Kristi Wharton
Prashanth Rangan
Kathryn Kavanagh
Mansi Srivastava
Lionel Christiaen
Anna-Katerina Hadjantonakis
Vivian Irish
Ken Birnbaum
Carrie Adler
Marcos Simoes-Costa
Mara Schvarzstein
Ben Martin

# NESDB 2017

## APRIL 27-30, 2017
## Marine Biological Laboratory
## Woods Hole, MA

### Keynote Speakers

**Monica Driscoll**
Rutgers University

**Len Zon**
Harvard Medical School

http://you.stonybrook.edu/NESDB2017

**Thursday April 27th**

**Education Session: 6:30-8:30**

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|---|---|
| 6:30-6:45 | **Welcome and Introductory Remarks by Cathy Savage-Dunn and David Matus** |
| 6:45-8:15 | **Alan Alda Center for Communicating Science, Nancy Serrell** |
| 8:15-8:30 | Fast Track Talks |
| | **Jasmin Camacho**, Harvard University<br>**Sophie Chase**, Smith College<br>**Matthew Koslow**, University of Albany<br>**Megan Norris**, Harvard University<br>**Jocelyn Steinfeld**, University of Massachusetts Boston |

**Poster Session and Mixer: 8:30-10:30 (odd number posters present)**

**Friday April 28th**

**Breakfast: 7:00-8:30**

**Session I: Morphogenesis and Motility 9:00-12:00**

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|---|---|
| *Chair:* | *Mansi Srivastava* |
| 9:00-9:20 | **Anna-Katerina Hadjantonakis**, Memorial Sloan Kettering Cancer Center, Member, FGFR signaling and the emergence of pluripotency in the mouse embryo. |
| 9:20-9:35 | **Natalia Shylo**, Yale University, Graduate Student, Tmem107 mouse models provide key insights into the phenotypic variability of cilia-mediated developmental patterning. |
| 9:35-9:50 | **Elizabeth Bearce,** Boston College, Graduate Student, TACC3, a microtubule plus-end tracking protein, regulates neural crest cell motility in vitro and in vivo. |
| 9:50-10:05 | **Mayu Inaba**, University of Connecticut Health, Assistant Professor, Cellular protrusion mediated niche-stem cell communication. |
| 10:05-10:30 | Coffee Break |
| *Chair:* | *Lionel Christiaen* |
| 10:30-10:50 | **Kathryn Kavanagh**, University of Massachusetts, Dartmouth, Assistant Professor, Shared developmental rules predict patterns of size evolution in vertebrate segmented structures. |
| 10:50-11:05 | **Tyler Huycke**, Harvard Medical School, Graduate Student, Genetic and mechanically mediated patterning of gut smooth muscle. |
| 11:05-11:20 | **Diana Rubel**, Stony Brook University, Undergraduate Student, Deletion of B3glct disrupts craniofacial, skeletal, and cardiac development in mice. |
| 11:20-11:35 | **Amanda Baumholtz**, McGill University, Graduate Student, Claudins regulate cell shape and localization of signaling proteins at the apical cell surface during neural tube closure. |
| 11:35-11:50 | **Jenny Lanni**, Wheaton College, Assistant Professor, Essential function of ion pump Slc12a7a/KCC4a in regulating zebrafish fin proportion and pigment stripe formation. |

**Lunch: 12:00-1:30**

**Session II: Genomics and Gene Regulation 1:45-5:15**

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| *Chair:* | *Carrie Adler* |
| 1:45-2:05 | **Marcos Simoes-Costa,** Cornell University, Assistant Professor, Gene regulatory control of neural crest axial identity and cell fate. |
| 2:05-2:25 | **Cesar Arenas-Mena**, CUNY College of Staten Island, Associate Professor, The origins of developmental gene regulation. |
| 2:25-2:40 | **Sushma Teegala**, Queens College, CUNY, Graduate Student, Tbx2 is required for the suppression of mesendoderm during early Xenopus development. |
| 2:40-3:00 | **Kenneth Birnbaum**, New York University, Associate Professor, The link between injury and development in plant regeneration. |
| 3:00-3:30 | Coffee Break (sponsored by Nightsea) |
| *Chair:* | *Mara Schvarsztein* |
| 3:30-3:50 | **Lionel Christiaen**, New York University, Associate Professor, Regulation of cardiopharyngeal fate specification in a simple chordate. |
| 3:50-4:05 | **Jeffrey Farrell,** Harvard University, Postdoctoral Fellow, A pre-gastrulation damage response uncovered by single-cell RNAseq. |
| 4:05-4:20 | Fast Track Talks<br><br>**Casey Kimball**, Keene State College<br>**Abraham Q. Kohrman**, Stony Brook University<br>**Uday Madaan**, Queens College, CUNY<br>**Daniel McIntyre**, NYU Medical Center<br>**Ashley Waldron**, University of Vermont<br>**Rachael Norris**, UConn Health |
| 4:20-4:50 | Coffee Break |

**Keynote Address: 4:55-6:00**

**Monica Driscoll**, Rutgers University, Professor, Neurons Can Take Out the Trash: A Novel Facet of Proteostasis and Mitochondrial Quality Control.

**Dinner: 6:15-7:30**

**Poster Session and Mixer: 8:00-10:00 (even number posters present)**

**Saturday April 29th**

**Breakfast: 7:00-8:30**

**Session III: Germline, Stem Cells and Regeneration 9:00-12:00**

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|---|---|
| *Chair:* | *Benjamin Martin* |
| 9:00-9:20 | **Mansi Srivastava**, Harvard University, Assistant Professor, The evolution of mechanisms for animal regeneration. |
| 9:20-9:35 | **Austen Barnett**, Harvard University, Postdoctoral Fellow, The role of Hox genes in germ cell development in a basally-branching insect. |

9:35-9:50 **Amelie Raz,** MIT, Graduate Student, Acoel regeneration mechanisms indicate ancient and widespread role for muscle in regenerative patterning.

9:50-10:10 **Mara Schvarsztein**, CUNY Brooklyn College, Assistant Professor, Chromosome inheritance in gamete and development.

10:10-10:35 Coffee Break

*Chair:* *Chitra Dahia*

10:30-10:50 **Prashanth Rangan**, SUNY Albany, Assistant Professor, RNA secondary structure regulates translation control of a germ line RNA in *Drosophila*.

10:50-11:05 **Nicholas Palmisano**, Queens College, CUNY, Graduate Student, The recycling GTPase, RAB-10, regulates autophagy flux in *Caenorhabditis elegans*.

11:05-11:20 **Nicholas Leigh,** Harvard Medical School, Postdoctoral Fellow, von Willebrand Factor D and EGF-Domains is essential for axolotl limb regeneration.

11:20-11:40 **Carolyn Adler**, Cornell University, Assistant Professor, A divergent neurexin-1 homolog controls muscle regeneration in planarians .

**Lunch: 12:00-1:30**

**Session IV: Signaling and Organogenesis 1:45-4:45**

*Chair:* *Anna-Katerina Hadjantonakis*

1:45-2:05 **Kristi Wharton,** Brown University, Professor, The varied BMP signaling output critical for development requires regulated proprotein processing.

2:05-2:20 **Matthew Harris**, Harvard Medical School, Graduate Student, When fish fly: using mutational phenocopy and phylogenetics to understand allometry in evolution.

2:20-2:35 **Jennifer Fish**, University of Massachusetts, Lowell, Assistant Professor, Tissue interactions and differing threshold requirements for Fgf8 contribute to variation in disease penetrance.

2:35-3:00 Coffee Break

*Chair:* *Kenneth Birnbaum*

3:00-3:20 **Benjamin Martin,** Stony Brook University, Assistant Professor, Combinatorial signaling interactions pattern the dorsal-ventral mesodermal axis by controlling bHLH transcription factor activity.

3:20-3:35 **Margherita Perillo**, Boston College, Postdoctoral Fellow, Positioning of nuclei at the neuromuscular and myotendinous junctions in the developing muscle.

3:35-3:50 **Tessa Montague**, Harvard University, Graduate Student, Vg1-Nodal heterodimers are the endogenous inducers of mesendoderm.

3:50-4:10 **Vivian Irish**, Yale University, Professor**,** Petal Development: a twist in fate.

4:10-4:45 Coffee Break

**Keynote Address: 4:45-5:50**

**Leonard Zon**, Harvard Medical School, Professor, Pathways Regulating Stem Cell Induction, Self-Renewal and Engraftment.

**Business Meeting: 5:50-6:10**

**Dinner: 6:30-8:30**

**Student and Postdoc Presentation Awards: 8:00-8:30**

**Sunday April 30th**

**Breakfast: 8:00-9:00**

**Departure**

**Education Session:**

Alan Alda Center for Communicating Science, Stony Brook University

**Nancy Serrell**

In this 90 minute workshop participants will learn general principles in how to craft short, clear, conversational statements, intelligible to non-scientists, about what you, as scientists, do and why it matters. The session will consist of an interactive presentation and discussion on interpreting technical material using examples and analogies to illuminate unfamiliar concepts to your audience. The plenary will address problems and solutions in public interactions as well as peer-to peer communication. Participants will practice clarity in speaking to non-scientists about their work and will be actively engaged in improvisation exercises or explaining scientific material to lay people.

**Abstracts:**

**Invited speaks and Selected talks**

### FGFR signaling and the emergence of pluripotency in the mouse embryo

Anna-Katerina Hadjantonakis, Minjung Kang, Vidur Garg
*Sloan Kettering Institute, USA*

FGF4 is the signal regulating specification of primitive endoderm (PrE) versus pluripotent epiblast (EPI) within the inner cell mass (ICM) of the mouse blastocyst. To gain insight into how FGF receptors (FGFRs) mediate a response to FGF4 within individual ICM cells, we combined single-cell-resolution quantitative imaging with single-cell gene expression profiling. Our data reveal that despite the PrE-specific expression of *Fgfr2*, it is *Fgfr1,* a related receptor expressed by all ICM cells, that is critical for the establishment of a PrE identity. Signaling through FGFR1 is required to constrain levels of the pluripotency-associated factor NANOG. However, stable ICM lineage commitment requires the activity of both receptors. Gene expression profiling of >500 cells isolated from wild-type and mutant embryos identified distinct downstream targets associated with each receptor. These data lead us to propose a model whereby the distinct and additive roles of two FGF receptors coordinate lineage divergence within the ICM.

