain atlas for the blind Mexican cavefish and coupled the atlas with automated computational tools to directly assess brain region shape and volume variability across all populations. We measured the volume and shape of every neuroanatomical region of the brain and assess correlations between anatomical regions in surface, cavefish and surface to cave F_2 hybrids, whose phenotypes span the range of surface to cave. We find that dorsal regions of the brain are contracted in cavefish, while ventral regions have expanded. Interestingly, in hybrid fish the volume and shape of dorsal regions are inversely proportional to ventral regions. This trend is true for both volume and shape, suggesting that these two parameters share developmental mechanisms necessary for remodeling the entire brain. Given the high conservation of brain anatomy and function among vertebrate species, we expect these data to studies reveal generalized principles of brain evolution and show that *Astyanax* provides a system for functionally determining basic principles of brain evolution by utilizing the independent genetic diversity of different morphs, to test how genes influence early patterning events to drive brain-wide anatomical evolution.

405 - Regionalized protein localization domains in the hair cell kinocilium.

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Sensory hair cells are the receptors for auditory, vestibular, and lateral line sensory organs in vertebrates. These cells are distinguished by hair-like projections from their apical surface that transduce mechanical stimuli into an electrical signal. Most of these "hairs" are actin-filled stereocilia arranged in a staircase fashion to form the characteristic hair bundle. Additionally, the hair bundle features a single, non-motile, true cilium called the kinocilium. The kinocilium plays important roles in hair bundle development and the mechanics of sensory detection. As such, characterization the kinocilium is fundamental to our understanding of sensory hair cells.

Transcriptomic analyses reveal that zebrafish hair cells express many uncharacterized cilium-associated genes. For three of these genes - *ankef1a*, *odf3l2a*, and *saxo2* - the human and mouse orthologs are either associated with sensorineural hearing loss or are located near deafness loci for which the causative gene has not been identified. To begin characterizing these three genes, we made transgenic fish that express fluorescently-tagged versions of their proteins, demonstrating their localization to the kinocilium of zebrafish hair cells. Furthermore, we find that Ankef1a, Odf3l2a, and Saxo2 exhibit distinct distribution patterns, suggesting regionalization along the length of kinocilia. Lastly, we report a novel overexpression phenotype for Saxo2. These results set the groundwork for future studies using gene knockouts to understand the role of these kinocilial proteins in hair cell structure and function.

406 - Engineering a transgenic zebrafish line for synaptic plasticity tracking

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Synaptic plasticity is involved in numerous neurological phenomena, including brain disorders and the mechanisms underlying memory and learning. A transgenic zebrafish line is here developed to study synapse development and synaptic organisation by expressing fluorescent biomarkers targetting synapses and neuronal membranes. FingRs (Fibronectin Intrabodies generated with mRNA display) are genetically encoded proteins able to target the post-synaptic proteins gephyrin or PSD95 located at inhibitory and excitatory synapses respectively. When associated with a fluorescent protein, FingRs act as biomarkers for gephyrin or PSD95, and thus for synapses. To express these biomarkers in zebrafish, plasmid DNA encoding FingRs coupled to a fluorescent protein is injected in fertilised zebrafish eggs. Integration of the plasmid DNA into the genomic DNA is allowed by the presence of a Tol2 transposon system encoded in the plasmid DNA. Confocal and 2-Photon microscopy techniques are used for fluorescence imaging in live zebrafish and allow synaptic organisation tracking across the brain. Additionally, changes in the size and shape of the synapses will be resolved using STED (stimulated emission depletion) microscopy which enable high-resolution molecular-scale imaging (~1µm). Experiments involving exposome manipulation will be carried out on transient and transgenic zebrafish to investigate their influence on synaptic plasticity. These notably include behavioural assays (e.g. social assays, stress exposure), learning assays, and gut microbiota manipulation. Alterations of synaptic development and plasticity are expected to be observed following these experiments.

407 - Defining the dynamic 3D genome during hearing regeneration in the adult zebrafish inner ear

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Unlike mammals, zebrafish can regenerate the mechanosensory hair cells (HCs) located in the inner ear after damage-induced cell death. We aim to reveal key genetic switches that will lead to approaches capable to trigger HC regeneration programs in humans. HC regeneration is a balancing act of supporting cells and HC progenitors (HCPs), that oscillate between self-renewal, proliferation, and terminal differentiation required to replace lost HCs. We developed a transgenic ablation system that allowed us to study HC regeneration in the zebrafish adult inner ear. Our initial studies focused on gene expression and local epigenetic changes at single-cell resolution during regeneration, however, sequencing-based genomic technologies have revealed that the genome has higher-order chromatin organization denoted by multilevel chromatin architectural features such as chromosome territories, A/B compartments, topologically associated domains (TADs), and long-range chromatin loops. We are now interested in identifying the changes in 3D genome architecture that initiate regeneration and instruct HC differentiation. TADs are thought to be mediated through CTCF, a DNA binding transcription and boundary factor along with the cohesin complex. Our unpublished results from bulk ATAC-seq on zebrafish inner ears detected CTCF as a top motif. Moreover, scATAC-seq data showed CTCF enrichment in HCPs emerging peaks as consequence of HC regeneration. I hypothesize that *de novo* formation of TADs and chromatin loops during HC regeneration will reveal a 3D “homeostasis vs. regeneration” profile, and any rearrangement will modify the HCP regenerative plasticity. Here, we adapted a method called single-cell split pool recognition of interactions by tag extension (scSPRITE) that leverages split-and-pool barcoding of thousands of individual cells to map the 3D genome organization in the adult inner ear comparing homeostatic and regenerating inner ears at the single cell level. Completing this project will help identify regeneration-specific TADs and depict putative regulatory regions underlying HC regeneration.

408 - Characterizing Human Disease Associated Mutations: A Zebrafish-Based Approach

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Next-generation sequencing has allowed for rapid advances in the identification of candidate disease-causing mutations. However, functional testing and validation of these mutations has lagged, particularly for rare mutations found only in single families or small populations. At the Hospital for Sick Children in Toronto (SickKids), we have established a Zebrafish Genetics and Disease Models Core Facility to allow for testing of candidate disease genes in zebrafish. Our facility makes use of the shared infrastructure and expertise present at SickKids, and provides services to efficiently generate mutations in zebrafish, including phenotypic analysis to validate zebrafish models and drug screening to help identify novel therapeutic agents. We make use of CRISPR-Cas9, along with High Resolution Melt (HRM) analysis, to generate mutations in zebrafish that are targeted to specific loci. We have developed zebrafish models for a diverse set of human diseases including inflammatory bowel disease, pediatric cancer, cardiac arrhythmia, and childhood muscle disease. In addition, we use Tol2-mediated transgenesis for overexpression studies. To date, we have worked with 20 labs and generated insertion-deletions in over 30 genes using CRISPR-Cas9, including at least 3 large deletions (several kb), as well as over 9 targeted mutation knock-ins based on candidate human disease genes. Here we present results of our high-throughput mutation generation efforts and summarize our pipeline approach.

