Boston Area Zebrafish Researchers Meeting

March 18, 2023
Higgins Hall, Boston College

9:00-9:45
Registration and Continental Breakfast Higgins Hall Atrium

9:45-10:00
Welcome and Introductory Remarks Higgins Hall 300

Talks Session I Chair: Larissa Patterson, Rhode Island College
10:00-10:15
Craig Ceol, University of Massachusetts Worcester Medical School
Getting back in the black: cellular transitions in melanocyte regeneration

10:20-10:30
Eric Surette, PhD student, McMenamin Lab, Boston College
Sonic hedgehog activity in the larval fin fold imprints shape of the adult caudal fin

10:35-10:45
Xibu Niu, Postdoc, Galloway Lab, Massachusetts General Hospital
A Conserved Transcription Factor Regulatory Program Promotes Tendon Fate

10:50-11:10 **Break**

Talks Session II Chair: Daniel Cifuentes, Boston University
11:10-11:25
Kellee Seigfried, University of Massachusetts Boston

Regulation of germ line stem cell function by the Adad1 RNA binding protein

11:30-11:40
Xuan Anita He, PhD Student, Fisher Lab, Boston University
Identification of conserved skeletal enhancers associated with craniosynostosis risk genes

11:45-11:55
Santiago Callegari, Postdoc, Nath Lab, Beth Israel Medical Center
Electrical Impedance Myography in Evaluating Aged-related Skeletal Muscle Deficits

12:00-12:30 **Community meeting**
12:30-1:25 **Lunch**

Talks Session III Chair: Stacy Nguyen (Boston College)
1:30-1:45
Juan Manuel Gonzalez Rosa, Massachusetts General Hospital
Optimization of methods for robust and reproducible generation of tissue-specific mutations in zebrafish

1:50-2:00
Shannon Paquette, PhD Student, Plavicki Lab, Brown University
Macrophage loss results in cardiac dysfunction and disrupts adult heart health

2:05-2:15
Dimitry Kretov, Postdoc, Cifuentes Lab, Boston University
The miR-144/Hmgn2 regulatory axis orchestrates chromatin regulation during erythropoiesis.

2:20-2:30
Kalki Kukreja, PhD Student, Megason and Klein Labs, Harvard University
Cell division dictates proportions but not identities of cell types across development

2:35-2:50 **Break**

Talks Session IV Chair: Anne Clatworthy (Harvard University)
2:55-3:10
Maria del los Angales Serrano, Boston University
Linking cardiovascular and brain development through KMT2D-dependent transcriptional regulation

3:15-3:25
Lydia Djenoune, Postdoc, Yuan Lab, Massachusetts General Hospital
Lux ad cilium: investigating the role of intraciliary calcium and fluid flow in left-right development

3:30-3:40
Chinyere Kemet, PhD Student, Feng Lab, Boston University
UFD1: A novel target against MYC/MYCN-driven cancers

3:45-3:55
Wade Sugden, Postdoc, North Lab, Boston Childrens' Hospital
Mechanisms of flow-driven transcriptional control of hematopoetic stem and progenitor cell development by Yap and Taz

Posters and Reception
4:00-4:30 **Posters**, Odd numbers
4:30-5:00 **Posters**, Even numbers
5:15-5:30 **Awards Ceremony**
5:30-6:00 **Reception**

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Invited Speakers

1. Craig Ceol, Ph.D. (UMass Medical School):

Getting back in the black: cellular transitions in melanocyte regeneration

2. Kellee Siegfried, Ph.D. (UMass Boston):

Regulation of germ line stem cell function by the Adad1 RNA binding protein

3. Juan Manuel Gonzalez Rosa, Ph.D. (Massachusetts General Hospital / Harvard Medical School):

Optimization of methods for robust and reproducible generation of tissue-specific mutations in zebrafish

4. Angie Serrano, Ph.D. (Boston University)

Linking cardiovascular and brain development through KMT2D-dependent transcriptional regulation

Oral Presentations

10. Eric Surette (Boston College)

Sonic hedgehog activity in the zebrafish larval fin fold imprints shape of the adult caudal fin

Eric Surette & Joan Donahue, Stephanie Robinson, Brendan Fitzgerald, Shahid Ali, Connor Murphy, Sarah McMenamin

Early developmental signaling pathways establish positional identities, which tissues read out to give rise to proper appendage shape. The zebrafish caudal fin is a promising model for studying shape and positional information, being composed of bony rays that each grow to distinct lengths. Peripheral-most rays grow relatively longer than central rays, resulting in a forked shaped fin. We discovered that transient overexpression of sonic hedgehog (shh) during a narrow window of larval development fundamentally alters the shape of the adult caudal fin. These fish develop central rays roughly the same length as the peripheral rays, resulting in a truncate shape. Furthermore, the truncate fin is reproduced upon regeneration, suggesting that positional memory has been fundamentally altered. We hypothesized that regional differences in larval Shh activity inform local cell proliferation rates, thus imprinting positional identity of ray length. Indeed, we observe higher Shh activity in peripheral

regions of the wild-type larval fin fold, which in turn undergo higher relative levels of proliferation. In developing truncate fins, proliferation is relatively decreased in peripheral regions, resulting in more uniform rates of proliferation. Our data support a model wherein regional shh regulates local proliferation rates, which in turn inform ultimate fin shape.

11. Xubo Niu, Ph.D. (Massachusetts General Hospital / Harvard Medical School)

A Conserved Transcription Factor Regulatory Program Promotes Tendon Fate

Xubo Niu, Delmy Melendez, Suyash Raj, Junming Cai, Dulanjalee Senedeera, Joseph Mandelbaum, Ilya A. Shestopalov, Scott D. Martin, Leonard I. Zon, Thorsten M. Schlaeger, Lick Pui Lai, Andrew P. McMahon, April M. Craft, Jenna L. Galloway

Tendons, which transmit force from muscles to bones, are highly prone to injury. An understanding of mechanisms driving tendon fate would impact efforts to improve tendon healing, yet this knowledge is limited. To find direct regulators of tendon progenitor emergence, we performed a high-throughput chemical screen in zebrafish. We established Forskolin as a tenogenic inducer across vertebrates, functioning through Creb1a, which is required and sufficient for tendon fate. Enhancers containing cAMP response elements (CRE) in human, mouse and fish, drove specific expression in zebrafish cranial and fin tendons. Mutation of CRE sites significantly disrupted enhancer activity and specificity in tendons. Analysis of these vertebrate enhancers identified motifs for Ebf/EBF transcription factors, which were sufficient to induce tendon activity. Notably, Creb1a/CREB1 and Ebf1a/Ebf3a/EBF1 overexpression facilitated tenogenic induction in zebrafish and human stem cells. Together, our work reveals conservation of activity of two novel transcription factors and enhancers in promoting tendon fate.