**Tmem107 mouse models provide key insights into the phenotypic variability of cilia-mediated developmental patterning.**

Natalia Shylo, Scott Weatherbee
*Yale University, USA*

Cilia are near ubiquitous cellular organelles in mammals. Disruptions of cilia lead to profound defects in multiple organs and tissues during embryonic and postnatal development. Numerous mouse models have been created to study ciliopathies - diseases of cilia. However, a limited number of mutations on inbred backgrounds provide a poor snapshot of the complex genetics of ciliopathies, which present as a continuous spectrum of diseases in highly diverse human population. Examining an allelic series of mouse mutations on different inbred backgrounds should identify key modifiers of ciliopathy phenotypes and provide critical insight into the function of cilia in development and disease. We previously reported on hypomorphic *Tmem107 schlei* mice, which survive to e18.5, exhibit exencephaly and polydactyly, but no left-right abnormalities or kidney cysts - common phenotypes of severe ciliopathies. In contrast, *Tmem107 null* mice display heterotaxy and develop kidney cysts as early as e16.5. The cystic phenotype is associated with a strong reduction of specific proteins within kidney cilia, including complete loss of ciliary Pkd2 localization, a key player in renal cystogenesis that accounts for 15% of human polycystic kidney disease. We migrated the *schlei* allele onto the C57BL6J background to more closely match the *Tmem107 null* allele, already on that background. To our surprise, we saw alleviation of some phenotypes - reduced frequency of exencephaly - and increased severity of others, like renal cystogenesis in *schlei*-B6 embryos. Defining the genetics underlying the range of cilia-related phenotypes we observed between *Tmem107 null* and *Tmem107 schlei* mouse mutations will help us better understand the molecular mechanisms of kidney development, will elucidate key modifiers of cilia-mediated organogenesis, and identify new diagnostic tools for human diseases.

**TACC3, a microtubule plus-end tracking protein, regulates neural crest cell motility in vitro and in vivo**

Elizabeth Bearce, Erin Rutherford, Andrew Francl, Matt Evans, Laura Anne Lowery
*Boston College, USA*

Coordinated cell migration is critical during embryogenesis. During development, cells from multiple lineages delaminate from their point of origin to travel through the embryo, providing foundations for the heart, gut, vasculature, & nervous system. Cell motility relies on dynamic coordination of the F-actin and microtubule (MT) cytoskeletons. These systems function together to respond to chemical cues, directionally polarize, and generate force and traction for motility. Therefore, cytoskeletal proteins that impact motility are critical during embryogenesis, and disruption of these genes can give rise to developmental disorders. Our work focuses on how MT plus-end regulators impact migration of cranial neural crest (CNC) cells, a multipotent cell that differentiates to form muscle, cartilage, bone, & nerves of the face. We identified one MT regulator, transforming acidic coiled-coil 3 (TACC3), as a putative effector of CNC motility. We previously showed that TACC3 functions as a MT plus-end tracking protein and regulates MT growth velocities in CNCs. Interestingly, TACC3 is one of 5 genes deleted in Wolf-Hirschorn syndrome, a genetic disorder that presents craniofacial defects consistent with disrupted CNC migration. Using *in situ* hybridization, we show that TACC3 is highly-expressed in motile CNCs in *X. laevis*. Manipulation of TACC3 protein levels is sufficient to alter CNC velocity *in vitro*. Overexpression (OE) drives increased single-cell migration velocities and explant dispersion. TACC3 KD results in marked motility defects, with fewer cells able to migrate persistently. In order to assess how TACC3 manipulation impacts cell velocity, we use confocal microscopy to examine localization of GFP-TACC3 in live cells, & effects of TACC3 manipulation on MT stabilization, adhesion turnover, and chemotaxis. Finally, KD but not OE of TACC3 significantly impacts pharyngeal arch morphology *in vivo*. Together, these data support a role for TACC3 in embryonic cell motility.

**Cellular protrusion mediated niche-stem cell communication**

Mayu Inaba
*Department of Cell Biology, UConn Health, United States*

Adult tissue stem cells produce highly differentiated but short-lived cells throughout life, contributing to the tissue maintenance and repair. Specialized environments called “niches” help to maintain stem cells by producing signals essential for stem cell maintenance. Stem cell-niche signaling has to be carefully regulated, since an excess of signal activation can lead to tumorigenic overproliferation of stem cells, while its shortage can deplete stem cells, causing tissue degeneration. Thus, niche signal has to meet two criteria 1) sufficient signal activation in stem cells, 2) no (or lower than threshold) signal activation in non-stem cells. Stem cells and their non-stem cell daughters are often juxtaposed each other, and thus how specificity of spatially confined niche signaling is achieved has been a mystery. It has been postulated that the secreted niche ligands “diffuse” only in a short-range, but how the range of diffusion can be tightly regulated remained unknown. We recently discovered previously unrecognized cellular protrusions, termed MT (microtubule-based)- nanotubes, that are specifically formed by stem cells and extend into the hub cells, the major niche component in the Drosophila testis. Our preliminary studies indicate that MT-nanotubes promote BMP signaling (Dpp ligand-Tkv receptor), a niche ligand required for stem cell maintenance. Based on our preliminary studies, we hypothesize that MT- nanotubes function to mediate productive niche signaling such that only stem cells experience enough niche-dependent signal transduction, providing a mechanistic basis for the short-range nature of the niche signaling. We further explore molecular and cellular mechanisms of MT-nanotube- mediated niche-stem cell signaling.

**Shared developmental rules predict patterns of size evolution in vertebrate segmented structures**

Kathryn Kavanagh
*University of Massachusetts, Dartmouth, USA*

Phenotypic diversity is not uniformly distributed, but how biased patterns of evolutionary variation are generated and whether common developmental mechanisms are responsible remains debatable. High-level “rules” of self-organization and assembly are increasingly used to model organismal development, even when the underlying cellular or molecular players are unknown. One such rule, the inhibitory cascade, predicts that proportions of segmental series derive from the relative strengths of activating and inhibitory interactions acting on both local and global scales. Here we demonstrate that this developmental design rule explains population-level variation in segment proportions, their response to artificial selection and experimental blockade of putative signals, and macroevolutionary diversity in limbs, digits and somites. Together with evidence from teeth, these results indicate that segmentation across independent developmental modules shares a common regulatory “logic”, which has a predictable impact on both the short and long-term evolvability.

**Genetic and mechanically mediated patterning of gut smooth muscle**

Tyler Huycke, Clifford Tabin
*Harvard Medical School, USA*

Beginning as a simple tube of endoderm surrounded by mesenchyme, the gut is patterned early in development to generate discrete layers of smooth muscle with distinct cellular alignments. Correct organization of these muscle layers into circumferential and longitudinal bands is required for directional peristalsis and to generate the appropriate physical forces that drive buckling morphogenesis of the gut lumen. How this muscle is patterned along the radial axis of the gut tube and the mechanisms controlling its alignment across the length of the tissue are poorly understood. We find that the precise radial pattern of the circumferential muscle layer is controlled by threshold levels of Hedgehog and BMP signaling, which act to place the muscle at an exact distance away from the endoderm. Differentiation of the later-forming longitudinal muscle layer is initiated by localized BMP inhibition, mediated by Noggin derived from both the circumferential muscle layer and enteric neural crest cells. The fact that the layers of smooth muscle form sequentially allows them to be exposed to unique mechanical environments present in the growing embryo at distinct times during development. In the early gut tube, differential growth along the radial axis places the mesenchyme under circumferential tension, and this stress is necessary for circumferential alignment of cells in the early-forming muscle layer. Once formed, cells of this circumferential layer begin to spontaneously contract, and these contractions are necessary and sufficient to align cells in the later-forming longitudinal layer. Together, our findings provide a model for how gene-regulated patterning is coupled with mechanical forces to control the development of gut smooth muscle.

**Deletion of B3glct disrupts craniofacial, skeletal, and cardiac development in mice**

Diana Rubel[1], Richard C. Grady[1], Sardar MZ Uddin[2], David E. Komatsu[2], Simon D. Bamforth[3], Jurgen E. Schneider[4], Takashi Sato[5], Hisashi Narimatsu[5], Robert S. Haltiwanger[6], Bernadette C. Holdener[1]

*[1]Department of Biochemistry and Cell Biology, Stony Brook University, USA; [2]Department of Orthopaedics, Stony Brook University, USA; [3]Institute of Genetic Medicine, Newcastle University, UK; [4]British Heart Foundation Experimental Magnetic Resonance Unit (BMRU), Wellcome Trust Centre for Human Genetics, UK; [5]National Institute of Advanced Industrial Science and Technology, Japan; [6]Complex Carbohydrate Research Center, University of Georgia, USA*

Peters plus syndrome (PPS) is a rare genetic disorder characterized by the presence of anterior eye segment abnormalities, short stature, brachydactyly, and developmental delay. In addition, cleft palate, congenital heart defects, and/or urogenital defects are present in 50% of patients. The disease is caused by loss-of-function mutations in beta-3-glucosyltransferase (B3GLCT). B3GLCT transfers a molecule of glucose to O-fucosylated thrombospondin type I repeats (TSRs). TSRs with O-Fucosylation consensus sequences are tandemly repeated within 49 predominantly extracellular matrix (ECM) associated proteins. The ADAMTS class of proteins (A Disintegrin and Metalloproteinase with ThromboSpondin motifs) makes up nearly 50% of these proteins, and is implicated in controlling the structural properties of the ECM, influencing cell migration, organogenesis, tissue organization and cell signaling. We used a mouse B3glct knockout to gain insight into the developmental origin of PPS and identify B3GLCT targets responsible for PPS anomalies. B3glct mutants showed reduced neonatal viability. MicroCT and MRI imaging identified potential ventricular septal and myocardial wall defects in some homozygotes. The survivors were runted and had broadened and domed heads. Skeletal preparations, 3D microCT renderings, and histological analyses identified defects in cranium structure, endochondral ossification, and hydrocephalus. Finally, reducing the copies of Adamts9 in B3glct homozygotes resulted in 100% neonatal lethality. These results provide evidence that defects in PPS patients result, at least in part, from abnormalities in ADAMTS9 function, and demonstrate that the B3glct mutant mouse will provide an invaluable resource for understanding how changes in the ECM structure or composition can lead to the collection of common congenital abnormalities seen in PPS patients. Funding sources: NIH GM061126 to RSH, and BHF FS/11/50/29038 and RE/08/004 to JES, and NIH RO1HD070888 to DEK.