409 - Heterogeneous pdgfrb+ cells regulate coronary vessel development and revascularization during heart regeneration

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pdgfrb expression marks mural cells and is required for the mural cell association with the blood vessels. In a developing zebrafish heart, *pdgfrb* expression precedes the coronary vessel formation and begins expression in the epicardium around the atrioventricular canal where coronary vessels emerge. *pdgfrb*+ mural cells co-develop with the nascent coronary vessels and are essential for their development. In adult zebrafish hearts, *pdgfrb*+ cells form two separate clusters of cells, the epicardial derived cells (EPDC) and the mural cells, based on single-cell RNA sequencing analysis. The mural cells around zebrafish coronary arteries also express *cxcl12b* and smooth muscle cell markers. Interestingly, these mural cells remain associated with the coronary arteries even in the *pdgfrb* mutant hearts. *pdgfrb* expression dynamically changes in injured zebrafish hearts in which *pdgfrb*+ cells express genes important for heart regeneration. Our results demonstrate that heterogeneous *pdgfrb*+ cells are essential for coronary development and heart regeneration.

410 - Single Cell analysis of Tendon and ligament diversification

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Tendons and ligaments are related connective tissues that attach skeleton to muscle or skeleton to skeleton, respectively. While both connective tissue types appear to arise from a *scleraxis-a* (*scxa*)-expressing progenitor, the regulatory mechanisms that specify tendons versus ligaments have remained elusive. To better understand tendon and ligament development, as well as more generally regional specification of these tissues, we have performed single-cell RNA expression and chromatin accessibility sequencing (scRNAseq and snATACseq) on purified *scxa:mcherry*$^{+}$ cells from the larval and juvenile zebrafish head. At the transcriptomic level, we find multiple genes whose expression distinguish tendons and ligaments, and we use in situ hybridization to validate that *thbs4a* and *mkxa* mark ligaments and *thbs4b* marks tendons in the head. We also uncovered a number of selectively accessible chromatin regions that drive transgene expression in distinct connective tissue patterns in the head. These include enhancers for *thbs4a*, *sparc*, and *mkxa* that drive transgene expression specifically in ligaments, and an *ecrg4a* enhancer that drives expression in tendons. We are currently mining this data to understand the distinct upstream transcription factors driving tendon versus ligament expression. In addition, analysis of the *scxa* locus has revealed a diversity of region- and cell-type-specific enhancers for head and trunk tendons and ligaments, suggesting that diverse regulatory machinery converge to regulate this master transcription factor of tendon and ligament fate. Altogether our single-cell analysis is revealing some of the first molecular and regulatory differences that distinguish tendons from ligaments, as well as region-specific mechanisms for inducing these fates in the head versus trunk.

411 - Whole-body replacement of primordial myofibers supports post-embryonic muscle growth in zebrafish

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Drastic increases in myofiber number and size are essential to support vertebrate post-embryonic growth. However, the collective cellular behaviors that enable these increases have remained elusive. Here, we created a fluorescent reporter system, *palmuscle*, for *in toto* imaging of the growth and fates of ~5000 fast myofibers in developing zebrafish. Using a whole-body, three-dimensional live scanning approach, we tracked the spatiotemporal changes in myofiber number, size, and nuclear number as animals grow. Remarkably, long-term surveillance of individual myofibers revealed massive elimination of pre-existing populations and gradual wholesale replacement by *de novo* births. This turnover process eradicated the vast majority of primordial myofibers before adult stages. We therefore propose that SWAP (Systematic Wipeout of All Primordial myofibers) occurs as an inherent yet under-appreciated mechanism of muscle growth during vertebrate development.

412 - Novel Insights into the Function of As3mt in the Zebrafish Liver

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Arsenic is one the world's most pervasive toxicants and nearly every organism has evolved a dedicated detoxification mechanism specific for this metalloid. *Arsenite methyltransferase* (*as3mt*) is an evolutionarily conserved enzyme that bio-converts trivalent arsenic into methylated species and is essential for cellular clearance of toxic arsenic. However, it is unclear if conservation of a dedicated arsenic-detoxifying enzyme accurately reflects the ongoing risk of arsenic exposure or if *as3mt* has moonlighting functions in other pathways. To explore this, we generated transgenic zebrafish that overexpress As3mt in hepatocytes under the *fabp10a* promoter and *as3mt* mutant zebrafish and evaluated whether either impacted liver biology or altered the response to arsenic exposure. There were no significant differences in phenotypes or gross liver size or hepatocyte morphology in larvae at 120 hours post fertilization. However, during inorganic arsenic challenge (iAs), RNA-seq analysis showed reduction in gene expression in several well-characterized iAs-induced stress responses, including the unfolded protein response and oxidative stress response. This supports a model where overexpression of *as3mt* aids in exporting arsenic, thereby reducing the magnitude of stress responses. Additionally, *as3mt* mutant zebrafish are more sensitive to iAs exposure, supporting the role of As3mt in arsenic detoxification. We next hypothesized that if As3mt has moonlighting roles in methylation then overexpression or knockout of As3mt at baseline may alter total DNA and RNA methylation levels. Slot blot analysis showed hepatic overexpression or knockout of As3mt did not alter total DNA methylation. Interestingly, basal hepatic overexpression of As3mt increased m6a modifications on total RNA. Taken together, these data suggest that *as3mt* is essential for arsenic metabolism and adaptation and point to a potential role for As3mt in RNA modification.