12. Xuan Anita He (Boston University)

Identification of conserved skeletal enhancers associated with craniosynostosis risk genes

Xuan Anita He (何璇), Anna Berenson, Michelle Bernard, Chris Weber, Juan I. Fuxman Bass, and Shannon Fisher

Cranial sutures are important sites of bone formation and allow flexibility during rapid brain growth in infancy and childhood. In craniosynostosis (CS), which occurs in 1/2000 infants, cranial sutures are prematurely replaced by bone. A genome-wide association identified a risk locus for CS within BBS9, adjacent to BMPER, encoding an extracellular modulator of BMP signaling. We hypothesized that distal regulatory elements for BMPER accounted for the CS risk. Using zebrafish transgenesis, we screened conserved sequences in the risk region and identified an enhancer that harbors a risk-associated variant and is active in the cartilage closely associated with frontal bone initiation. We used an enhanced yeast one-hybrid assay to identify transcription factors that interact with the enhancer, including interactions specific to the risk allele. To address the variability of Tol2-based transgenesis that hinders direct comparison of allele-specific effects on enhancer activity, we are testing the feasibility of CRISPR-mediated-integration of the enhancer in an enhancer-desert genomic region. Our findings support a specific genetic mechanism to explain the contribution of a risk locus to CS. More broadly, our combined in vivo approach is applicable to many complex genetic diseases to build a link between association studies and specific genetic mechanisms.

13. Santiago Callegari (Beth Israel Deaconess Medical Center)

Electrical Impedance Myography in Evaluating Aged-related Skeletal Muscle Deficits: A-first-in-Zebrafish Study

Anjali K. Nath, Santiago Callegari, Tyler Mourey, Janice A. Nagy, Seward B. Rutkove

Sarcopenia affects up to 40% of the elderly; is associated with higher risk of death; and there are no effective treatments nor biomarkers. Electrical Impedance Myography (EIM) has been extensively validated as a simple and effective method to assess muscle, although not in Zebrafish. A platform combining these offers unprecedented opportunities in the field. Two EIM experiments were performed: A pilot study using a 1-mm needle EIM probe, and a validation study using anesthetized animals and a surface probe. Epaxial muscles were assessed anterior and lateral to the dorsal fin. Open-field test and histology were used to evaluate muscle function and structure respectively, and correlated with EIM parameters. Casper Zebrafish aged 6 (n=8) and 33 months (n=7) were assessed. A reduced velocity, maneuverability, and mobility was noted, along with marked muscle atrophy in aged animals. EIM parameters at 2 kHz revealed decreased phase angle (5.3±2.1 Vs. 10.7±1.5 kHz; p=0.0002)

and reactance (89.0±3.9 Vs. 172.2±54.8 kHz; p=0.007). Phase values strongly correlated with functional and structural muscle properties (r=0.725 p<0.05; r = 0.685 p<0.05). The validation cohort of 25 young (6-8 months) and 20 aged (>3 years) WT animals replicated EIM findings. In summary, EIM successfully detects aged-related muscle deficits in Zebrafish.

14. Shannon Paquette (Brown University)

Macrophage loss results in cardiac dysfunction and disrupts adult heart health

Shannon Paquette*, Cliff Oduor, Amy Gaulke, Peter Bronk, Vanny DaFonseca, Cadence Lee, Jeffrey Bailey, Bum-Rak Choi, Alan Morrison, and Jessica Plavicki

Macrophages are well-characterized as sentinel immune cells that coordinate cellular responses to injury and infection. However, recent developments in cardiac macrophage biology have broadened our understanding of this critical cell type in heart development and function. Still, it is not known how macrophage loss affects heart health into adulthood. Using a combination of optical mapping, histology, echocardiograms, electrocardiograms (ECG), and physiological stress tests, we demonstrate that macrophage loss significantly disrupts adult heart health and function, leading to arrhythmia and fibrosis. At 4 months post-fertilization (mpf), optical voltage mapping experiments reveal that irf8(st96/st96) macrophage knockout zebrafish have significantly prolonged AV delay and abnormal voltage patterning. At 12 mpf, mutants have reduced cardiac muscle density and significant epicardial effects. Longitudinal analysis of echocardiographic measurements reveals several significant age- and sex-specific changes in irf8 mutant heart function, in addition to the consistent presence of arrhythmia, prolonged relaxation time, and disrupted aortic ejection. ECG traces of macrophage mutants following a 1-hr swim tunnel endurance test also reveal a significant increase in incidence of arrhythmias. We are now performing scRNAseq on both 4 and 12 mpf WT and mutant hearts to identify changes in immune cell populations, inflammatory factors, and signatures of myocardial stress and ion channel dysfunction. Overall, these data reveal significant cardiac abnormalities following macrophage loss and expand our knowledge of critical macrophage functions governing homeostatic heart health.

15. Dmitry Kretov, Ph.D. (Boston University School of Medicine)

The miR-144/Hmgn2 regulatory axis orchestrates chromatin regulation during erythropoiesis.

Dmitry Kretov, Leighton Folkes, Alexandra Mora-Martin, Isha Walawalkar, Noreen Syedah, Kim Vanuytsel, George J. Murphy, Simon Moxon, Daniel Cifuentes

Differentiation of hematopoietic stem and progenitor cells (HSPCs) is a highly regulated process that involves the coordinated action of multiple layers of regulation. Here we show how the post-transcriptional regulatory layer interfaces with the chromatin regulation level via miR-144 and its target Hmgn2 to orchestrate chromatin condensation during terminal erythropoiesis. Electron microscopy and ATAC-Seq analysis showed an increase in open chromatin regions in miR-144 mutant erythrocytes compared to wild-type cells, accompanied by an increase in transcription. Single-cell sequencing revealed that miR-144 mutant erythrocytes do not progress to final differentiation stages and resemble erythroid precursor cells. Among the several targets of miR-144 that influence chromatin organization, the miR-144-dependent regulation of Hmgn2 is conserved from fish to humans. Our genetic probing of the miR-144/Hmgn2 regulatory axis established that intact miR-144 target sites in Hmgn2 3'UTR are necessary for proper erythrocyte maturation in both zebrafish and human iPSC-derived erythroid cells while loss of Hmgn2 rescues in part the miR-144 null phenotype. Altogether, our results uncover miR-144 and its target Hmgn2 as the backbone of the genetic regulatory circuit that controls terminal differentiation of erythrocytes in vertebrates and expands miR-144 target repertoire. Overall, we show how microRNA can expand their targeting network and amplify their regulatory potential via targeting a master regulator of chromatin organization.