**Claudins regulate cell shape and localization of signaling proteins at the apical cell surface during neural tube closure**

Amanda Baumholtz[1,2], Annie Simard[1,2], Evanthia Nikolopoulou[3], Joerg Piontek[4], Nicholas D.E. Greene[3], Aimee Ryan[1,2]

*[1]McGill University, CA; [2]Research Institute of the McGill University Health Centre, CA; [3]Birth Defects Research Centre, UCL Institute of Child Health, UK; [4]Institut für Klinische Physiologie, Germany*

Morphogenetic remodeling of the neural plate into a closed neural tube requires synchronization of cell shape changes with cell movements within the neural and non-neural ectoderm. Throughout neural tube closure, the integrity of these epithelial cell layers is maintained by intercellular junctions, the most apical of which are tight junctions. We discovered that members of the claudin family of integral tight junction proteins regulate molecular and morphological changes that are essential for both early and late events during neural tube morphogenesis. Claudins regulate paracellular permeability, apical-basal cell polarity and cell adhesion, and link the tight junction to the actin cytoskeleton. Removal of Claudin-3, -4, and -8 from tight junctions of the chick ectoderm using the C-terminal domain of *Clostridium perfringens* enterotoxin (C-CPE) resulted in folate-resistant neural tube defects in 100% of treated embryos that are caused by defective apical constriction and convergent extension. Open neural tube defects were also observed in mouse embryos treated with C-CPE. Removal of Claudin-3 from the non-neural ectoderm using a Claudin-3-specific C-CPE variant affected only the final phase of epithelial remodeling that joins the apposed neural tube folds to form a closed tube. Molecular analyses revealed that apical-basal polarity was maintained. However, apical accumulation and/or localization of Rho-GTPase, planar cell polarity and Par polarity signalling components were dramatically reduced/altered. We hypothesize that the cytoplasmic tails of claudins uniquely interact with components of Rho-GTPase signalling and polarity complexes to coordinately regulate changes in cell movements and cell shape that are required for neural tube closure.

**Essential function of ion pump Slc12a7a/KCC4a in regulating zebrafish fin proportion and pigment stripe formation**

Jenny Lanni[1,3], David Peal[2,3], Caroline Stanclift[1], Ethan Fitzgerald[1], Kathryn Henrikson[1], Margot Bowen[2,3], Kristopher Kahle[4], Matthew Harris[2,3]

*[1]Wheaton College MA, USA; [2]Department of Genetics, Harvard Medical School, USA; [3]Department of Orthopaedic Research, Boston Children's Hospital, USA; [4]Center for Mendelian Genomics, Yale School of Medicine, USA*

Regulation of proportion, or allometry, is a critical but poorly understood aspect of vertebrate development. How growth in complex organs and scaling for size is coordinated is also largely unknown. We have identified a dominant zebrafish mutant with altered proportion of the adult fins and barbs; this mutant, *schleier*, is named after its flowing fins. When compared to wildtype, s*chleier* mutants display an increased rate of fin growth throughout development, leading to overall positive allometry. Analysis of fin rays in mutant individuals revealed that long fins were accompanied by changes in skeletal patterning. Specifically, bone segments were elongated in mutant fin rays, but a similar number of segments was retained per ray. *Schleier* homozygous animals also show disruption of the zebrafish stripes into leopard-like spots. Using massively parallel sequencing methods, we linked the *schleier* mutation to a single missense variant in *slc12a7a* encoding the potassium-chloride transporter *Kcc4a*. Functional analysis of this variant using a *Xenopus* oocyte assay revealed that it abrogated function of the channel and co-transport. We designed specific guide RNAs against the *slc12a7a* gene and injected them with Cas9 mRNA into embryos. The resulting mosaic adults showed local overgrowth of fins and barbules. Our findings suggest that that *schleier* is due to haploinsufficiency of *slc12a7a/kcc4a*. Further, these data suggest that the change in potassium regulation is essential to mediate local effects on growth and proportion, distinct from systemic effects. Our previous data implicated the role of increased, or potentially super-physiological potassium conductance in regulating proportion. Importantly, these data implicate that bioelectric signaling is normally necessary to regulate appropriate proportion of the adult. In addition, we find that similar gene function is essential to regulate pigment pattern during postembryonic development.

**Gene regulatory control of neural crest axial identity and cell fate**

Marcos Simoes-Costa
*Cornell University, USA*

The neural crest is a progenitor cell population in vertebrate embryos that engages in extensive migration and differentiates into multiple cell types, including chondrocytes, osteocytes and the neurons and glia of the peripheral nervous system. Cranial neural crest cells give rise to the skeletal elements of the face and the cranial ganglia, playing a central role in craniofacial development. To scrutinize the molecular program controlling development and differentiation of this cell population, we employed a combination of state of the art developmental biology techniques with systems-level approaches. Cis-regulatory analysis of neural crest genes, transcriptional profiling and loss-of-function assays in avian embryos allowed for efficient identification of regulatory sub-circuits that are unique to the cranial neural crest. Our results highlighted how signaling and transcriptional inputs are integrated to drive cranial neural crest formation, and revealed novel genetic interactions that endow these cells with their unique features, such as the ability to differentiate into chondrocytes. The discovery of a gene regulatory circuit that supports formation of particular neural crest derivatives holds the promise of enabling engineering and replacement of specific neural crest derived cell types.

**The origins of developmental gene regulation**

Cesar Arenas-Mena
*College of Staten Island/City Univ of NY, USA*

It is proposed that developmental enhancers evolved from unicellular inducible promoters that diversified the expression regulatory genes during metazoan evolution. In short, during metazoan evolution, constitutive-type promoters of regulatory genes would have acquired novel receptivity to distal regulatory inputs from promoters of inducible genes that eventually specialized as enhancers. Promoters and enhancers are functionally similar; both can regulate the transcription of distal promoters and both direct local transcription. Additionally, enhancers have experimentally characterized structural features and motifs, such as the TATA box, that reveal their origin from inducible promoters. The distal cooperative regulation among promoters identified in unicellular opisthokonts possibly represents the precursor of distal regulation of promoters by enhancers. Our genome-wide ATAC-seq, Chip-seq and PRO-seq analysis combined with previous and our own functional *cis*-regulatory analyses in sea urchin embryos confirm that active enhancers are transcribed. In addition, we have proposed that metazoan evolution involved the developmental suppression of cell proliferation and transcriptional potency. Histone variant H2A.Z promotes chromatin accessibility at transcriptional regulatory elements and it is developmentally regulated in indirectly developing sea urchins and polychaetes; my original hypothesis of a transcriptional multipotency role of H2A.Z has been experimentally confirmed. We are currently characterizing the transcriptional and post-transcriptional regulation of *H2A.Z* in the purple sea urchin *Strongylocentrotus purpuratus*. Our results reveal that embryonic expression of *H2A.Z* is primarily driven by a single cis-regulatory module, and that undetermined Myb-family and GATA transcription factors contribute to control the transcriptional regulation of *H2A.Z* during the maternal to zygotic transition.

**Tbx2 is required for the suppression of mesendoderm during early Xenopus development**

Sushma Teegala[1], Riddhi Chauhan[2], Daniel Weinstein[1,2]
*[1]City University of New York - The Graduate Center, United States; [2]City University of New York - Queens College, United States*

The T-box family proteins are DNA-binding transcriptional regulators that play crucial roles during early vertebrate embryogenesis. Well-characterized members of this family, including the transcriptional activators Brachyury and VegT, are essential for the proper formation of mesoderm and endoderm, respectively. No studies published to date have demonstrated a role for T-box proteins in the regulation of ectoderm during early *Xenopus* development. Studies in our lab have identified a member of the T-box family, Tbx2, that is both sufficient and necessary for the formation of dorsal ectoderm. Tbx2 is expressed zygotically in the presumptive ectoderm during blastula stages. Ectopic expression of Tbx2 represses mesoderm and endoderm, while loss of Tbx2 leads to inappropriate expression of mesoderm and endoderm in the region fated to give rise to ectoderm. We also find that misexpression of Tbx2 induces neural tissue in animal cap explants, suggesting that Tbx2 plays a role in both the establishment of ectodermal fate and its subsequent dorsoventral patterning. Our studies further suggest that Tbx2 functions as a transcriptional repressor during germ layer formation, and mediates this activity through repression of target genes that are stimulated elsewhere in the embryo by activating T-Box proteins. Taken together, our results point to a critical role for Tbx2 in limiting the potency of blastula-stage progenitor cells during vertebrate germ layer differentiation.