413 - Effects of Folate Analogues on the Development of Liver in Zebrafish

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Methotrexate (MTX) is a folate antagonist widely used to treat several diseases, especially cancer, rheumatoid arthritis and psoriasis. However, one of the side effects of MTX treatment is known to be hepatotoxicity. The biological mechanisms by which folate metabolism interferes with liver development are poorly understood. To disrupt folate metabolism, we treated zebrafish larvae with MTX, a competitive inhibitor of dihydrofolate reductase (DHFR). Exposure of larvae zebrafish at 3 days post fertilization (dpf) to MTX for 72 hr caused significant increases of liver size at 6 dpf, although there were no other developmental abnormalities. Treatment with folinic acid, a folate derivative that enter the folate pathway without the enzymatic activity of DHFR, reversed the MTX-induced liver enlargement. To investigate the cause of liver enlargement, it was confirmed that the size of nuclear was increased in hepatocyte of zebrafish. Our results indicated that the MTX increased liver size accompanied by enlarged hepatocytes in zebrafish larvae. These findings provide insight for understanding the mechanism of MTX-induced hepatomegaly and that folate metabolism is essential for liver development in zebrafish. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2021R1F1A1062649)

414 - Using zebrafish to dissect the genetic drivers of epilepsy-related comorbidities

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Children with epilepsy suffer from a spectrum of debilitating comorbidities, including cognitive impairment, neurodevelopmental deficits and motor dysfunction, which can at times be more burdensome than the seizures themselves. To truly improve these patients' quality of life, ideally, we need therapies that reduce or eliminate seizures in addition to comorbid symptoms. Unfortunately, such therapeutics remain largely elusive.

Here we utilized our previously published zebrafish models of early-onset epilepsies (*Griffin & Carpenter et al., 2021*) to further understand the functional consequences of single gene mutations in the development of clinically observed comorbidities. Using a series of light and vibrational startle, light-dark preference and social choice assays we demonstrated that *arxa*, *stxbp1b* and *scn1lab* deficient larval zebrafish exhibited altered motor responses, cognition and/or exploratory behaviors compared to wild-type siblings. We investigated whether agents with known anti-seizure properties (clinically and preclinically) could modify these phenotypes and found that stiripentol and clemizole, but not valproic acid, corrected deficits observed in the *scn1lab*$^{-/-}$ larvae. Employing a custom MATLAB algorithm, we demonstrated that these behaviors are distinct from seizure-like movements observed in this line. Our study supports the use of zebrafish as a robust, dual platform to understand and discover novel therapeutics for epilepsy and its associated comorbidities.

415 - Developmental origin of the Axon Cap Glia which surround the initial segment of the Mauthner axon in zebrafish

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Mauthner cells are giant excitatory interneurons that exist in pairs on the left and right sides of the metencephalon's fourth segment in teleost. They control C-type escape behavior, in which the body moves away from the stimulus input to initiate escape behavior. The initial segment of the Mauthner cell is surrounded by axons of excitatory spiral neurons and axons of inhibitory feed-back neurons. This special structure is called Axon Cap. In addition, in many teleost species, a specialized group of glial cells, called Axon Cap Glia, surround the Axon Cap, forming an electrical insulator, and are involved in unusually rapid ‘electrical inhibition’ from the inhibitory feedback neurons (Furukawa and Furshpan, 1963; Hatta and Korn, 1998). Until recently, there were no markers for these glial cells, and research had not progressed.

In this study, we show that a transgenic fish (Ohno et al. 2021) expresses GFP in these Axon Cap Glia. We observed the development of Axon Cap Glia in the fish from 1.5 days post fertilization (1.5 dpf) to 5 dpf by live Imaging and immunofluorescence. We found that the Axon Cap Glia precursor cells appeared to migrate along the Radial Glia from the ventricular zone to the initial segment of the Mauthner cells at 2.5 dpf, surrounding the Axon Cap by 3 dpf. We are currently studying further development of the Axon Cap Glia and their roles in the fish behavior.

416 - Functional Imaging and Photo-manipulation of Cells Derived from Three Germ Layers in the Larval Zebrafish Gut

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Zebrafish larval gut could be a good model to investigate the motor function of digestive organs. Previously we reported strong periodic Ca^{2+} events in the circular smooth muscles and neurons during peristaltic reflex] in the distal intestine (Okamoto and Hatta, 2022). Meanwhile other types of movements more regular and higher in frequency have been observed including retrograde waves in the proximal intestine (PI), anterograde waves in the mid-and distal intestine (MI, DI) and rapid contractions in the distal end of intestine (DE). In order to investigate the mechanism of their formation we express Ca^{2+} indicator GCaMP3 in various cell types and search cells which become active in association with each wave. A distinctive non-neuronal cell group acts strongly in conjunction with anterograde waves in MI and DI which may correspond to the interstitial cells of Cajal or pacemaker cells. We also use photoconversion of Kaede to examine cell shapes.

Here we demonstrate that in addition Ca^{2+} events were found in the endodermal tissues some of which were associated with local gut wall movement. When endodermal cells expressing ChR2 were stimulated by irradiation with blue light in MI or DI peristalsis reflex-like activity was induced. On the other hand, in PI amplification of the retrograde waves was observed without changing its frequency by stimulation of endoderm, a pace-maker like cell or a neuron. Meanwhile a strong local contraction perpendicular to the duct-axis were induced by stimulation of a neuron or a smooth muscle. Activation of nitrergic neurons inhibited rapid regular contractions in DE. Thus, various types of neurons and non-neuronal cells are associated with gut movements and optogenetic stimulation of a single to small number of cells could manipulate such movements in vivo. Accumulation of such data should elucidate the circuits which regulate the gut function at single cell resolution.

417 - Olfactory stimuli alter spontaneous activity in the optic tectum and change visual perception

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Input from multiple sensory systems interact to drive perception. For example, olfactory cues can influence visual perception and vice versa. We hypothesize that these influences occur at the level of altering predictions, which are represented by spontaneous activity. To test this, we performed two-photon calcium imaging in *Tg(elavl3:H2B-GCaMP6s*) zebrafish. Visual stimuli elicit activity in the olfactory bulb, while odours elicit responses and changes in spontaneous activity in the optic tectum. To test whether radial glia could contribute to these changes, imaging was carried out in fish expressing JrGECO1a under the control of the *slc1a3b* promoter. Our observations are consistent with the proposal that olfactory and visual perceptions can be mutually altered through a change in expectation, potentially with the involvement of signalling via glial cells.