16. Kalki Kukreja (Harvard University)

Cell division dictates proportions but not identities of cell types across development

Kalki Kukreja, Sean Megason, Allon Klein

As tissues develop, cells divide and differentiate concurrently. Conflicting evidence shows that cell division is either dispensable for differentiation, or plays a role in regulating transcription factor accessibility, concentration, and activity. To determine the role of cell division in differentiation, we block cell division during early gastrulation in zebrafish embryos using two

independent approaches and profile them at single cell resolution. We show that cell division is dispensable for differentiation of all major cell types from early gastrulation to end of segmentation, however the proportions of cells across cell types are not robust to division block. These differences can partly be explained by differential proliferation of different cell types. We also observe that without cell division, differentiation slows down non-uniformly across multiple cell types, with the strongest effect in blood cells. Our work sheds light on the role of division in making an embryos and also showcases the importance of combining embryo-wide perturbations with single-cell RNA sequencing to uncover the function of biological processes across multiple tissues.

17. Lydia Djenoune, Ph.D. (Massachusetts General Hospital / Harvard Medical School)

Lux ad cilium: investigating the role of intraciliary calcium and fluid flow in left-right development

Lydia Djenoune, Mohammed Mahamdeh, Thai V. Truong, Christopher T. Nguyen
Scott E. Fraser, Martina Brueckner, Jonathon Howard, Shiaulou Yuan

In most vertebrates, the left-right (LR) body axis is specified during early embryogenesis by a cluster of cells in the left-right organizer. In this organizer, motile cilia move rapidly to create a leftward directional flow of extracellular fluid that first breaks bilateral symmetry in the developing embryo. However, how this flow is sensed and transduced into phenotypic LR asymmetry remains unclear. By developing and utilizing a novel optical toolbox that combines light sheet microscopy, optical tweezers, genetically encoded calcium indicators, and deep learning, we show that immotile cilia function as mechanosensors that convert flow forces into calcium signals. The mechanical manipulation rescues cardiac laterality and is instructive: left- or right-sided cardiac looping is induced by targeting one cilium on the left or right side of the organizer, respectively. Our results establish cilia as mechanosensitive cellular levers that convert biomechanical forces into calcium signals that build the LR axis and present a model to decipher mechanisms leading to proper situs of the early heart.

18. Chinyere Kemet (Boston University)

UFD1: A novel target against MYC/MYCN-driven cancers

Xiaodan Qin1, Andrew Lam1, Chinyere Kemet, Xu Zhang, Hui Feng

Cancer is the second leading cause of death in the US with a predicted 600,000 deaths in 2023. Aberrant activity of MYC or MYCN, oncoproteins regulating gene transcription and protein synthesis, contributes to tumor formation and aggressiveness in a broad spectrum of human cancers. Targeting MYC/MYCN is currently unsuccessful due to high toxicities to normal tissues. Due to their increased protein synthesis, MYC/MYCN-driven cancer cells often harbor increased misfolded/unfolded proteins in the endoplasmic reticulum (ER), referred to as ER stress. The ER-associated degradation (ERAD) pathway is critical in mitigating ER stress. A key mediator of ERAD is the P97-UFD1-NPL4 complex, which retrotranslocates ubiquitinated substrates from the ER and presents them to the proteasome for degradation. Cancers are more susceptible to protein folding mistakes, relying on the activation of the unfolded stress response and enhanced ERAD for their survival. Although these cellular pathways are initially self-preservative, unresolved stress leads to apoptosis, which is recently an objective for cancer therapeutics. Current inhibitors targeting ERAD have been unsuccessful due to toxicities. In zebrafish models of MYC/MYCN-driven cancer, Ufd1 depletion does not impact tumor onset but significantly decreased tumor burden. On the other hand, overexpression of UFD1 led to increased tumor burden and decreased survival of zebrafish. We developed an experimental agent to target UFD1 and found that MYC/MYCN-driven cancer cell lines were sensitive to this agent, indicating that UFD1 is a promising target for the treatment of these cancers.

19. Wade Sugden, Ph.D. (Boston Children's Hospital)

Mechanisms of flow-driven transcriptional control of hematopoietic stem and progenitor cell development by Yap and Taz

Wade W. Sugden, Zachary C. LeBlanc, Mayuri Tanaka-Yano, Ran Jing, Maria Gonzalez di Tillio, Mohamad Najia, Yang Tang, Elizabeth Molnar, Stephan George, Brittney Love, Caroline Kubaczka, Nan Liu, Nah-Young Shin, Thorsten M. Schlaeger, Edroaldo Lummertz da Rocha, Alan B. Cantor, Stuart H. Orkin, Grant Rowe, Wolfram Goessling, George Q. Daley, Trista E. North

Hematopoietic stem and progenitor cells (HSPCs) emerge from artery-derived hemogenic endothelium (HE), driven by the Runx1 transcription factor (TF). Physical forces from blood flow are required to generate HSPCs from HE, but how these forces are sensed and converted

into a “stemness” regulatory module remains unclear. Using the zebrafish model, we show that the YAP TF is responsible for maintenance, not initiation, of the hematopoietic program in newly-specified HE. By employing a heat shock-inducible dominant negative YAP zebrafish line, we show the YAP paralogue TAZ can promote CD41+ and Flk+/Myb+ HSPC production upon reduced YAP function. YAP/TAZ initiate transcriptional responses downstream of mechanical stimuli and require DNA binding cofactors to regulate target genes. Surprisingly, luciferase assays in HEK293 cells demonstrated a potent synergistic effect of TAZ/RUNX1, but not YAP/RUNX1, in transcriptional regulation at RUNX enhancers. Finally, we identify the stretch-gated ion channel Piezo1 as a regulator of flow-induced YAP/TAZ mechanotransduction in HE. Zebrafish stimulated with Piezo1-small molecule agonist Yoda1 increase HSPC number and YAP target gene expression in a YAP- and Piezo1-dependent fashion. These results provide a ‘membrane-to-nucleus’ mechanism for force-sensing in HE, and offer a stretch-Piezo1-YAP axis that could potentially be pharmacologically tuned in vitro to enhance HSPC differentiation.