**The link between injury and development in plant regeneration**

Idan Efroni[1], Alison Mello[1], Tal Nawy[1], Pui-Leng Ip[1], Nicholas DelRose[1], Ashley Powers[2], Rahul Satija[1,2], Kenneth D. Birnbaum[1]
*[1]Center for Genomics and Systems Biology, Biology Department, New York University, 10003*
*[2]New York Genome Center, N.Y. 10013*

Highly regenerative organisms can respond to injury by initiating developmental programs that lead to the organogenesis that originally takes place in the embryo. Plants have both embryonic and post-embryonic development, as they can initiate roots, for example, in the early stages of embryogenesis and from several different adult structures. Which developmental programs do they use during regeneration? We use a combination of lineage tracing, single-cell RNA-seq, and marker analysis to test different models of regeneration ontology. We have shown that rapid cell-identity transitions lead to the formation of a new stem cell niche that is recruited from multiple remnant tissues. Stem cell activation is preceded by embryo-like cellular states that also exhibit the distinct hormone signaling dynamics of early development. Embryonic mutants that could escape to form normal adult meristems recapitulated embryonic defects in root tip regeneration. I present some new findings on the signals that lead to the induction of embryonic programs after injury as we now focus on the role of injury-responsive cis-regulatory control of key embryonic genes. Our findings suggest plant organ regeneration occurs through rapid activation of embryonic patterning programs hardwired to injury response.

**Regulation of cardiopharyngeal fate specification in a simple chordate**

Lionel Christiaen[1], Wei Wang[1], Xiang Niu[1,2], Robert Kelly[3], Rahul Satija[1,2]
*[1]New York University, USA; [2]New York Genome Center, USA; [3]CNRS, Aix-Marseilles Université, France*

In vertebrates, mounting evidence point to the existence of multipotent cardiopharyngeal progenitors that produce second-heart-field-derived cardiomyocytes, and branchiomeric skeletal head muscles. However, the cellular and molecular mechanisms underlying these early fate choices remain largely elusive. The tunicate *Ciona* has emerged as an attractive model to study early cardiopharyngeal development at high spatial and temporal resolution: through two asymmetric and oriented cell divisions, defined multipotent cardiopharyngeal progenitors produce distinct first and second heart precursors, and pharyngeal muscle (aka atrial siphon muscle, ASM) precursors. I will present an extensive analysis of lineage-specific transcriptome dynamics using single cell RNA-seq. We characterized molecular features of multipotent progenitors, such as extensive multilineage transcriptional priming, defined regulatory inputs governing differential gene expression dynamics, and finally, a novel regulator of second heart field-specification.

**A pre-gastrulation damage response uncovered by single-cell RNAseq**

Jeffrey Farrell[1], Andrea Pauli[2], Alexander Schier[1]
*[1]Harvard University, USA; [2]Institute of Molecular Pathology (IMP), Austria*

Developing embryos endure constant challenges from their environment, yet nearly always produce viable and phenotypically wild-type embryos. This suggests that embryos have remarkable capacity to recover from errors and damage. One major challenge faced by early embryos is DNA damage. In most situations, cells with unrepaired DNA damage would be eliminated through apoptosis to prevent deleterious genomic changes that can result in uncontrolled growth and potentially tumorigenesis. However, embryos across many phyla, including zebrafish embryos, are incapable of committing apoptosis until during gastrulation. This raises the question: what happens to damaged cells from pre-gastrulation embryos? Through single-cell RNAseq of zebrafish embryos just prior to gastrulation, we uncovered a previously undescribed cellular expression program that may hold the answer, which we call 'seven-sleeper.'

The 'seven-sleeper' program combines expression of developmental regulators with genes associated with cellular stress, p53 activation, and apoptosis. We have found that several different sources of DNA damage activate the expression of the 'seven-sleeper' program, but not general stresses. Surprisingly, though 'seven-sleeper' cells express apoptotic genes (*e.g. caspase 8*), live cell tracking shows that the 'seven-sleeper' cells do not commit apoptosis during gastrulation, when apoptosis becomes active. Additional data suggests these cells remain alive and contribute to many tissues. The 'seven-sleeper' cells seem to temporarily arrest their cell cycle, and perhaps are more likely to differentiate into enveloping layer cells, a cell type that is terminally differentiated unusually early in development. We are currently pursuing the role of developmental regulators in this cell type. We hypothesize 'seven-sleeper' either preserves cells until the full complement of DNA repair pathways are activated during gastrulation or acts as a memory of which cells have experienced damage.

**Neurons Can Take Out the Trash: A Novel Facet of Proteostasis and Mitochondrial Quality Control**

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Toxicity of misfolded proteins and mitochondrial dysfunction are pivotal factors that promote age-associated functional neuronal decline and neurodegenerative disease. Moreover, misfolded human disease proteins and mitochondria can move into neighboring cells via unclear mechanisms, which may promote pathology spread. To deal with these challenges, neurons invest considerable "internal" cellular resources in chaperones, protein degradation, autophagy, and mitophagy to maintain proteostasis and mitochondrial quality.

We have found a previously unrecognized capacity of *Caenorhabditis elegans* adult neurons to extrude large (~4um) membrane-surrounded vesicles called "exophers" that can harbor protein aggregates and organelles. Inhibiting chaperone expression, autophagy, or the proteasome, as well as compromising mitochondrial quality, enhances exopher production, suggesting exopher-genesis as response to stress. Proteotoxically stressed neurons that extrude exophers subsequently function better than those that do not, revealing a neuroprotective capacity. The extruded exopher transits through a surrounding tissue where some contents appear degraded, but some non-degradable materials can be subsequently found in remote cells.

Our observations suggest that exopher-genesis is a potential "garbage-removal" response to challenged proteostasis and organelle function—an external type of homeostatic control. We propose that exophers may be components of a conserved mechanism that constitutes a fundamental, but formerly unrecognized, branch of neuronal proteostasis and mitochondrial quality control, which, when dysfunctional or diminished with age, might actively contribute to pathogenesis in human neurodegenerative disease and brain aging.

**The evolution of mechanisms for animal regeneration**

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Many extant lineages of animals can regenerate any missing cell type, raising the possibility that the earliest animals to evolve were capable of "whole-body" regeneration. Functional studies of multiple species in phylogenetically informative positions relative to each other are needed to evaluate how the gene regulatory networks (GRNs) underlying regeneration have evolved. Acoels represent the earliest lineage of animals with bilateral symmetry (Bilateria), and therefore, comparisons of acoels and other bilaterians can reveal the evolution of GRNs over 550 million years of evolution. Previous work in the acoel *Hofstenia miamia*, a highly regenerative species that is amenable to mechanistic studies of regeneration, had uncovered a shared role for Wnt and Bmp signaling in patterning of new tissue during regeneration in acoels and planarians, the well-established model system for studies of regeneration. Therefore, we seek to assess whether shared or divergent mechanisms underlie other aspects of regeneration, such as wound signaling and the regulation of stem cells. *Hofstenia* is a powerful new model system that offers many advantages including accessible embryos and inhibition of gene function via soaking in dsRNA. We are combining high throughput sequencing approaches with functional studies to identify gene regulatory networks activated early during regeneration in *Hofstenia*. Additionally, we are utilizing the embryos of *Hofstenia* to investigate the developmental regulation of regenerative cell types.

**The role of Hox genes in germ cell development in a basally-branching insect.**

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Hox genes encode transcription factors that play an ancient and conserved role in establishing the regional identities of animal body plans, with a specific role in arthropods (chelicerates, myriapods, crustaceans and hexapods) in establishing segmental identities. In the cricket *Gryllus bimaculatus*, which is a member of the early-deriving insect group Orthoptera, the induction of a subset of mesodermal cells to form the primordial germ cells (PGCs) is restricted to the second through the fourth abdominal segments (A2-A4). It has been shown that in numerous insect species, the Hox genes *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*) jointly regulate the identities of abdominal segments, suggesting that these genes may restrict PGC formation to specific abdominal segments. To explore this possibility, we use RNA interference (RNAi) to study the role of *Gryllus Antp*, *Ubx* and *abd-A* in the context of PGC induction. Our RNAi results suggest that *Gb-Ubx* does not play a role in determining the segmental position of the PGCs. Our results also suggest that *Gb-Antp* and *Gb-Abd-A* act to restrict PGC number in A2-3, and are needed for proper PGC clustering in A3-4. These results provide, to our knowledge, the first data that suggest a role for these ancient genes in regulating the embryonic germ line in an animal.

**Acoel regeneration mechanisms indicate ancient and widespread role for muscle in regenerative patterning**

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Regeneration - the replacement of lost body parts – is widespread in the Metazoa. The formation and patterning of new tissues during regeneration requires positional information. How this positional information is harbored in adult tissues is poorly understood. In the planarian *Schmidtea mediteranea*, positional control genes (PCGs) are hypothesized to convey positional information during regeneration, and are regionally and constitutively expressed in adult planarians. Planarian PCGs are predominately expressed in a single differentiated tissue type: the musculature. PCGs exist in non-planarian regenerative species as well. Acoels are early-diverging bilaterians, cladistically distinct from both deuterostomes and protostomes and separated from all other bilaterians by over 550 million years of evolution. We demonstrate here that PCGs expressed in the basal acoel *Hofstenia miamia* coexpress with one another in a common differentiated, subepidermal cell type, consistent with a single primary source of adult positional information. Strikingly, analysis by both *in situ* hybridization and single-cell qRT-PCR demonstrates that all known *Hofstenia* PCGs are specifically expressed in muscle cells during both homeostatic tissue maintenance and regeneration. The vast majority of *Hofstenia* muscle cells express one or more PCGs, suggesting expression of positional information is a major feature of adult *Hofstenia* muscle. PCG expression changes dynamically in pre-existing muscle cells after injury, consistent with the known roles for many of these genes in guiding regeneration outcomes. This data demonstrating an instructive positional role for muscle in *Hofstenia* suggests that true muscle originated at the base of the bilateria as not only a contractile tissue, but also as the source of positional information guiding adult regeneration in the Bilateria.

**Chromosome inheritance in gamete and development**

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Errors in chromosome partitioning in meiosis result in aneuploid gametes that form embryos that are unviable or developmentally abnormal. In meiosis, each maternal homologous chromosome becomes connected to its paternal counterpart by crossover (CO) recombination. These connections are key to enable the ordered partitioning of the genome during the two meiotic divisions. Studies have focused on understanding the meiotic steps leading to formation of COs. However it is understudied how perturbations in different steps of meiosis that abrogate CO formation translate into specific chromosome partitioning defects. Observations of the product of meiotic mutant divisions by Severson et al. suggest that mutants lacking COs segregate homologous chromosomes in the first division in one of two different patterns. Our live imaging analysis of these meiotic mutants revealed the pattern of chromosomes segregation in the two meiotic divisions.