418 - Assessing the differential neurodevelopmental toxicity of environmental chemicals in zebrafish larval development

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The neurodevelopmental toxicity of environmental chemicals has been barely understood due to the complicated nature of the brain and spinal cord and the limited tools to analyze them. Fungicides are widely used and contaminate the aquatic systems through drift or runoff after application in agriculture. To understand the cellular and molecular mechanism of the neurodevelopmental toxicity of the fungicides against non-target organisms, we took advantage of zebrafish as a vertebrate animal model. We screened the fungicides that cause microcephaly and the aberrant motor behavior in the zebrafish larval development. At the cellular level, our state-of-the-art imaging analyses identified the differential toxic effects on the formation of specific neurons and the neural connections in the brain, which may affect the modulation of the neurotransmissions and neural circuits in the brain of humans and wildlife animals. Molecular studies demonstrated the altered gene expressions in neuronal and glial development and the mitochondrial damage underlying the neurodevelopmental toxicity of the selective fungicides. Our assessment may contribute to understanding the basis of neurodevelopmental disorders derived from the unseen toxicity of the environmental chemicals.

419 - Development of a SETX zebrafish model to study cerebellar ataxia

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Des mutations du gène Senataxin (SETX) ont été liées à deux maladies neurodégénératives distinctes, une ataxie cérébelleuse connue sous le nom d'apraxie oculomotrice de type 2 (AOA2) et une forme de sclérose latérale amyotrophique juvénile. Cependant, les mécanismes sous-jacents aux défauts neurologiques dus aux mutations SETX dans l'AOA2 (et dans la SLA) restent inconnus, probablement en raison de l'absence d'un modèle SETX approprié. Peut-être plus important encore, il n'existe toujours pas de traitement ou de remède efficace pour les défauts neurologiques de l'AOA2 liée à SETX. La découverte des mécanismes pathogènes se produisant dans le système nerveux central (SNC) sera essentielle pour comprendre et traiter les déficits neurologiques dus au déficit en SETX dans l'ataxie cérébelleuse. Ici, nous avons utilisé CRISPR/Cas9 pour générer un mutant de poisson zèbre setx KO. Quatre ARNg ont été conçus et synthétisés pour cibler le poisson zèbre setx. En utilisant, extraction optimisée de l'ADN génomique suivie d'un test de fusion à haute résolution (HRM), nous avons montré une grande efficacité de la génération d'indels induite par CRISPR. Nous caractérisons actuellement le modèle setx CRISPR pour évaluer tout déficit morphologique et comportemental. Le développement d'un modèle de poisson zèbre SETX sera utile pour étudier la pathogenèse de l'ataxie cérébelleuse et pour le criblage in vivo de composés thérapeutiques potentiels.

420 - Elucidating the role of the Mctp proteins: novel calcium sensors of the endoplasmic reticulum and endosomes

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Multiple C2 and Transmembrane Domain Proteins (Mctp) have been proposed as novel endoplasmic reticulum calcium sensors with phospholipid affinity; however, their function remains unknown. MCTPs are evolutionarily conserved from invertebrates to mammals. The zebrafish genome possesses four mctp genes (*mctp1a*, *mctp1b*, *mctp2a* and *mctp2b*) that are expressed from embryonic development to diverse adult tissues. CRISPR/Cas9 KO´s of the mctp zebrafish genes showed that *mctp2b* is essential for normal development while the other three mutant genes do not generate an apparent phenotype. The *mctp2b* KO showed a low rate of viability at 24 hpf and abnormal phenotypes that do not survive after 5 dpf.

In this study, we explored the effect of the overexpression of *mctp2b* and systematic deleted-versions of its C2 domains. In addition, a comparative analysis of the promoter regions of the zebrafish mctp genes is presented.

Overexpression of *mctp2b* resulted in embryonic lethality similar to the KO, which points to the importance of Mctp2b function on early stages of development. Mctp´s possess three C2 domains (C2A, C2B and C2C) that have been suggested to bind calcium with different affinities. Overexpression of selective deletion mutants of each C2 domain produced different rates of lethality and embryonic malformations that did not survive after 5dpf, indicating that other functional domains of the protein may also be responsible of the lethal phenotype.

in silico analysis of the promoter region of the four mctp zebrafish genes showed that they share transcription factors binding-sites involved in embryonic development but, interestingly, mctp2b lacks of a TATA box, which makes it a candidate for constitutive expression.

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421 - MITF restricts the melanocyte stem cell lineage by silencing default neuronal fates

Alessandro Brombin[1], Jana Travnickova[1], Daniel J. Simpson[1], Hannah Brunsdon[1], Yuting Lu[1], Zhiqiang Zeng[1], Adelaide I.J. Young[1], Tamir Chandra[1], E. Elizabeth Patton[1]

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Melanocytes, our pigment-producing cells, can emerge directly from the neural crest during development, and are replenished from multiple stem cell niches in adult tissues. In mammals, distinct McSC populations serve as reservoirs for melanocytes that pigment the growing hair shaft, or for skin pigmentation in response to UV-irradiation or wound healing. In zebrafish, nerve-associated McSCs are an on-demand regenerative population at all stages, and the cell-of-origin for multiple pigment cell types in adult skin. We recently showed that the transcription factor Tfap2b specifies multipotent McSCs originating from the neural crest. Accordingly, *tfap2b* fate mapping demonstrated that McSCs gave rise to melanocytes, two other pigment cell types, and nerve-associated cells in the adult pattern. While the master melanocyte transcription factor, Mitfa, is expressed in McSCs and has a well-known function in melanocyte specification and differentiation, here, we present an unexpected role for Mitfa in suppressing alternative McSC cell fates. We employ scRNA-seq coupled with live imaging in *mitfa* mutant embryos and find that rather than being simply deficient for McSC function, *mitfa* mutant McSCs generate numerous, ectopic daughter cells that line nerves and acquire new neuronal-like identity. These experiments suggest that Mitfa activity is critical for both promoting melanocyte fates, as well as repressing a default neuronal-like identity. These findings have implications well beyond pattern formation because in both human and zebrafish melanomas (cancer of the melanocyte) *Tfap2b* is expressed in residual disease following therapy, and melanomas with low MITF activity are enriched for neuronal-like signatures. Thus, we propose that a previously unappreciated MITF-TFAP2b mechanism is co-opted in melanoma to represent a dysregulated McSC state. Our approach illustrates the power of zebrafish melanocyte developmental biology to inform dysregulated cell states in cancer.