Poster Presentations

20. Sahar Tavakoli, Ph.D. (Harvard University)

Zebrafish chemical compound screen uncovers inducers of skeletal muscle engraftment across species

Sahar Tavakoli, Isaac Adatto, Sara Ashrafi Kakhki, Victoria S Chan, Haleh Fotowat, Eric Gahwiler, Margot E Manning, Kathleen A Messemer, Apoorva Rangan, Song Yang, Leonard I Zon, Amy J Wagers

Genetic muscle disorders compromise muscle function through increased inflammation and impaired muscle regeneration. Transplantation of exogenous muscle progenitor cells could be an approach to enhance muscle function and repair; but this approach has been limited due to the typically poor engraftment efficiency of cultured progenitors. To define regulators of muscle engraftment, we developed a novel cross-species platform, employing zebrafish and mouse, to discover chemical compounds that promote muscle progenitor engraftment in vivo. Muscle cells derived from zebrafish blastomeres were treated for 4 hours with biomolecules and transplanted into the flanks of adult zebrafish (n=15/ biomolecule). We focused our screening on a well-annotated library of 230

lipids; since lipids are known to enhance cell migration and to regulate the homeostasis and regenerative function of muscle and blood stem cells. Using limit-dilution assays, potential “hits” from the primary screen were identified and re-evaluated in replicate transplantation experiments. We discovered two lipids that promote muscle progenitor cell engraftment in vivo: lysophosphatidic acid (LPA) and niflumic acid (NFA). Using a bioluminescence imaging, we further ascertained that both NFA and LPA enhance satellite cell engraftment in mouse (mean BLI radiance ± SEM- NFA: 27.2E+6 ± 6.8 E+6 p/s; LPA: 25.6 E+6 ± 4.4 E+6 p/s; vehicle-treated cells: 8.2 E+6 ± 1.4 E+6 p/s; n=15, 1-way ANOVA, $p \leq 0.05$), indicating conservation of the promyogenic activities of these compounds across vertebrate species. Studies in sapje-like fish (dystrophin mutant) transplanted with NFA or LPA treated cells showed higher engraftment efficiency, significantly better swimming performance and ability to swim against a water current, compared to fish engrafted with vehicle-treated cells. Mechanistically, the promyogenic activities of LPA and NFA appear to be associated with increased cytoplasmic Ca2+ and down-regulation of muscle development genes. The RNA sequencing analysis also identified the upregulation of myoblast fusion regulating genes, including myomaker (Tmem8c) and Ccl8 in the LPA-treated satellite cells. The success of this cross-species approach to uncovering evolutionary conserved pathways regulating muscle regeneration suggests new potential opportunities for treating muscle disease by enhancing myogenic contributions of transplanted muscle progenitors.

21. Shiaulou Yuan, Ph.D. (Massachusetts General Hospital / Harvard Medical School)

How the embryo distinguishes right from left: cilia as force sensors

Lydia Djenoune, Mohammed Mahamdeh, Thai V. Truong, Christopher T. Nguyen, Scott E. Fraser, Martina Brueckner, Jonathon Howard, Shiaulou Yuan

The breaking of bilateral symmetry in most vertebrates critically depends upon the motile cilia of the embryonic left-right organizer (LRO), which generate a directional fluid flow, but how this flow is sensed remains unclear. Here, we demonstrated that immotile LRO cilia are mechanosensors for shear force using a methodological pipeline that combines optical tweezers, light sheet microscopy and deep learning to permit in vivo analyses in zebrafish. Mechanical manipulation of immotile LRO cilia activated intraciliary calcium transients that required the cation channel polycystin-2. Furthermore, mechanical force applied to LRO cilia

was sufficient to rescue and reverse cardiac situs in zebrafish that lack motile cilia. Thus, LRO cilia are mechanosensitive cellular levers that convert biomechanical forces into calcium signals to instruct left-right asymmetry.

22. Larissa Patterson, Ph.D. (Rhode Island College)

Cryptic role for alx4b in zebrafish iridophore development

Jakob Mastalerz, Melanie Cragan, Katie Sutton and Larissa Patterson

The colors and patterns of tropical fish are visually striking traits that also serve important ecological roles. These patterns are composed of thousands of individual pigment cells called chromatophores. During development, chromatophores migrate from their origin in the embryonic neural crest to distant locations throughout the body, ultimately forming stunning patterns. The horizontal stripes of the zebrafish, Danio rerio, are composed of three pigment cell types: yellow xanthophores, black melanophores and iridescent iridophores. Previous studies suggested that embryonic melanophores and iridophores arise from a bipotent precursor, but the mechanisms of specification and lineage restriction are not well understood. We hypothesized that the *ALX homeobox 4* genes (*alx4a* and *alx4b*) play a role in iridophore fate specification. To better understand the mechanisms of pigment cell lineage restriction, our lab used CRISPR/Cas9 to isolate loss of function alleles for homeobox transcription factors *alx4a* and *alx4b,* known to be expressed by differentiated iridophores, but not melanocytes. We found that *alx4a* mutants do not develop body iridophores whereas *alx4b* mutants were indistinguishable from wildtype, suggesting that *alx4a* but not *alx4b* is required for iridophore development. Interestingly, while *alx4a* mutants lacked all body iridophores, they still developed iridophores in their eyes. To test the hypothesis that Alx4b is able to compensate for the loss of Alx4a during eye iridophore development, we generated dihybrid crosses and quantified eye iridophores across a range of genotypes. Our results suggest that both Alx4a and Alx4b are required for eye iridophore but only Alx4a is required for body iridophores.