Meiotic mutants that lack the meiosis cohesin component REC-8 segregate sister chromatids away from each other in the first division, as they would in the second division in wild type meiosis. These mutants fail to partition chromatids in the second division resulting in the formation of diploid gametes. These findings are the basis of a scheme designed to derive viable and stable tetraploids from any C. elegans strain in order to query the roles of genome size on cell division, development, and evolution.

Meiotic mutants with REC-8 include him-3, syp-1, syp-2 and spo-11 that are defective at different steps in CO formation. These mutants keep sister chromatids together as the wild type yet fail to partition in the first meiotic division. Their centrosomes, however, continue to progress through the cell cycle giving rise to spermatocytes with transient tetrapolar spindles. The chromatids eventually segregate to each of the four spindle poles yielding aneuploid sperm. Interestingly, analysis of altered karyotype and special meiotic mutant spermatocytes suggests that a single bi-oriented homologous chromosome pair is sufficient to suppress the formation of the transient tetrapolar spindles. We will report on the mechanism by which a single homologous chromosome pair might prevent formation of tetrapolar spindles.

Together these studies will lead to a better understanding of fundamental mechanisms promoting accurate chromosome inheritance in normal and pathological meiosis.

**RNA secondary structure regulates translation control of a germ line RNA in Drosophila**

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Translation repression is mediated by regulatory factors including RNA binding proteins (RBPs). These RBPs bind the 3' untranslated regions (3'UTRs) of messenger RNAs (mRNA) to mediate repression or activation. While several mRNAs have binding sites for RBPs, only a few are selected for regulation. Why some mRNAs but not others are chosen for regulation is not known. We hypothesized that secondary structure of 3'UTR plays an instructive role in target selection. *Drosophila melanogaster* female germ line is a powerful model system to address this question, as germline RNAs are highly regulated for maternal deposition to the egg. The maternally deposited *polar granule component* (*pgc*) is an excellent candidate to test our hypothesis, as its mRNA is present but is only translated in two short pulses. Using biophysical and structure probing analysis, we found that *pgc* 3'UTR forms a unique secondary structure. To determine if this secondary structure is important for RBP binding, we utilized two different approaches. First, we used circular permutation to alter the overall secondary structure of the RNA while maintaining sequence identity. *In vivo* analysis of reporter constructs revealed that changes in secondary structure due to circular permutation had profound effects on translation with one permutant causing loss of translation and one causing unrestricted translation. We have identified a gain of and loss of secondary structural elements in the RNA that cause these changes. Second, we focused on the role of the individual structural elements in *pgc* 3'UTR. We found that deletion of one conserved stem loop has causes loss of translation control during embryogenesis. We are currently identifying the protein that binds this stem loop to mediate this affect. Together our results suggest that secondary structure of 3'UTRs is pivotal for the control of gene expression.

**The recycling GTPase, RAB-10, regulates autophagy flux in *Caenorhabditis elegans***

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Autophagy and endocytosis are two quality control mechanisms employed by the cell to maintain homeostasis. Autophagy is an evolutionarily conserved process that involves the formation of a double-membrane vesicle called the autophagosome, which engulfs unneeded cellular cargo. Endocytosis involves the uptake and sorting of cargo throughout the cell, important for cell signaling and cell growth. Although both endocytosis and autophagy show crosstalk, the extent to which endocytic proteins and/or compartments contribute to autophagy is still unknown. To further elucidate the crosstalk between autophagy and endocytosis, we conducted an RNAi screen for endocytic genes that altered the autophagosome reporter, GFP::LGG-1, in *daf-2/IIR* mutants. *daf-2* encodes the insulin-like receptor (IIR) and *daf-2/IIR* loss of function mutants have elevated levels of GFP::LGG-1 foci in epidermal seam cells. RNAi-mediated knockdown of autophagy genes in *daf-2/IIR* mutants results in the formation of enlarged GFP::LGG-1 foci, indicative of defective autophagy. We reasoned that the depletion of endocytic genes involved in autophagy would also result in the formation of enlarged GFP::LGG-1 foci in seam cells. Thus, we screened for endocytic genes that when depleted by RNAi, altered GFP::LGG-1 expression in *daf-2* mutants. One gene identified was the small GTPase, RAB-10, a regulator of endocytic trafficking from yeast to mammals. *rab-10* alters the localization pattern of the autophagy reporter, GFP::LGG-1 in wild type and *daf-2(e1370)* mutants. We find that *rab-10* activity is required for autophagy flux, the dynamic process of autophagosome formation and degradation. We show that RAB-10 is needed for the localization of GFP::ATG-9 to punctate structures, which may represent the unique vesicle, called the “ATG-9 reservoir”. Lastly, we find that the GTPase cycling ability of RAB-10 is required for its role in autophagy.

**von Willebrand Factor D and EGF-Domains is essential for axolotl limb regeneration**

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Axolotls (*Ambystoma mexicanum)* have the remarkable ability to regenerative lost limbs. Regeneration competent species, such as the axolotl, rely on a pool of heterogeneous progenitor cells, called the blastema, to regenerate lost structures. However, the molecular events that govern progenitor cell formation and function remain mostly unknown. Therefore, we took an unbiased approach to begin to identify genes that may be important for blastema cell function. We used RNA-sequencing (RNA-seq) to define genes that were specific and highly enriched in the blastema. We found a previously unstudied gene, von Willebrand Factor D and EGF-Domains (*VWDE*), to be highly expressed in the blastema and enriched over 18-fold compared to a variety of other tissues sampled. Time course RNA-seq data revealed that *VWDE* expressionpeaks in the early to mid bud blastema. RNA *in situ* hybridization confirms this expression profile. To determine if *VWDE* plays a role in axolotl limb regeneration we injected a morpholino targeting *VWDE* to knockdown VWDE expression. We found that *VWDE*-targetingmorpholino significantly delayed limb regeneration, resulting in smaller blastemas compared to control morpholino injected blastemas. This delay in blastema growth is likely due to a reduction in proliferation as *VWDE*-targeting morpholino resulted in an almost 50% reduction in blastema cell proliferation (38% vs. 20%, $P<0.01$). Remarkably, this one time injection of *VWDE*-targeting morpholino resulted in the aberrant regeneration in 14/28 limbs, compared to 1/28 in controls. These results suggest that VWDE is required for axolotl limb regeneration. In addition, they suggest that this previously unstudied gene may provide an essential function as a growth factor during limb regeneration. Excitingly, this gene has a mammalian ortholog, potentially opening the door to the study of the function of VWDE in mammalian regeneration.

**A divergent neurexin-1 homolog controls muscle regeneration in planarians**

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Regeneration of body parts requires the replacement of multiple cell types. To dissect this complex process, we studied planarian flatworms that are capable of regenerating any tissue after amputation. An RNAi screen for genes involved in regeneration of the pharynx identified a novel gene, Pharynx regeneration defective-1 (PHRED-1) as essential for normal pharynx regeneration. PHRED-1 is a predicted transmembrane protein containing EGF, Laminin G, and WD40 domains, is expressed in muscle, and has predicted homologs restricted to other lophotrochozoan species. Knockdown of PHRED-1 causes abnormal regeneration of muscle fibers in both the pharynx and body wall muscle. In addition to defects in muscle regeneration, knockdown of PHRED-1 or the bHLH transcription factor MyoD also causes defects in muscle and intestinal regeneration. Together, our data demonstrate that muscle plays a key role in restoring the structural integrity of closely associated organs, and in planarians it may form a scaffold that facilitates normal intestinal branching.

**The varied BMP signaling output critical for development requires regulated proprotein processing**

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BMP signaling has many roles in development and its mis-regulation results in profound consequences for the developing organism. Differences in the amount or type of signaling output can be achieved in part by the existence of multiple ligands and a variety of receptor complexes. However, signaling output is also regulated at a number of other points in the pathway, the extracellular space and during signal transduction. Our findings reveal that signaling output is critically influenced by alternative processing of BMP proproteins required for the maturation of ligands and their ability to preferential activate different receptors. In addition to the conventional S1 proconveratse cleavage site that separates the highly conserved C-terminal ligand domain typical of TGF-b/BMP family members, from their prodomains, we identified an additional proconvertase processing site (NS) within the prodomain of the Drosophila melanogaster BMP5/6/7 ortholgue, Gbb. Mutating either the NS or the S1 site reduces signaling activity but surprisingly, mutating NS has more profound effects on development and viability. Mutations at the analogous site in BMP4, BMP15 and AMH/MIS are associated with cleft lip/palate (CL/P), ureteropelvic junction obstruction (UPJO), premature oovarian failure (POF), and persistent Müllerian duct syndrome (PMDS). Indeed, we find that mutating NS in hBMP4 reduces signaling activity in cell culture and sensitizes hBMP4 to environmental insults. Introduction of the same mutation into the mouse BMP4 locus by CRISPR resulted in compromised embryonic development and craniofacial abnormalities. In Drosophila, we find that the NS site in Gbb is critical for preventing ligand latency, as well as for generating a larger BMP ligand with a different activity and a different receptor signaling preference. The NS cleavage site is required for pupal ecdysis and viability. In the absence of NS processing the expression of a known neurosecretory hormone CCAP which is important in the progression of Drosophila through metamorphosis, is disrupted. The BMP type II receptor Wit is specifically required for this process, and NS-cleaved Gbb appears to be necessary for its activation. Together, these data highlight a mechanism that provides exquisite regulation of BMP signaling.