422 - Modelling ALS in zebrafish: Fishing for mechanisms and therapeutics

Kessen Patten[1]

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressing and fatal disorder with no effective treatment to meaningfully prolong survival and no biomarker. To understand ALS pathogenesis, my lab has been developing ALS models in zebrafish. In a loss-of-function (LOF) of the ALS associated gene *C9orf72*, we showed that C9-LOF mutants exhibit severe motor behavioural defects. Analysis of the neuromuscular junctions using specific presynaptic and postsynaptic markers SV2 and alpha-bungarotoxin respectively, revealed a marked reduction in the number of synaptic contacts in the C9-LOF fish. We observed a lower amplitude and frequency of the spontaneous miniature end-plate currents, indicating a presynaptic defect in C9-LOF zebrafish. Importantly, the C9-LOF zebrafish model recapitulates a major pathological hallmark of ALS - TDP-43 mislocalization. Using our zebrafish ALS models, we also performed drug screens in order to discover new therapeutics. In a large we identified a class of 13 neuroleptics that restored in zebrafish ALS models. The most potent was pimozide, prevented the reduction in neuromuscular transmission in zebrafish ALS model and enhanced transmission in a mouse model of ALS. In C9-LOF zebrafish, we found that pharmacologically increasing the activity of calpastatin in these fish ameliorates motor behaviour and neuromuscular morphology. In conclusion, we show that synaptic dysfunction at neuromuscular junctions in zebrafish ALS C9orf72 model and zebrafish ALS models can allow identification of promising compounds for the treatment of ALS, which may be a useful therapeutic approach to stabilize neuromuscular transmission and prolong survival in this disease.

423 - Blind cavefish retain functional connectivity in the tectum despite loss of retinal input

Evan Lloyd[1], Brittnee McDole[2], Martin Privat[3], James Jaggard[4], Erik Duboue[2], German Sumbre[3], Alex Keene[1]

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Sensory systems display remarkable plasticity and are under strong evolutionary selection. The Mexican cavefish, *Astyanax mexicanus*, consists of eyed river-dwelling surface populations, and multiple independent cave populations which have converged on eye loss, providing the opportunity to examine the evolution of sensory circuits in response to environmental perturbation. Functional analysis across multiple transgenic populations expressing GCaMP6s showed that functional connectivity of the optic tectum largely did not differ between populations, except for the selective loss of negatively correlated activity within the cavefish tectum, suggesting positively correlated neural activity is resistant to an evolved loss of input from the retina. Further, analysis of surface-cave hybrid fish reveals that changes in the tectum are genetically distinct from those encoding eye-loss. Together, these findings uncover the independent evolution of multiple components of the visual system and establish the use of functional imaging in *A. mexicanus* to study neural circuit evolution.

424 - Functional Genomics and Precision Medicine for Rare Neurological Disorders

Éric Samarut[1, 2], Uday Kundap[1, 2], Katarzyna Ochenkowska[1, 2], Aveeva Herold[1, 2], Meijiang Liao[1, 2]

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What makes a brain sick? What are the molecular consequences of disease-causing mutations and their aftermaths on neuronal circuits and brain activity? Working in close collaboration with clinicians and geneticists, **our lab seeks answers to these questions and focuses on *making sense* of genetic mutations associated with neurological diseases.** We postulate that improving our understanding of the underlying mechanisms of rare genetic neurological disorders will help develop innovative therapies, and our research programs tackle two main themes: **(1) Unravelling novel pathogenic mechanisms of neurological disorders:** We seek to unravel novel, yet undiscovered, aspects of genetic epilepsies, and yet undiagnosed related neurological diseases. To do so, we investigate the perturbations due to specific genetic mutations at multiple levels: behavioural, physiological, cell networks, and molecular. Ultimately, these findings will identify new actionable pathways and molecular targets that can be further validated by functional assays. **(2) Developing precision medicine in epilepsy -** It has been widely recognized that zebrafish models of epilepsy are exceptionally well suited to study neurological disorders, particularly in the context of epilepsy. We previously developed several genetic models of brain seizures and we now propose to determine specific treatment-responsiveness profiles associated with different genetic backgrounds causing epilepsy. Using patient-specific genetic avatars, our goal is to perform precision medicine to tailor the anti-epileptic treatment for each specific genetic background.

Our research will enable the discovery of novel functional roles for specific genetic variations associated with pathologies of the nervous system. Such studies are instrumental to understand the underlying causes of the disease and to unravel novel pathways that can be further targeted for therapeutic discovery and development. Our research bridges the gap between genetic diagnosis and the development of therapies for neurological disorders thus offering an innovative translational output.

425 - Reconstructing circuit development at the single-cell level

Yinan Wan[1, 2], Ziqiang Wei[1], Shaul Druckmann[1, 3], Philipp Keller[1]

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Animal survival requires a functioning nervous system to develop during embryogenesis. Newborn neurons must assemble into circuits producing activity patterns capable of instructing behaviors. Elucidating how this process is coordinated requires new methods that follow maturation and activity of all cells across a developing circuit.

We present an imaging method for comprehensively tracking neuron lineages, movements, molecular identities, and activity in the entire developing zebrafish spinal cord, from neurogenesis until the emergence of patterned activity instructing the earliest spontaneous motor behavior.

We found that motoneurons are active first and form local patterned ensembles with neighboring neurons. These ensembles merge, synchronize globally after reaching a threshold size, and finally recruit commissural interneurons to orchestrate the left-right alternating patterns important for locomotion in vertebrates. Individual neurons undergo functional maturation stereotypically based on their birth time and anatomical origin.

Our study provides a general strategy for reconstructing how functioning circuits emerge during embryogenesis. It also highlights the potential of light sheet microscopy for making novel discoveries in zebrafish neurodevelopment by combining long-term whole-embryo developmental imaging and circuit/brain-wide functional imaging.

426 - Non-cell autonomous effects of cardiomyocyte renewal

Kazu Kikuchi[1]

[1]National Cerebral and Cardiovascular Center Research Institute

Zebrafish regenerate the heart by cardiomyocyte renewal. We recently found that Klf1/Eklf, a Krüppel-like transcription factor essential for red blood cell development, is also necessary and sufficient in the myocardium to induce cardiomyocyte renewal in adult zebrafish hearts. Myocardial overexpression of Klf1 leads to a massive expansion of cardiomyocytes in the uninjured heart, providing a model to investigate cellular and molecular mechanisms of cardiomyocyte renewal. We analyzed the Klf1-overexpressed heart and found that not only cardiomyocytes but other cell types such as epicardial and endothelial cells are also activated and proliferated in response to the cardiomyocyte-specific overexpression of Klf1. Although the cardiomyocyte expansion was induced without injury, immune cells such as macrophages and T cells were also increased and activated in the Klf1-overexpressed heart. These data suggest that cardiomyocyte renewal accompanies a coordinated response of non-myocytes, which may be critical for cardiac development and regeneration. We are currently investigating the underlying cell-cell communication mechanisms of the coordinated response.