23. Natasha O'Brown, Ph.D. (Harvard Medical School)

Neuronal Spock1 Induces Blood-Brain Barrier Functional Development

Natasha O'Brown, Nikit Patel, Ursula Hartmann, Allon Klein, Chenghua Gu, Sean Megason

The blood vessels in the brain have uniquely restrictive properties, including specialized tight junction complexes and reduced transcytosis, that limit access to the brain, creating the so-called blood-brain barrier (BBB). These restrictive properties are actively induced and maintained by signals in the brain microenvironment. However, while the importance of these microenvironmental signals has been known for decades, what these signals are and which cells produce them remain poorly understood. To try to identify these signals, we turned to zebrafish, which offer a powerful tool to study the BBB dynamically in vivo, and initially characterized the developmental timeline and subcellular mechanism of BBB functional development. During the course of these studies, we identified a spontaneous mutant with regionalized BBB leakage, specifically in the forebrain and midbrain. We mapped this leaky mutant to the neuronally secreted proteoglycan Spock1 and validated that loss of Spock1 function results in the leaky phenotype. We found that Spock1 signals to the vasculature within a 10-20 um range by controlling critical vascular cell interactions that lead to increased vessel transcytosis. Finally, a single exogenous dose of human SPOCK1 partially restored BBB function in the spock1 mutants while also restoring vascular expression of BBB genes.

24. Emily Goering (Harvard University)

Defining Kynurenine Pathway Control of Salmonella Typhimurium Infection in Zebrafish Larvae

Emily Goering, Anne Clatworthy, Margarita Parada-Kusz, Deborah Hung

Antibiotic-resistant bacterial infections remain a persistent cause of hospitalization and death around the world. In a whole organism screen for anti-infectives, we found that exogenous application of the kynurenine pathway metabolite 3-hydroxy-kynurenine (3-HK) rescued zebrafish larvae from lethal Salmonella Typhimurium infection. Exogenous 3-HK did not act like a typical antibiotic in that it did not inhibit bacterial growth in vitro but did restrict bacterial growth in vivo. Further analysis of endogenous production of 3-HK through the kynurenine pathway revealed that kynurenine 3-monooxygenase (Kmo), the enzyme responsible for catabolism of kynurenine to 3-HK, is required for the normal immune response to systemic Salmonella Typhimurium infection. Inhibition of Kmo and the endogenous production of 3-HK resulted in decreased survival to

sub-lethal infection. Sensitivity to infection upon Kmo inhibition correlated with more rapid expansion of bacterial burden and, paradoxically, enhanced ROS production and pro-inflammatory cytokine induction. While macrophage cell numbers are not altered following Kmo depletion, their ability to control intracellular bacterial replication is impaired and Kmo depleted macrophages have a decreased ability to acidify engulfed particles. Taken together, this work provides a novel mechanism by which Kmo and the endogenous production of 3-HK impacts macrophage function.

25. Zachary LeBlanc (Boston Children's Hospital)

The Role of Sepp1a in HSPC formation

Zachary C. LeBlanc, Mariam Hachimi, Trevor Bingham, Wade Sugden, Thorsten Schlaeger, George Q. Daley, Trista E. North

Hematopoietic stem and progenitor cells (HSPCs) arise from hemogenic endothelium (HE) lining the dorsal aorta. These cells proliferate rapidly while maintaining multilineage potential, supporting lifelong hematopoiesis of the organism. Production of HSPCs in vitro would improve outcomes and access to curative treatments for patients with hematological disease. However, incomplete understanding of the extrinsic factors governing their emergence and expansion have thus far prevented the generation of long-lived multipotent HSPCs from iPSC precursors. We have previously shown that inflammatory cues are important for HSPC production, but excessive inflammation may exhaust the stem cell pool. Selenoproteins are a diverse class of antioxidant peptides that signal oxidative state and balance inflammation. Here, we show that the secreted selenoprotein, Sepp1a, acts as a positive regulator of HSPC production. Genetic knock-down of sepp1a by morpholino and knock-out by CRISPR/Cas9 based gene editing decreased HSPC number as determined by in situ hybridization for conserved markers runx1 and cmyb. Flow cytometry of CD41+ HSPCs confirmed and quantified this reduction. Interestingly, this effect was independent of cellular selenium concentration and reactive oxygen stress. These results suggest that Sepp1a has an important function in regulating HSPC number in vivo which may translate to in vitro differentiation protocols.

26. Gregory Teicher (University of Massachusetts, Amherst)

Marigold: Deep learning software for automated pose tracking of embryonic and larval zebrafish

Gregory Teicher, R. Madison Riffe, Wayne Barnaby, Gabrielle Martin, Benjamin E. Clayton, Josef G. Trapani, Gerald B. Downes

Zebrafish embryos and larvae are ideally suited for high-throughput behavioral studies in the context of drug discovery, toxicology, and modeling neurological disorders such as autism and epilepsy. However, existing software for video-based behavioral analysis can be incompatible with recordings that contain dynamic backgrounds or foreign objects, lack support for multiwell formats, require expensive hardware, or demand considerable programming expertise. We introduce Marigold, a deep learning program for automated pose tracking of embryonic and larval zebrafish. Marigold tracks up to 10 user-defined body keypoints, supports both single- and multiwell formats, and exports a range of kinematic parameters as well as publication-quality visualizations. By leveraging recent advances in ultra-compact convolutional neural network design and an efficient curriculum learning-based training process, Marigold achieves respectable speeds on most current laptop and desktop computers. Marigold features an intuitive graphical user interface supporting a streamlined workflow, is a standalone, cross-platform application requiring little to no setup, and will be released in the coming weeks as free and open source software. At BAZaR, we will provide a brief live demonstration as well as present results from two sets of biological experiments to showcase Marigold's ease of use, robust pose tracking, and amenability to diverse experimental paradigms.

27. Rikki Garner, Ph.D. (Harvard Medical School)

Biomechanical regulation of adhesion-mediated cell patterning

Rikki M. Garner, Tina Chen, Aleksandra Denisenko, and Sean G. Megason

The self-organization of cells into patterns is essential for proper development. One widespread mechanism that is critical for patterning dozens of embryonic tissues across diverse organisms is adhesion-mediated sorting. In this scheme, distinct adhesion molecules are differentially expressed on the surface of different cell types, driving cells to group together based on compatible adhesion molecule expression (see our recent review on this topic: Tsai, Garner, & Megason, ARCDB, 2022). However, while there is ample evidence that cells can sort by adhesion compatibility, almost nothing is known about how cells move

through the tissue, find partners with similar adhesion protein expression, and eventually stop moving to lock in the sorted pattern. In particular, we fundamentally lack an integrative understanding of how cell mechanical and migratory properties control the ability of cells to sort.
Here, I present my progress towards developing a combined experimental and theoretical approach to address this gap, using developing zebrafish embryos as a model system. First, I will discuss the “in toto” imaging and image analysis pipeline I have established to (1) visualize the dynamics of cells and their interfaces at high resolution, (2) perform automated 3D segmentation and tracking of all of the cells within a developing tissue, and (3) quantify cellular rearrangements in the tissue over time. Second, I will show how I utilized this approach to measure how perturbations to adhesions and actin biomechanics affect the ability of cells to move within the tissue and exchange neighbors. Finally, I will present my efforts to develop a physically-realistic computational model of cellular patterning in 3D multicellular systems, which integrates both adhesion-mediated sorting and actin biomechanics.