**When fish fly: using mutational phenocopy and phylogenetics to understand allometry in evolution**

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The material basis of evolutionary change has been a long lasting, and contentious, focus of genetics. We argue that that one can leverage the constraint in development to help define developmental mechanisms associated with character change in evolution. We outline an efficient method to isolate and interrogate sequence variation from large clades of species using cross-hybridization of DNA to oligos designed from one or more known reference sequences targeting conserved coding and/or non-coding regions of interest. We have found that the evolutionary distance between organisms used for reference and species under analysis can be up to 70 million years distant and still permit efficient data recovery. This 'Phylomapping' strategy can capture species-specific variation across large taxonomic diversity. We have applied this analysis to study variation in proportion in Beloniformes fishes, a clade encompassing intriguing shifts in skeletal allometry, such as the large paired-fins of flying fish and the rostrum outgrowths of needlefish and halfbeaks. We used the Phylomapping approach to isolate over 70-80% of coding sequence from a phylogeny-wide distribution of 43 species of Beloniformes. Through analysis phylogenetic patterns of nucleotide variation within the clade, we identify genes that are associated with the evolution of overgrowth phenotypes in this group. Importantly, we show that function-altering changes to genes that have an intriguing phylogenetic signature within the dataset can be sufficient to reproduce evolved Beloniformes phenotypes in the zebrafish. Specifically, combination of two mutant loci in the zebrafish is sufficient to phenocopy the pattern of fin overgrowths in flying fishes. Thus, constraint in development can be used to guide clade-wide comparative genetic analysis to distill mechanisms underlying natural variation.

**Tissue interactions and differing threshold requirements for Fgf8 contribute to variation in disease penetrance**

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Generation of variation is an inherent property of development with important implications for disease and evolutionary processes. However, mechanisms generating variation in development are poorly understood. Fgf8 is a secreted signaling factor that contributes to the growth and development of many tissues, including the limbs and jaw. Using an allelic series of *Fgf8* mutant mice to generate embryos of differing *Fgf8* dosages (100%-15% WT expression), we investigated the effects of reductions in *Fgf8* on jaw and limb morphogenesis. Fgf8 levels have no obvious effect on phenotype until they drop below 40% of WT, when jaw and facial defects are observed. Notably, jaw defects exhibit directional asymmetry, with the left side more severely affected. In contrast, no apparent asymmetry or morphological defects were observed in the limbs. To investigate mechanisms underlying differential susceptibility to *Fgf8* reduction, we compared Fgf expression by qPCR in E10.5 mandibles and limbs. We found that Fgf expression in limbs is much higher overall than in the jaw. Further, Fgf4 is upregulated in limbs as Fgf8 is reduced, providing compensation as previously reported. Thus, the limbs are more buffered against Fgf8 perturbation than is the jaw. To investigate mechanisms underlying directional asymmetry, we evaluated cell death and jaw patterning. *Fgf8* mutants exhibit increased cell death in jaw progenitors at E9.5 relative to WT, however, there is no difference in L-R asymmetry. We do, however, observe asymmetry in jaw patterning (e.g, *Dlx5* expression) in mutant embryos at E10.5. We also find asymmetry in first pharyngeal pouch (PP1) morphology. We hypothesize that interactions between the heart field (a bilaterally asymmetric source of *Fgf8*) and prospective pharyngeal endoderm contribute to asymmetry in PP1 development, generating asymmetry in jaw morphogenesis. Our data also suggest that different tissues have different threshold requirements for activation of Fgf signaling.

**Combinatorial signaling interactions pattern the dorsal-ventral mesodermal axis by controlling bHLH transcription factor activity**

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During gastrulation, the mesodermal germ layer is patterned into dorsal-ventral subtypes through combinatorial interactions of the BMP and FGF signaling pathways. How these signals are integrated to induce specific dorsal-ventral cell fates is not well understood. We used post-gastrulation neuromesodermal progenitors (NMPs), which undergo a binary dorsal-ventral patterning decision, as a simplified model to understand how these signals impart cell fate. Using zebrafish and mouse NMPs, we identify an evolutionarily conserved mechanism of BMP and FGF mediated dorsal-ventral mesodermal fate patterning that occurs through modulation of bHLH transcription factor activity. We extend our analysis to the gastrula stages of zebrafish development to show that bHLH activity is responsible for the dorsal-ventral patterning within the entire mesodermal germ layer. Unexpectedly, this mechanism only affects dorsal-ventral mesodermal patterning and has no effect on anterior-posterior pattern, providing evidence for the molecular uncoupling of dorsal-ventral and anterior-posterior patterning downstream of FGF and BMP signaling.

**Positioning of nuclei at the neuromuscular and myotendinous junctions in the developing muscle**

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Skeletal muscles are multinucleated cells that are generated by the fusion of myoblast precursors. A crucial feature of muscle development is that myonuclei are actively moved in the growing myotube, a mechanism that involves microtubules and motors, to eventually position nuclei at the periphery of the muscle. Here, two subsets of nuclei can be identified because of their proximity to defined structures: the post-synaptic myonuclei (**PSM)**, close to the neuromuscular junction, and the myotendinous junction myonuclei (**MJM**), at the junction between muscle and tendon cells. However, how these subsets of nuclei are positioned at these specialized regions is not understood. To elucidate the mechanism by which PSM and MJM are uniquely positioned, we have examined *Drosophila* larval muscles that have been depleted of several cytoskeletal proteins. Specifically, we specifically depleted the muscle of kinesin heavy chain (KHC), dynein heavy chain (DHC), ensconsin, pins, and proteins of the dynactin complex. We found that PSM are precisely positioned by the action of dynein and pins at the cell cortex. Distances between PSM neighbors are regulated by the opposite action of dynein and kinesin. Moreover, we noticed that depletion of such cytoskeletal proteins specifically in the muscle affects the morphology of the neuromuscular junction in distinct ways. We then analyzed the position of MJM. We found that the MJM at the posterior end of the muscle are closer to the myotendineous junction than MJM at the anterior end, and this distance is regulated by dynein. Together, our work reveals that in the developing muscle nuclei are not all equally moved, and that muscles show distinct regionalized organization. While the first steps of myonuclei movements are known, we defined that the position of nuclei at specialized junctions is finely regulated, an important and still unexplored step of muscle development.

**Vg1-Nodal heterodimers are the endogenous inducers of mesendoderm**

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Nodal is considered the key inducer of mesendoderm in vertebrate embryos and embryonic stem cells. Other TGF-beta signals, such as Vg1, have also been implicated in this process but their roles have been unclear or controversial. Here we report that zebrafish embryos without maternally provided *vg1* fail to form endoderm and head and trunk mesoderm, and closely resemble *nodal* loss-of-function mutants. Although Nodal is processed and secreted without Vg1, it requires Vg1 for its endogenous activity. Conversely, Vg1 is unprocessed and resides in the endoplasmic reticulum without Nodal, and is only secreted, processed and active in the presence of Nodal. Co-expression of Nodal and Vg1 results in heterodimer formation and mesendoderm induction. Thus, mesendoderm induction relies on the combination of two TGF-beta signals: maternal and ubiquitous Vg1, and zygotic and localized Nodal. Modeling reveals that the pool of maternal Vg1 enables rapid signaling at low concentrations of Nodal.

**Petal Development: a twist in fate**

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Helical growth in some plant species causes spiral patterns, such as those of pea tendrils. This helical patterning often has a particular handedness. A considerable amount of experimental work in Arabidopsis has shown that alterations in the organization of cortical microtubules, or in microtubule associated proteins, can lead to handed helical growth. We have identified mutations in *RHAMNOSE BIOSYNTHESIS 1 (RHM1)* that cause dramatic left-handed helical growth of Arabidopsis petal epidermal cells, leading to twisted petals. *RHM1* encodes a UDP-L-rhamnose synthase; rhamnose is a major component of pectin, a major structural component of the cell wall. The *rhm1* mutants display decreases in the levels of the pectic polysaccharide rhamnogalacturonan-I. *rhm1* mutant roots also display left-handed helical growth and, unlike other mutants with a similar phenotype, *rhm1* does not alter the orientation of microtubule arrays. Our findings reveal a novel source of left-handed growth in plants caused by changes in cell wall composition that is independent of microtubule orientation; we propose that an important function of rhamnogalacturonan-I is to suppress helical twisting of expanding plant cells. We suggest that many instances of helical growth in nature may be due to naturally occurring alterations in the levels or organization of cell wall components.

**Pathways Regulating Stem Cell Induction, Self-Renewal and Engraftment**

Leonard I. Zon

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Hematopoietic stem cell transplantation involves the homing of stem cells to the marrow, an active process of engraftment, and the self-renewal of the blood stem cells. We have been using the zebrafish as a model to study the molecular biology of this process. Blood stem cells are born in the dorsal aorta of the developing embryo. By imaging RUNX1 GFP+ cells arriving in the next site of hematopoiesis (the caudal hematopoietic territory), engraftment can be visualized. This process involves an attachment phase and then an extravasation to the abluminal side of the endothelial cells. The endothelial cells cuddle the hematopoietic stem cell and the stem cells have the ability to be maintained in a quiescent fashion or to divide symmetrically or asymmetrically. Using chemical screens, we have found small molecules that can enhance engraftment or suppress engraftment. We have also been studying the role of macrophages in the mobilization of stem cells in the aorta region. The macrophage attaches physically to stem cells, and breaks down extracellular matrix to allow the release. Our studies have uncovered stages of stem cell engraftment that are altered by chemicals, which could have therapeutic value in marrow transplantation procedures.

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**Poster abstracts**

**1. Slit-Roundabout signaling functions to guide the formation of a heterogeneous glial bridge that supports commissure formation in the zebrafish forebrain**

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Connections between the two hemispheres of the central nervous system are enabled by commissures, which form when pathfinding growth cones cross the embryonic midline. Little is known about the cellular behaviors exhibited between commissural axons and the cells of the midline growth substrate in the vertebrate forebrain nor the guidance mechanisms regulating these neuron-glial interactions. We take advantage of the simple system of the zebrafish forebrain to simultaneously visualize midline crossing commissural axons and their astroglial growth substrate. Previously, we showed that cells expressing the astroglial marker Glial fibrillary acidic protein (Gfap) establish a bridge-like structure across the diencephalic and telencephalic midlines prior to and throughout commissure formation. Using *gfap* labeling transgenic lines paired with cell transplantation procedures, we show *gfap*+ astroglial cells display radial glial, mesenchymal, and multi-branching cell morphologies. Live cell imaging suggests these astroglial morphologies may exhibit different behaviors during commissure formation. To further characterize the astroglia of this glial bridge, we show that the Zebrafish radial fiber antibodies (Zrf1-4) demarcate distinct domains within the “diencephalic glial bridge”. We introduce new programing to quantitatively analyze the differential expression of these four Zrfs in all 3-dimensions with statistical significance. Lastly, using loss and gain of function approaches we test how manipulation of the Slit signaling ligands influence the formation of the heterogenous populations of astroglia in the forebrain. We show that Slit1a acts distinctly from Slit2/3 in patterning the positions of these different Zrf markers in the glial bridge. We conclude that the diencephalic glial bridge is a subdivided population of astroglial cells that show similar but distinct positioning in this bridge as well as differential responses to Slit signaling.