427 - Oncogenic RAS drive Cancer Hallmarks during preneoplastic initiation

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Abigail Elliot[1], Isabel Ribeiro Bravo[1], Henna Myllymaki[1], Jeanette Astorga Johansson[1], Lisa Kelly, Yi Feng*[1]

My lab uses an *in vivo* live imaging approach and zebrafish cancer models to investigate the earliest events of tumour initiation. We wish, through our work to find novel cancer preventative strategies. Using a translucent zebrafish larval stage pre-neoplastic cell (PNC) development model, our research has revealed that upon $HRAS^{G12V}$ expression, basal keratinocytes undergo de-differentiation followed by a rapid divergent cellular state transition toward either an EMT or a more differentiated phenotype within 24 hours. Emerging PNCs elicit an inflammatory response, which progresses toward a chronic inflammation state. More importantly, recruited innate immune cells play a Trophic role in promoting PNC proliferation, resembling a key cancer hallmark "tumour promoting inflammation". Our data has shown that the NFKB pathway is activated both in PNCs and recruited innate immune cells and blocking its activation in either cell type leads to reduced PNC growth. In addition, we have been investigating changes in metabolism during PNC initiation, and as with established tumours, we found PNC metabolic adaption to be important for initial growth and our data has revealed the importance of mitochondrial functional change during PNC initiation which exposes a vulnerability of PNCs to be targeted for cancer prevention.

428 - Comparing the effects of preconsumer and environmentally sourced microplastics in two generations of fathead minnows (Pimephales promelas)

Kennedy Bucci[1], Malak Bayoumi[1], Kathleen Stevack[2], Trudy Watson-Leung[2], Chelsea Rochman[1]

[1]University of Toronto, [2]Ministry of the Environment Conservation and Parks

The global ubiquity of plastic pollution has caused scientists, decision-makers, and the public to ask whether microplastics (plastic < 5 mm) are a threat to wildlife. Over the past decade, research has shown that virgin microplastics do cause effects to a variety of organisms at different levels of biological organization. However, more research is needed to understand the effects of microplastics with an environmentally relevant mixture of contaminants. In this experiment, we exposed fathead minnows to polyethylene microplastics purchased from a manufacturer or collected from the shoreline of a lake in a heavily polluted region. Our goal was to compare the effects of microplastics with and without an additional chemical cocktail sorbed from the environment. We exposed fathead minnows to microplastics for a full life cycle and raised a subset of their offspring in clean water. The results of this experiment showed that both microplastic types imposed a physical stress on the fish, impacting growth, energy storage, and energy allocation. Only the environmental microplastics, however, caused additional stress on the endocrine system, causing eggs to be more fragile with a thinner eggshell, delaying reproduction, and increasing the rate of malformation in the offspring. The results of our study suggest that microplastics in the environment may already be causing population-level effects in wildlife. Furthermore, testing with environmentally realistc microplastics is critically important to understanding the true effects of microplastic pollution.

429 - Creating zebrafish patient avatars to personalize treatment options for hard-to-treat cancers

Nicole Melong[1], Nadine Azzam[1], Jason Berman[2]

[1]CHEO Research Institute, [2]CHEO Research Institute/University of Ottawa

There has been dramatic improvement in treatment outcomes for many pediatric cancers over the last three decades. However, for the population of young people with relapsed or refractory cancer, the prognosis remains grim. When no clear treatment options are available, a rapid *in vivo* preclinical platform is needed to prioritize potential therapeutic candidates. Our lab has generated a larval zebrafish xenograft platform, which can be utilized to provide rapid drug response data in a clinically actionable timeframe. This scalable platform has been employed for a variety of applications, including single and combination drug studies, novel radiotherapy trials, and chemotherapeutic-induced toxicity evaluation. Currently, this platform is being leveraged both nationally within Canada's PRecision Oncology For Young peopLE (PROFYLE) program and internationally with the comparable Australian Zero Childhood Cancer (ZCC)Program.

Highlights of this model include using a focused chemical genomic approach to demonstrate that xenografted patient-derived T-cell acute lymphoblastic leukemia harbouring mutations in the NOTCH and PI3K/AKT pathways respond concordantly to their targeted therapies; and evaluation of a novel radiotherapy target beam, which improved the accuracy of the radiation dose and decreased off target effects *in vivo* when combined with Gold nanoparticles. Lastly, we have created a national pipeline for tumour sample acquisition and the generation of functional drug response data to complement mutational analysis by next generation sequencing to aid clinicians facing complex or limited treatment decisions. This approach is currently being employed in a pilot study to compare retrospective matched ZCC patient and mouse PDX therapeutic response data with prospective zebrafish larval PDX data as a proof-of-principle that drug efficacy signals are representative and conserved across model systems. These studies demonstrate the robustness and feasibility of the zebrafish larval PDX model as a preclinical tool for patient-specific therapeutic decision-making, providing novel treatment opportunities and renewed hope for these vulnerable patients.

430 - Test

John John[1]

[1]IZFS

This is the abstract

431 - Abstract Title

Alyssa Czerwinski[1]

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XYZ

432 - Using evolution and development of Antarctic fishes to understand adaptation to climate changes past and present

Jacob Daane[1], Matthew Harris[2], Bill Detrich[3]

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Climate change is expected to rapidly disrupt established weather patterns and to shift habitat boundaries, putting intensive pressure on species to either migrate or adapt to their changing environment. Here, we utilize the evolution of Antarctic notothenioid fishes combined with the power zebrafish genetics to explore mechanisms of adaptation to climate change. Notothenioids underwent an adaptive radiation in response to sustained global cooling over the last 30 million years, enabling a retrospective analysis of adaptation to past climate change events. Additionally, as notothenioids have adapted to extremely cold and thermally stable waters, these fishes are particularly sensitive to ocean warming and act as sentinel species in the modern era. Here, we discuss the usage of comparative genomics to unravel past instances of notothenioid trait evolution and discuss our efforts to assess the evolvability of notothenioids to ongoing warming. For ancient climate change, we focus on reduction in skeletal density, a buoyancy adaptation that enabled notothenioids to efficiently forage in the water column. We show that there was a transient increase in mutation rate and a reduction in skeletal density prior to the global cooling events that presented the ecological opportunity for adaptive radiation. These genomic and morphological primed notothenioids to adaptively diversify in response to paleoclimate change. Further, we identify a gene in drift within notothenioids, *trip11*, and show that loss of this gene in zebrafish results in an adult-viable reduction in skeletal density, like that observed in cryonotothenioids. Finally, to assess adaptation to ongoing climate change, we discuss our initial efforts to model the impact of warming seas on a crucial bottleneck in fish thermal adaptation: embryonic development. These data provide a framework for predicting adaptability of species in the Anthropocene. Supported by US NSF 1955368 (JMD, HWD) and 2001584 (MPH).