28. Stephanie Tsai, Ph.D. (Massachusetts General Hospital)

Endogenous Tenocyte Activation Underlies the Regenerative Capacity of the Adult Zebrafish Tendon

Stephanie L. Tsai, Steffany Villasenor, Rishita R. Shah, Jenna L. Galloway

Tendons are essential, frequently injured connective tissues that transmit forces from muscle to bone. Their unique highly ordered, matrix-rich structure is critical for proper function. While adult mammalian tendons heal after acute injuries, endogenous tendon cells, or tenocytes, fail to respond appropriately, resulting in the formation of disorganized fibrovascular scar tissue with impaired function and increased propensity for re-injury. Here, we show that unlike mammals, adult zebrafish tenocytes activate upon injury and fully regenerate the tendon. Using a full tear injury model in the adult zebrafish tendon, we defined the hallmark stages and cellular basis of tendon regeneration through multiphoton imaging, lineage tracing, and transmission electron microscopy. Remarkably, we observe that the zebrafish tendon can restore normal collagen matrix ultrastructure by 6 months post-injury. We show that regeneration progresses in three main phases: early inflammation, proliferation and formation of a cellular bridge between the severed tendon ends, and differentiation/matrix remodeling. Importantly, we demonstrate that pre-existing tenocytes are the main cellular source of regeneration. Collectively, our work debuts the zebrafish tendon as one of the only

reported adult tendon regenerative models and positions it as an invaluable comparative system to identify regenerative mechanisms that may inspire new therapeutic strategies.

29. Michelle Bernard (Boston University School of Medicine)

Evaluating pathogenicity of SMAD6 variants

Michelle Bernard, Xuan Anita He, Shannon Fisher

Smad6 is an intracellular downstream inhibitor of bone morphogenetic protein (BMP) signaling that is crucial in regulating skeletal development. An abundance of deleterious SMAD6 variants has been identified in several distinct congenital disorders including craniosynostosis (CS), bicuspid aortic valve (BAV), and radioulnar synostosis (RUS). There is also a high prevalence of tolerated SMAD6 variants in the human genome, raising the possibility that SMAD6 mutations confer a selective advantage. To evaluate the pathogenicity of SMAD6 missense variants identified in patients from CS, BAV, and RUS cohorts, we developed a functional assay based on dorsal-ventral (DV) patterning in zebrafish embryos. Injection of wild–type SMAD6 RNA severely dorsalizes embryos. We hypothesized that non-pathogenic SMAD6 variants would have a similar effect, while pathogenic variants would less severely dorsalize embryos due to loss of protein function. Based on our assay, we observed different degrees of pathogenicity, with some variants having a strong dorsalizing effect and others having an intermediate or minimal effect. This efficient functional assay can be used to verify pathogenicity of sequence variants identified in human patients and predict the functional consequences of variants of uncertain pathogenicity.

30. Shuning He, Ph.D. (Dana-Farber Cancer Institute)

In vivo identification of active drugs and drug combinations for high-risk neuroblastoma using zebrafish models

Shuning He, Megan Martel, Mark W. Zimmerman, A. Thomas Look

Most children with high-risk neuroblastoma respond initially to chemotherapy, but a large proportion will experience therapy-resistant relapse. In addition, due to intensified therapies, survivors of high-risk neuroblastoma often face long-term toxicities such as skeletal dysplasia, cardiac dysfunction, and premature mortality. We aim to identify drugs and

drug combinations with selectively activity against high-risk neuroblastoma cells in vivo, without undue toxicity to normal tissues. We capitalize on the advantages of zebrafish as a model organism to address pressing questions relevant to the generation of effective and selective therapies for high-risk neuroblastoma. We have developed zebrafish models of refractory neuroblastomas. These zebrafish develop aggressive neuroblastoma with up to 80% tumor penetrance by 3 weeks of age, ideal for the rapid analysis of small-molecule drugs by adding the drugs to fish water in 12-well plates. We screened a panel of kinase inhibitors and identified entrectinib (a RTK inhibitor that is active against NTRK1/2/3, ALK and ROS1) as the most active candidate drug. We validated its anti-neuroblastoma activity in human neuroblastoma cell lines. Our data showed that entrectinib is more active than the ALK inhibitor lorlatinib or the NTRK inhibitor larotrectinib, and less toxic than the multi-RTK inhibitor crizotinib. Importantly, we found that the activity of entrectinib is independent of MYC/MYCN, ALK, NF1, P53 mutation status, suggesting entrectinib as a potential anti-neuroblastoma drug.

31. Xiaodan Qin, Ph.D. (Boston University)

In vivo targeting of xenografted human cancer cells by functionalized fluorescent silica nanoparticles in zebrafish

Xiaodan Qin, Fabrice J.F. Laroche, Saquib Ahmed M. A. Peerzade, Andrew Lam, Igor Sokolov, Hui Feng

Developing nanoparticles capable of detecting, targeting, and destroying cancer cells has caused great interest in the field of nanomedicine. The bridging of nanotechnology to its biomedical application requires in vivo animal models. The zebrafish has emerged as a powerful model for cancer research. Due to their optical transparency and rapid development, the zebrafish embryos are well suited for real-time monitoring of the behavior of cancer cells and their interactions with the tumor microenvironment. Here, we developed a method that sequentially introduces human cancer cells and functionalized nanoparticles in transparent Casper zebrafish embryos to monitor the targeting of these cancer cells real-time. Our data shows that fluorescently labeled nanoparticles, which are conjugated with folate groups, can specifically recognize and target metastatic human cervical epithelial cancer cells. The recognition and targeting process can occur as early as 30 min post-injection of the nanoparticles tested. We also demonstrated that the ultrabright particles showed faster tumor detection and higher relative fluorescent contrast of cancer cells. Hence, the utility of zebrafish model enables the testing of nanoparticles on

various types of human cancer cells, facilitating the selection of optimal nanoparticles in each specific cancer context for future testing in mammals and the clinic.