**2. Development of an Eftud2 knock-out mouse model to uncover the etiology of mandibulofacial dysostosis with microcephaly (MFDM).**

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Splicing and alternative splicing affects approximately 95% of the mammalian genes and is a crucial process during development. *Eftud2* (elongation factor tu GTP binding domain containing 2) is a core component of the spliceosome and haploinsufficiency of *EFTUD2* is associated with MFDM. MFDM is characterized by developmental delay, microcephaly, micrognathia, malar hypoplasia and small or dysplastic pinnae. We hypothesized that splicing of a subset of developmentally important genes are disturbed by reduced levels of a general splicing factor such as *EFTUD2*. To evaluate this, we developed an *Eftud2* knock-out mouse model using the CRISPR/Cas9 system to study the requirement of this splicing factor on the transcriptome and developmental processes associated with craniofacial development. Guide RNAs were designed flanking exon 2 of *Eftud2* and were micro-injected with Cas9 mRNA in zygotes. Among six borned mice, one male had a deletion of exon 2 of the *Eftud2* gene by PCR screening. Sanger sequencing of genomic DNA from offspring of this mutant mouse confirmed deletion of exon2 of *Eftud2*. *Eftud2* heterozygous animals were viable and fertile. However, a 32% reduction of *Eftud2* mRNA expression was observed by RT-qPCR in heterozygous embryos compared to wild-type (P=0.001, t-test). *Eftud2* heterozygous males were mated to their heterozygous littermates for embryo collection. To date, of 250 embryos collected from e8.5 to e16.5, expected numbers of wild type (70) and heterozygous (122) embryos were found, while 5 homozygous mutant embryos and 53 resorbed embryos were found ($\chi$ =54.1, $p<0.001$), suggesting that most homozygous mutant embryos arrest post-implantation. Conditional knock-out mouse for *Eftud2* is being generated and transcriptome of heterozygous embryos will be analyzed. Our observations so far indicate that reduced mRNA levels of *Eftud2* does not result in craniofacial abnormalities in mice and that *Eftud2* homozygous mutant mice are not viable.

**3. RNA Helicases Involved in Ribosome Biogenesis Promote Cell Cycle Progression and GSC Differentiation**

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Germline stem cells (GSCs) are able to both self renew as well as differentiate into gametes. Upon fertilization, gametes give rise to a zygote, which creates a complete adult organism, including germ line that launches the subsequent generation. The germ line relies heavily on RNA regulators such as small RNAs, RNA binding proteins (RBPs) and RNA helicases to maintain this cycle. The role of RBPs in the germ line have been well characterized, yet the variety of roles of RNA regulators in translation control are not fully known. To asses the roles of RNA helicases in the germ line, we conducted a forward genetic screen, targeting RNA helicase genes for depletion using RNA interference (RNAi). Our data reveals crucial, non-redundant roles of 21 RNA helicases in maintaining the germ line. Specifically, three of these helicases are known through homology to be involved in biogenesis of the small 40S ribosomal subunit. We find that these conserved helicases are required for germline development, maintenance and surprisingly, proper differentiation of GSCs. RNAi depletion of these helicases disrupts the GSC differentiation and halts the cell cycle at the G1/S checkpoint, leading to an accumulation of undifferentiated cells and a characteristic GSC abscission defect. Through the implications in ribosome biogenesis, these RNA helicases promote progression of the cell cycle, ensuring the completion of cytokinesis after cell division in the germ line. We plan to fully describe the downstream targets of these helicases, rescue this phenotype by modulating cell cycle proteins and show the distinct role of these helicases in ribosome processing.

**4. The Role of Cypin in Xenopus Embryonic Development**

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Cypin, a cytosolic protein that has guanine deaminase activity, has been shown to be an important factor in proper neural development by modulating the activity of postsynaptic density protein 95 (PSD-95) and regulating dendrite branching. Cypin has been shown to promote microtubule (MT) assembly *in vitro* by binding directly to tubulin heterodimers. However, the effect of cypin on microtubule dynamics has never been assessed *in vivo*. Moreover, the *Xenopus laevis* homolog of cypin has not been well characterized. We sought to further elucidate the effect of cypin on microtubules in living cells and assess the expression of cypin during *Xenopus* development. We found that cypin does not localize to the microtubule lattice or plus end, unlike well-characterized plus end tracking proteins (+TIPs) or other microtubule associated proteins (MAPs). Furthermore, overexpression of human, but not *Xenopus* cypin, had a significant effect on microtubule dynamics in vivo as measured by the velocity, lifetime, and length of growing MT plus ends. Human cypin overexpression resulted in decreased microtubule growth velocity and length, indicating decreased MT polymerization. These data suggest that the critical tubulin-binding CRMP homology domain in cypin may not be functionally conserved between human and *Xenopus*. Finally, we examined cypin expression in *Xenopus* using whole-mount in situ hybridization and found cypin to be expressed in the otic vesicle, a progenitor of the inner ear. Taken together, these results suggest a more complex, upstream function for cypin in regulating MT dynamics and a new role for cypin in the development of the *Xenopus* inner ear.

**5. Here be monsters: patterns of phylogenetic and ontogenetic diversity in skull shapes of phyllostomid bats**

Jasmin Camacho
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The New World leaf-nosed bats (phyllostomids), the most ecologically diverse clade of mammals, display exemplary morphological adaptations associated with specialized modes of feeding. Phyllostomid bat skulls underwent significant alteration, most notably in the cranial base, cranial vault, and the length of the face. These changes, among others, occurred over a very short evolutionary interval, which brings into focus underlying developmental mechanisms behind those changes. Despite the wealth of studies into bat evolution virtually nothing is understood about the developmental events responsible for the emergence of cranial diversity. In this study, we describe, quantify and compare morphological events in the evolution and development of phyllostomid bats and examine signaling molecules required for cartilage and bone development. We found that the skulls evolved along five distinct orientations and these evolutionary changes appeared progressively later during development. Significant gene expression changes during skeletal growth between key bat species will provide the foundation for testing hypothesis about the link between ontogeny and phylogeny. Specifically, gene expression may be experimentally replicated in the mouse embryo and subsequent phenotype examined to evaluate if evolutionary patterns are mimicked. This will reveal causative connection between morphological and molecular changes.

**6. The TACC protein family and their unique regulation of the dynamic microtubule plus-end**

Garrett Cammarata

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The TACC (Transforming Acidic Coiled-Coil) family of proteins plays an important role in the regulation of the plus-end of microtubules. It is at this plus-end that the characteristic dynamic nature of microtubules allows for constant tubulin heterodimer addition and removal, thereby affecting the overall extent of microtubule growth. In addition to affecting the dynamicity of microtubules, the TACC family of proteins is known to interact discretely with other +TIPs (plus end tracking proteins), such as the microtubule polymerase, XMAP215. Although such protein interactions have been described before, the knowledge of the TACC family's complete mechanism affecting the plus-end is not known. In addition, how phosphorylation of TACC proteins can play a role in a non-mitotic environment has not been fully defined. Recent in-vitro evidence as well as live cell data from our lab supports the idea that one such member, TACC3, may possess a role in directly affecting the polymerization of microtubules. As members of this family each possess unique characteristics affecting plus-end dynamics, we have employed high resolution time lapse imaging, in-vitro reconstitution assays, and biochemical techniques to look further into how these TACC proteins differentially regulate the overall dynamic nature of the microtubule.

**7. Knockdown of WHSC1 perturbs normal craniofacial development in *Xenopus laevis***

Rachael Cella

*Boston College, USA*

Cranial neural crest cells are multipotent stem cells that form above the embryonic neural tube during neurulation, and subsequently undergo an epithelial to mesenchymal transition (EMT) in order to migrate into the organism's peripheral region to form various craniofacial structures. When the migration of these cells goes awry during embryonic development, intellectual, craniofacial, and other abnormalities may arise. Wolf-Hirschhorn Syndrome, a neurodevelopmental disorder caused by mutations on the short arm of chromosome four, exhibits the aforementioned phenotypes through distinct features such as wide nasal bridges, high foreheads, and other deformities. The goal of my current research is to study one of the genes commonly mutated in this disorder, WHSC1 (Wolf-Hirschhorn Syndrome Candidate 1), in the model organism *Xenopus laevis* (frog). This gene is 90 kilobases long and located within the WHS critical genomic region, an 165 kilobase stretch, that is typically subject to deletions, and sometimes even microduplications. It can be found on chromosome one in *Xenopus*. Recently, I have used morphometry to obtain a quantitative analysis of a tadpole's craniofacial region during stage 42-43 of embryonic development after the WHSC1 protein levels undergo knockdown. Thus far, the data collected suggests that WHSC1 knockdown increases facial width and midface area, but further analysis is still needed. In the future, neural crest cell motility will be analyzed using migration assays to help elucidate which cell biological mechanisms are affected when WHSC1 is knocked down.