433 - A molecular network of conserved factors keeps ribosomes dormant in the egg

Andrea Pauli[1], Laura Lorenzo-Orts[1], Carina Pribitzer[1], Irina Grishkovskaya[1], Manuel M Matzinger[1], Elisabeth Roitinger[1], Susanne Kandolf[1], Katarina Belacic[1], Tzi-Yang Lin[1], Anton Meinhart[1], Karl Mechtler[1], David Haselbach[1]

[1]Vienna Biocenter

Molecular machines like the ribosome are produced in large quantities during oogenesis and stockpiled in the quiescent egg to be readily available during embryogenesis. However, the egg and early embryo are translationally repressed. How ribosomes are stored for extended amounts of time in the mature egg, and whether the ribosome itself contributes to the change in translational activity during embryogenesis, is currently unknown.

Here, we discovered a new mechanism of translational regulation at the ribosome itself during the egg-to-embryo transition. Using polysome gradients, mass-spectrometry and cryo-EM analyses of ribosomes isolated from zebrafish and *Xenopus* eggs and embryos, we provide molecular evidence that ribosomes transition from a dormant to a translationally active state during the first hours of embryogenesis. Dormant ribosomes are associated with four conserved factors that form two modules and occupy functionally important sites of the ribosome: a Habp4-eEF2 module binds to the mRNA channel and the A-site, and a Dap1b/Dap-eIF5a module blocks the polypeptide exit tunnel and the E/P-site. Functional analyses of zebrafish knockout mutants and *in vitro* translation assays reveal a key role of the Habp4-eEF2 module in increasing ribosome stability in the egg and of the Dap1b-eIF5a module in inhibiting translation. Dap1b is a newly discovered translational inhibitor that stably inserts into the polypeptide exit tunnel and is sufficient to block translation in an *in vitro* mammalian translation extract. Thus, a developmentally programmed, conserved ribosome state plays a key role in ribosome storage and translational repression in the egg.

434 - Generation and phenotypical characterization of new emilin1a and emilin1b mutant zebrafish models

Paola Braghetta[1], Paolo Bonaldo[1], Dario Bizzotto[1], Giulia Pigato[1], Nicola Facchinello[1]

[1]University of Padova

Elastin microfibrils interface located protein-1 (Emilin-1) is an extracellular matrix protein that associates with microfibrils and elastin, and is involved in elastogenesis, cell adhesion and proliferation, cytokine bio-availability. Most of the studies have been performed in mouse models, where the role of this glycoprotein in the cardiovascular system and skin has been thoroughly investigated. In situ hybridization revealed that trascription of zebrafish *emilin1a* and *emilin1b* genes is regulated in a spatiotemporal manner during embryonic development, being mostly expressed by blood vessels, heart, and mesenchymal cells of various organs. *Emilin1*.Here, we report the generation by CRISPR/Cas9 genome editing technique and characterization of *emilin1a/1b* double mutant zebrafish, both in larval and in adult stages. A series of morphological measurements and molecular analyses were performed to systematically characterize the molecular changes in *emilin1a/1b* mutant zebrafish. No gross phenotypic abnormalities were evident in *emilin1a/1b* knockout fish. According to the expression pattern, we decided to focus on trunk vasculature and neuronal motor system development, taking advantage of several reporter zebrafish lines available in our facility and of established techniques. Morphological and morphometric analyses of the *emilin1a and emilin1b* knockout fish revealed that both dorsal aorta and intersegmental vessels are significantly smaller in diameter. Preliminary data obtained by immunofluorescence using acetylated tubulin-antibody suggested that downregulation of *emilin1a* is associated with motor neuron defects, showing an overall abnormal development of axons, with decreased arborization. Moreover, *emilin1a* knockout embryos exhibit a strong reduction in the labelling of synaptic vesicle glycoprotein 2a. We believe that these new mutant zebrafish lines will be of great help to understand the role of Emilin-1 in early stages of cardiovascular and neurological development and will become an essential tool to identify novel therapeutic target and perform drug screenings that will be of great value to the understanding Emilin-1 function.

435 - The effects of abiotic factors and ageing on nanofertilizer toxicity in zebrafish embryos.

Aaron Schultz[1]

[1]School of Life and Environmental Sciences, Deakin University, Australia

Nanoscale fertilisers (e.g. phosphorous, iron and zinc) are being explored at the research scale to overcome the challenges of the low efficacy of conventional bulk fertilizers in agriculture. After application to agricultural crops, these nanofertilizers may enter aquatic environments from agricultural runoff events. It is, therefore, important to understand their interactions with abiotic factors such as natural organic matter and sunlight (UV exposure) and their potential effects on different aquatic organisms. In this study, the potential acute and sublethal toxicity effects of phosphorous-based (P) and iron oxide (FeO) nanomaterials (NMs) were examined in zebrafish embryos. The embryos were exposed to pristine NMs in the presence of humic acid (HA) and/or UV exposure or were exposed to 3-month aged NMs. Zebrafish embryo survival, hatching rate, developmental deformities, and heart rate were recorded up to 96 hpf. Alkaline phosphatase staining was used to examine NM effects on blood vessel formation and the expression of several genes involved in development, hatching and oxidative stress were also analysed. It was observed that no LC_{50} (survival) and IC_{50} (hatch and heart rates) were obtained when the zebrafish embryos were exposed to the aged NMs or when embryos were exposed to NMs in the presence of HA and/or UV. Interestingly, several of the P-based NMs caused an increase in zebrafish hatch rate at 48 and 54 hpf that appeared to be due to an increase in gene expression of zhe1. Overall, the results suggest that P-based NMs and FeO NMs cause no acute toxicity and minimal sub-lethal toxicity to zebrafish embryos in environmentally realistic experimental conditions.

436 - IZFS Environmental Sustainability Committee Activities

Viviana Vedder[1, 2, 3]

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Climate change and waste management are topics, that should be on the top of everyone's agenda, before a climate crisis cannot be avoided anymore. Therefore, the Environmental Sustainability Committee (ESC) of the IZFS has taken a more active role in keeping the discussion alive. Here, we will give insights into our work of the past year and take the initiative to invite you to our workshop.