32. Ellie Meader, Ph.D. (Boston Children's Hospital)

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

Eleanor Meader, Mindy Leder, Morgan Walcheck, Zachary LeBlanc, Isaac Oderberg, Paul Wrighton, Eleanor Quenzer, Tim Long, Sung-Eun Lim, Mariam Hachimi, Jenna Frame, Wolfram Goessling, Trista North

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 examined the initiation and regulation of mitophagy in HSPCs, this being a key process by which adult HSCs regulate ROS by removing damaged mitochondria. Oxidative stress begins to limit HSPC numbers immediately upon their colonization of the caudal hematopoietic tissue (CHT), and imaging with a transgenic mitophagy reporter shows that this coincides with the onset of mitophagy in HSPCs. scRNAseq analysis and in situ hybridization data suggest that mitophagy is induced in these cells via the bnip3lb receptor. We confirmed this by morpholino directed knockdown of bnip3lb, which reduces HSPC marker expression in the CHT via ROS-induced apoptosis. In contrast, induction of mitophagy elevated detection of HSPC markers. 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.

33. *Canceled*

34. Joanna Yeh, Ph.D. (Massachusetts General Hospital)

Instantaneous visual genotyping and facile site-specific transgenesis via CRISPR-Cas9 and phiC31 integrase

Junyan Ma, Weiting Zhang, Zhengwang Sun, Saba Parvez, Randall T. Peterson, Jing-Ruey Joanna Yeh

Here, we introduce 'TICIT', Targeted Integration by CRISPR-Cas9 and Integrase Technologies, which utilizes the site-specific DNA recombinase – phiC31 integrase – to insert fluorescent markers into CRISPR-Cas9-generated mutant alleles. It allows instantaneous determination of a zebrafish's genotype simply by examining its color. This technique, which relies on first knocking in a 39-basepair phiC31 landing site via CRISPR-Cas9, enables researchers to insert large DNA fragments at the same genomic location repeatedly and with high precision and efficiency. We demonstrated that TICIT could also be used to create reporter fish driven by an endogenous promoter. Additionally, we created a landing site located in the tyrosinase gene that could support transgene expression in a broad spectrum of tissue and cell types, acting as a putative safe harbor locus. Hence, TICIT can yield predictable and reproducible transgene expression, facilitate diverse applications in zebrafish, and may be applicable to cells in culture and other model organisms.

35. Mohamed El-Brolosy, Ph.D. (Harvard Society of Fellows/Whitehead institute)

Further characterizing transcriptional adaptation; a novel mode of genetic robustness

Mohamed A. El-Brolosy, Atharv Oak, An Hoang, Jingchuan Luo, Reuben Saunders, Olivia Corradin and Jonathan S. Weissman

Transcriptional adaptation (TA) is a recently identified genetic robustness mechanism through which cells can compensate for mutations by transcriptionally upregulating functionally related genes. Mechanistically, we identified mutant mRNA degradation as the trigger of TA. We proposed a model whereby following mutant mRNA decay, RNA degradation intermediates can regulate the expression levels of genes in a sequence-dependent manner (El-Brolosy et al., Nature, 2019). First discovered in zebrafish, additional studies identified other models of TA in mouse, worms and human cells. Despite identifying mRNA degradation as the trigger, the underlying molecular machinery remained poorly understood. In this presentation, I will go over the discovery of TA and discuss how it impacts the design of mutant alleles. I will also share unpublished work on the progress we made using CRISPR screens and functional genomic approaches to characterize the molecular machinery,

including the identification of a conserved RNA binding protein that modulates TA.

36. Isabella Ranieri (Boston College)

Thyroid hormone relieves Thrab-mediated repression of pectoral fin ray patterning, but regulates endoskeleton radials in a Thrab-independent manner

Isabella Ranieri, Shahid Ali, Sarah McMenamin

Pectoral fins are the predecessors of tetrapod fore-limbs, and understanding the genetic and hormonal factors regulating their development is of great interest in biomedicine and evolution. To test the role of thyroid hormone (TH) during pectoral fin development, we examined development of these appendages under hypothyroid (hypoTH) and euthyroid wild-type (WT) conditions. Fins of WT fish have 12 external rays, and an endoskeleton composed of 4 proximal and 8 distal radials. HypoTH fins developed supernumerary rays, ~38% more distal radials, and showed elongation in the most posterior proximal radials. In WT fish, fin rays form bifurcations at ~75% the total length; we found that these bifurcations required TH, because under hypoTH conditions the bifurcations formed very distally or failed to form. Since shh is required for fin ray growth and ray bifurcation, we hypothesized that TH promotes shh expression. We imaged a shh:GFP reporter in multiple TH contexts. In HypoTH fins, shh was expressed in the same location, but at ~50% of normal expression levels. Nuclear TH acts by binding to dual-action receptors, including Thrab, which represses or activates gene expression depending on ligand (TH) binding. When Thrab was absent in HypoTH fish, we found that the bifurcation placement was rescued, but the endoskeleton remained disrupted. This suggests that TH regulates ray morphology by relieving repression of Thrab, and the hormone regulates development of the endoskeleton through Thrab-independent mechanisms.

37. Nina Weichert-Leahey, Ph.D. (Dana-Farber Cancer Institute)

Genetic predisposition to neuroblastoma results from a regulatory polymorphism promoting the adrenergic cell state in zebrafish and human

Nina Weichert-Leahey, Hui Shi, Ting Tao, Derek A Oldridge, Adam D Durbin, Brian J. Abraham, Mark W Zimmerman, Shizhen Zhu, Andrew C

Wood, Deepak Reyon, J Keith Joung, Richard A Young, Sharon J Diskin, John M. Maris, A Thomas Look

Neuroblastomas exhibit plasticity between a neural crest-like mesenchymal and a more differentiated sympathetic adrenergic cell state. The adrenergic cell identity of neuroblastoma requires LMO1 as a transcriptional co-factor. LMO1 expression levels and the risk of developing neuroblastoma are associated with the single nucleotide polymorphism rs2168101 G>T that affects a GATA motif in the first intron of LMO1. Here, we sought to define the causal mechanism underlying the association between rs2168101 and the risk of developing neuroblastoma in vivo. Using genome editing, we introduced the precise TATA allele into the first intron of lmo1 in our MYCN-driven neuroblastoma model in zebrafish and found that the GATA wild-type zebrafish have much higher neuroblastoma penetrance than zebrafish with the TATA allele, mirroring the protective effects of the TATA allele in human. In both human and zebrafish with a germline GATA allele, neuroblastomas employ the adrenergic cell state, whereas tumors with the TATA allele employ the mesenchymal cell state, independent of LMO1. Thus, not only the increased risk associated with the regulatory GATA motif in the first intron of LMO1 is conserved over 400 million years of evolution that separate zebrafish and humans, but also the oncogenic regulatory circuitries involved in the initiation of neuroblastoma.

38. Bill Zong Jia (Harvard Medical School)

A bioelectrical phase transition patterns the first beats of a vertebrate heart

Bill Z. Jia, Yitong Qi, Jaime David Wong-Campos, Sean G. Megason, Adam E. Cohen

The heart is among the first organs to function in vertebrate development, but its transition from silent to beating has not been directly characterized. Using all-optical electrophysiology, we captured the very first zebrafish heartbeat and analyzed the development of cardiac excitability around this singular event. The first beats appeared suddenly and propagated coherently across the primordial heart. Targeted optogenetic perturbations mapped the development of excitability and conduction before and after the first heartbeats. Measured bioelectrical dynamics support a noisy saddle-node on invariant circle (SNIC) bifurcation as the critical phase transition that starts the heart. Simple models of this bifurcation quantitatively capture cardiac dynamics in space and time through early development, including coherent beating before transcriptional

specification of pacemakers. Our work shows how gradual and largely asynchronous development of single-cell bioelectrical properties produces a stereotyped and robust tissue-scale transition from quiescence to coordinated beating.

39. Gabriella Petrullo (Boston College)

Evaluation of craniofacial diversity in the danionin subfamily and across differing thyroid hormone conditions

Gabriella Petrullo, Stacy Nguyen, Rachel S. Lee, Emma Mohlmann, John O. Blythe, Isabella Ranieri, Sarah McMenamin

Zebrafish (Danio rerio) belong to the danionin subfamily of over 100 identified species. To better understanding craniofacial adaptation, we assessed the craniofacial diversity across the danionin subfamily. We obtained individuals from 9 danionin species and scanned representatives of each using microCT. Using 3D geometric morphometrics, we identified two major morphological groups: 1) D. rerio and 4 closely related species (D. nigrofasciatus, D. kyathit, D. aesculapii and D. albolineatus) showed shorter, more elongated heads, and 2) C. erythromicron, D. margaritatus and D. aequipinnatus, showing taller, more triangular heads. Thyroid hormone is known to modulate craniofacial proportions, and we hypothesized that some of the species differences might be due to interspecific differences in hormone metabolism or downstream pathways. We compared the head shapes of TH-deficient D. rerio and D. albolineatus, asking if these phenocopied the craniofacial morphology of other danionin species. We found that the hypothyroid Danios occupied a unique region of morphospace not occupied by other species, and that each species had a unique morphological response to hypothyroidism. Our results reveal considerable natural diversity in danionin head shape and suggest that alterations to thyroid hormone have the capacity to create unique overall craniofacial phenotypes.

40. Melody Harper (Boston College)

Aspects of bone ray axis patterning are autonomously remembered by fin tissue

Melody Harper & Sarah McMenamin

Regenerating tissues must remember or interpret their spatial position, using that positional identity to restore original size and patterning. The

zebrafish caudal fin is composed of rays built of bony segments that taper and shorten distally, and the entire structure regenerates in three weeks. It remains unresolved whether positional identity informing ray regeneration is autonomous to the tissues, or else directed by extrinsic positional cues. To distinguish between these possibilities, we transplanted portions of rays into novel sites, then amputated above the graft site, causing tissues to regenerate in a new environment. Testing whether segment length is intrinsic to the tissues, we transplanted distal portions of rays (with short segments) to proximal locations (composed of longer segments). Our results suggest that segment length is informed by attributes intrinsic to the tissue, as distal regions maintained shorter segments despite being in a proximal context. Further, distal transplants regenerated at a different rate, requiring twice the time of native rays to return to isometric growth. Transplanting peripheral-most rays to center positions, we found transplanted rays grew significantly shorter, suggesting extrinsic cues influence ray length. Lastly, white leucophores exclusively appear on peripheral-most tissue and this pattern is maintained even when peripheral transplants regenerate in central positions.

41. Anjali Nath, Ph.D. (Beth Israel Deaconess Medical Center)

Illuminating the function of the druggable genome through human phenotypes and model organisms

Ebubechi Nwaubani, Santiago Callegari, Zsu-Zsu Chen, and Anjali Nath

GPCRs are widely recognized as an important class of druggable proteins in human biology. However, of the ~800 GPCRs in the genome, there are ~140 GPCRs for which the endogenous ligands and physiological functions are unknown. A systematic means of identifying connections between understudied GPCRs and human physiology could have a transformative effect on human health. One approach is to leverage emerging genomic datasets and mine for associations between specific gene sets and diseases or clinical traits. However, the molecular mechanisms by which variants contribute to disease are often unclear. Therefore, we integrate complementary omics methods to frame these genes into biochemical pathways. Subsequently, these data are used to direct further experimentation in zebrafish; an organism amenable to high-throughput screening. By integrating these three approaches, we are identifying previously unknown therapeutic agents for metabolic diseases.

42. Stephanie Robinson (Boston College)

Regulation of Sonic hedgehog during larval fin fold development determines caudal fin shape and supportive endoskeleton

Stephanie Robinson, Eric Surette, Joan Donahue, Brendan Fitzgerald, Connor Murphy, Sarah McMenamin

Vertebrate development involves precise regulation of morphogenetic processes. The shapes of medial fins are essential for swimming functions, and comprise both an external skeleton of rays and an internal supportive endoskeleton. We have found that upregulating shh expression during a brief temporal window of larval fin fold development fundamentally alters the shape of the caudal fin and causes the anal fin to be occasionally malformed or missing altogether. In wild-type zebrafish, the caudal fin grows into a distinct ‘forked’ shape, but larval shh upregulation causes the central rays to grow roughly the same length as peripheral rays, creating a triangular truncate fin shape. We asked if altered external fin shape originated during larval development and was underlain by malformations in the endoskeleton. We visualized spatio-temporal patterns of osteoblasts and chondrocytes during development of the endo- and exoskeleton . This image series demonstrated that shh overexpression causes a variety of malformations, including variably disorganized hypural bones and a lack of a hypural diastema. Our data support a model in which larval patterns of Shh pathway activity presage the ultimate shape of both the internal and external components of the caudal fin.