437 - Studying myelinated axon biology in vivo using zebrafish

David Lyons[1]

[1]University of Edinburgh

Myelinated axons are essential to nervous system formation, health and function. We use the zebrafish as a model organism to study myelinated axon biology, due to the suitability of zebrafish for detailed live imaging of cell behaviour, cell-cell interactions and neural circuit function in vivo over time, as well as their genetic conservation with mammals and experimental tractability, and their amenability for scalable drug screening, including in disease-relevant paradigms. In this plenary presentation I will provide an overview of ongoing projects that are investigating the growth in diameter of axons prior to myelination and mechanisms of myelination, particularly those of activity-regulated myelination. In addition I will describe our work studying the consequences of demyelination in vivo and our efforts to use zebrafish to identify potential therapeutics of relevance to promoting remyelination and neuroprotection. Finally, I will speak to the integration of studies in zebrafish with those in complementary mammalian models and human-based platforms.

438 - IZFS Environmental Sustainability Committee Activities - Finding Sustainable Solutions

Maximilian Breuer[1]

[1]Institute for Biochemistry and Biology, University Potsdam

We need to do our part and find solutions for sustainable research and improve waste management to reduce the impact of the on-going climate catastrophe. Therefore, the Environmental Sustainability Committee (ESC) of the IZFS is trying to find solutions for sustainable science in our aquatic research facilities. Here, we will give insights into our work of the past year, including scientific outreach, discussions with the community and interviews with problem solvers! We also would like to take the initiative to invite you to our workshop and ask you to actively take part in the discussion!

439 - The Tide is Turning on Plastic Pollution

Natasha Tucker[1]

[1]IZFS

Join Natasha Tucker, executive director of a Canadian environmental organization, for a presentation on the pervasive plastic pollution issues' impact on our oceans, and what you can do about it.

440 - Thisse for Ten: The Beauty of Pattern

Marnie E. Halpern[1]

[1]Geisel School of Medicine at Dartmouth

In 1992, Bernard Thisse, with a bit of help from family members, began to assemble a monumental collection of zebrafish gene expression patterns that transformed developmental biology research in this model. I will touch on our ten collaborative publications that illustrate Bernard's impact on diverse biological questions and the value of long-standing friendships in science.

443 - Introduction to the new plenary session, the ESC and its goals

Corinne Houart[1]

[1]KCL

The Environmental Sustainability Committee (ESC) has been created to develop IZFS activities related to climate issues and to the development of sustainable approaches to zebrafish research. The new Environmental Sustainability plenary session will

- Cover approaches and initiatives reducing pollution and waste generated by our research

- Give visibility to zebrafish research addressing impact of climate change on life forms.

444 - Prefollicle cell sentinels trigger macrophage activation and sex-reversal in zebrafish

Florence Marlow[1], Paloma Bravo[1, 2], Yulong Liu[3], Bruce Draper[3, 4]

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Formation of an initially indifferent bipotential gonad that later differentiates as ovary or testis is a co-mmon mechanism observed during reproductive development. Female zebrafish, but not males, retain this remarkable plasticity and can completely switch their phenotypic sex from female to male in response to environmental or genetic factors that impair fertility. Herein, we used genetic models to identify the cellular and molecular drivers of sex-reversal and show that ablation of definitive but not primitive macrophages completely blocks sex-reversal in the absence of the conserved fertility factor *bmp15.* Single cell sequence and genetic analyses identify a somatic gonad population as the source of macrophage-activating ligands. This study identifies the relevant ligands and receptors and establishes a germline-follicle cell-macrophage axis that mediates sex-reversal in zebrafish.

445 - Functional connectivity of the developing enteric nervous system

Rajlakshmi Sawale[1], Ethan Scott[2], Gilles Vanwalleghem[1, 3]

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The gut-brain axis is a physiological communication network between the microbiome, enteric and central nervous system. The gut microbiome can affect behaviour and cognition, and patients with psychiatric (anxiety, depression) or neurological disorders (autism spectrum disorders, Parkinson's disease) often show gastrointestinal comorbidities. Our goal is to understand the bidirectional communication between the gut microbiome and the nervous system, and how dysregulation of this communication can affect behaviour. Our first step is to characterize the functional development of the enteric nervous system in larval zebrafish and investigate the influence of the gut microbiome on its development. We are using light sheet microscopy to image the activity of the enteric neurons in 3 to 7 days post fertilization larvae using a nuclear-targeted GCaMP6s in wild-type and germ-free zebrafish larvae. We observed that the spontaneous neuronal activity increases from 3 to 5 dpf, before dropping suddenly at day 7. This recapitulates what has been observed in the optic tectum as the neuronal network develops and self-organizes. Finally, these parameters are affected in germ-free or fed animal, with germ-free animals showing an increase in activity. This study will lay the foundations for future studies of the gut-brain axis in disease models.

446 - Efficient generation of embryonic lethal knockin alleles by a simple microhomology-mediated strategy and surrogate reproduction

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In zebrafish embryos, microhomology-mediated end joining (MMEJ) is one of the major mechanisms responsible for the repair of DNA double-strand breaks (DSBs). The distance between the DSB end and the microhomology sequence is a key parameter that determines the frequency of MMEJ repair. In previous MMEJ-mediated knockin approaches, universal gRNA(s) was often used in donor vectors to induce DSBs for MMEJ repair, and this generally resulted in a 6-bp sequence between the DSB end and the homology sequence, which might severely compromise knockin efficiency. Here, we simply used the reverse complementary sequence of the upstream sequence of the endogenous Cas9 cutting site as the gRNA seed sequence of the donor vector, inducing two microhomology sequences with zero gaps to the Cas9 cutting sites in both genomic locus and the targeting vector. Using this method, we could visualize the knockin events in the injected F0 embryos with high efficiency and successfully generate precise knockin alleles in 10 different genomic loci. In some cases, the F0 knockin embryos showing tissue-specific EGFP expression could not survive, due to the disruption of endogenous protein. Thus we dissected a body piece containing the gonadal ridge from the F0 knockin embryos, and grafted it under the abdominal skin of an immunodeficient host fish at 2 months post-fertilization (mpf). After 1 month, the grafted tissues underwent rapid gonadal development and gametogenesis. Functional spermatozoa were obtained from the grafted gonads, which gave rise to heterozygous F1 embryos with a precise knockin allele. In this way, we have successfully generated embryonic lethal knockin alleles in zebrafish in a relatively short time by combining MMEJ-mediated knockin and surrogate reproduction.