**8. The adaptor protein Src Homology 2 Domain Containing Protein D (SHD) reversibly binds CrkL and is expressed in the developing zebrafish central nervous system**

Brendan Chandler, Ashley Waldron, Jaye Weinert, James Vincent, Alicia Ebert, Bryan Ballif
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One major mechanism of signaling in eukaryotes is through phosphorylation, the reversible addition of phosphate groups to target proteins on serine, threonine, or tyrosine residues. One result of tyrosine phosphorylation is the creation of docking sites recognized by proteins containing Src Homology 2 (SH2) domains, a modular protein domain that binds to specific motifs containing phosphotyrosine. The adaptor protein Crk and its close relative CrkL contain SH2 domains, and have been shown to play critical roles in several signaling pathways. During development of the vertebrate central nervous system, Crk/CrkL are responsible for propagating the Reelin signal from tyrosine phosphorylated Disabled-1 to downstream molecules controlling migration of neural progenitors. In focal adhesions, CAS is phosphorylated by Src family kinases in response to various cues, allowing the coupling of Crk/CrkL to CAS which leads to activation of GTPases and cytoskeletal rearrangement and alternations in cell motility. Given the diverse roles of Crk/CrkL, it is likely that there are phosphotyrosine-dependent Crk/CrkL binding partners which have not yet been identified. We identified several candidates using an *in-silico* screen for proteins enriched in the preferred Crk/CrkL SH2 domain binding sequence: YXXP. One of these proteins was SHD, a 340 amino acid adaptor protein containing 5 YXXP sites in humans, as well as a proline rich region and an SH2 domain. We found that indeed SHD showed a phosphorylation-dependent interaction with the SH2 domain of CrkL. It has been reported that *Shd* is expressed at high levels in the brain of mammals. Toward developing a model to study the role of SHD *in vivo* we characterized its expression in zebrafish by *in situ* hybridization. Indeed we found *Shd* highly expressed in the developing zebrafish nervous system. Together, these early findings suggest a possible role for SHD as a reversible interacting partner of Crk/CrkL in nervous system development.

**9. Wnt: The Spinal Frontier**

Sophie Chase, Julia Kim, Carla Valez, Daniel Wood
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During embryonic development, the central nervous system is built through the proper regulation of neural stem cell proliferation and differentiation. In vertebrate organisms, radial glial cells serve as the resident neural stem cell. Although much is still to be understood about neurogenesis, evidence shows that paracrine factors of the Hedgehog, Tgf-beta, and Wnt families play a major role in the proliferation and differentiation of radial glial cells across all axes within the spinal cord. Here, we are investigating the role of the non-canonical Wnt5b signaling protein in radial glial development. We hypothesize that Wnt5b cross talks with the canonical Wnt/β-catenin pathway and specifically functions to repress β-catenin signaling. We show that loss (mutants) and gain (heatshock induction) of wnt5b function results in the increase and decrease of radial glial cell numbers. The data suggest that Wnt5b- mediated attenuation of Wnt/β-catenin signaling serves to reduce the amount of radial glial proliferation during spinal cord development. Furthermore, we are employing mathematical modeling to guide our predictions of Wnt5b as a secreted morphogen that patterns neural stem cell proliferation and differentiation. We intend to use our results and this Wnt5b model to better understand the role of neural stem cell regulation in spinal cord development and disease.

**10. Identification of the role of Dnaaf2 in mouse embryonic development**

Agnes Cheong
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The Knockout Mouse Project (KOMP) is a globally funded project to generate a public resource of mouse embryonic stem cells with a null mutation in every gene within the mouse genome. Additional NIH projects are generating mutant mice from these ES cells to elucidate gene function and understand human diseases. These efforts are largely targeted at adult biomedically related phenotypes such as heart disease, diabetes and behavioral disorders. However, a large fraction of genes are essential resulting in embryonic lethality of homozygous knock-out mutants. Our lab is characterizing a large number of these early lethal phenotypes towards functional annotation of the genome. One of these early lethal alleles is a mutation in the gene, Dynein, Axonemal, Assembly Factor 2 (Dnaaf2), that encodes a highly conserved protein thought to be essential for the preassembly of dynein arms. Dnaaf2 homozygous embryos can be recovered at E9.5 and have cardiac and neural defects. The goal of this project is to understand the role of Dnaaf2 during mouse embryonic development.

**11. Intracellular modulations of cytokine signaling leading to successful central nervous system axon regeneration in *Xenopus laevis*.**

Rupa Choudhary, Ben Szaro
*University at Albany, USA*

Central nervous system (CNS) axons lose their capacity to regenerate following traumatic injury in adult amniotes, but they retain this capacity throughout life in anamniotes. In all vertebrates, CNS injury initially triggers cytokine signaling pathways, but how this inflammation response activates the repair mechanisms that lead to successful CNS axon regeneration in anamniotes is unknown. In vertebrates from fish to mammal, optic nerve injury triggers increased expression of *Suppressor of Cytokine Signaling 3* (*SOCS3*) mRNA. SOCS3 is an important modulator of cytokine signaling that inhibits CNS axon regeneration, yet, anamniotes can overcome this inhibition to regenerate optic axons successfully. To address this issue, we examined the *SOCS3* response to optic nerve injury in *X. laevis. In situ* hybridization and RT-qPCR confirmed that *SOCS3* mRNA expression increased in retina ganglion cells with injury, but immunostaining indicated that SOCS3 protein expression decreased. Polysome profiling showed that translation of *SOCS3* mRNA was at least as efficient in the injured eye as in the uninjured eye, arguing that the decrease seen in SOCS3 protein was due to increased protein degradation rather than decreased mRNA translation. In tumor cells, another member of the SOCS gene family, SOCS2 is known to mediate SOCS3 degradation by targeting it to the Elongin B/C complex for ubiquitination and proteasomal degradation, and we observed that SOCS2 protein expression increased in *Xenopus* retinal ganglion cells with injury. These results suggest that a similar mechanism could be acting in *Xenopus* retina to alleviate SOCS3-inhibition of successful optic axon regeneration. *Supported by NSF (IOS 1257449) and a predoctoral fellowship to R.C. (NY SCIRB DOH01-C30861GG).*

**12. An Emerging Role for DBL-1/BMP in Lipid Metabolism in C. elegans**

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Transforming Growth Factor-beta (TGF-β) Superfamily is a large family of peptides that control cell functions such as differentiation, proliferation, and regulation of the immune system. Misregulation of TGF-β family members is implicated in many diseases. Recently, roles in lipid metabolism have been emerging for TGF-βs, BMPs, and GDFs. In *C. elegans*, DBL-1/BMP is a major regulator of body size, cell patterning, and innate immunity. However, a role in lipid metabolism has not been shown. To analyze the function of DBL-1 in lipid metabolism, we observed overall lipid stores, biochemical analysis, lipid droplet dynamics. Using Oil Red O, a neutral lipid dye, we observe an overall decrease in the levels of triglycerides in DBL-1 pathway mutants, *dbl-1(lf)*, *sma-3(lf)*, and *dbl-1++(oe)*. Genetic epistasis analysis places the DBL-1 pathway upstream of DAF-2/IIR, a well-known regulator of lipid metabolism. The Oil Red O results were confirmed via preliminary gas chromatography-mass spectrometry data. Using a GFP tagged lipid droplet associated protein, DHS-3, we analyzed the lipid droplet dynamics within *dbl-1* and *dbl-1++* mutants. We show that lipid droplet size positively correlates with the level of DBL-1 signaling in the worm. A decrease in lipid droplet size is also seen in electron micrographs of *sma-3* and *sma-9* mutants, both components of the DBL-1 pathway. However, any change in DBL-1 signaling decreases the overall number of lipid droplets in the worm. Finally, we show that proper lipid levels are dependent on expression of DBL-1 in the epidermis, as epidermal expression of DBL-1 is necessary and sufficient to rescue the low-fat phenotype of *sma-3* mutants. Together these data describe a definite role in lipid regulation for the DBL-1/BMP pathway in *C. elegans*. These findings implicate that DBL-1/BMP signaling plays a role in maintenance of proper metabolic homeostasis and development.

**13. Developing a CRISPR/Cas9-mediated gene knockout protocol for *Xenopus laevis***

Quinn Coughlin
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The rise of automated DNA sequencing and bioinformatics has provided biologists with a growing bank of information that correlates clinically observed phenotypes with specific DNA sequences. Although informative, these relationships must be considered non-causal until confirmed empirically. The ability to synthetically alter gene expression in vivo is essential to such reverse genetics research, although our tools used to do so are often imperfect. One example are morpholinos (MOs), synthetic nucleotide oligomers used to knock down/out gene expression in vivo by blocking translation start sites or pre-mRNA splice sites. Despite their widespread use, unidentified off-target effects and difficult oligomer design have historically plagued MO-based experiments. CRISPR/Cas9-mediated mutagenesis, however, is a new and promising tool used to alter gene expression with higher specificity than MOs. The Lowery Lab is currently developing an X. laevis CRISPR/Cas9 gene knockout protocol to replace MOs in its reverse genetics experiments. The technique is based on a similar protocol developed in X. tropicalis that knocks out gene expression by inducing frameshift mutations via the non-homologous end joining (NHEJ) DNA repair pathway. Here we describe concepts underlying the CRISPR/Cas9-mediated mutagenesis technique, pros and cons in comparison to other gene knock down/out methods, and data supporting the protocol's efficacy collected thus far.

**14. The recovery of vertebrate vision examined in microplates**

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Regenerative medicine is a rapidly changing field of medicine with potentially ground-breaking treatments of blindness and low vision. However, as new methodologies are developed, a critical question will need to be addressed: how do we monitor *in vivo* for functional success? In the present study, we developed novel behavioral assays to examine the vertebrate visual system. In the assays, zebrafish larvae are imaged in multiwell or multilane plates while various red, green, blue, yellow or cyan objects are presented to the larvae on a computer screen. The assays were used to examine a loss of vision at 4 or 5 days post-fertilization and a gradual recovery of vision in subsequent days. The developed assays are the first to measure the loss and recovery of vertebrate vision in microplates and provide an efficient platform to evaluate novel treatments of visual impairment.

**15. Shh is a Key Pathway for Maintenance of Adult Mouse Intervertebral Disc and its Regeneration**

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Disc degeneration and associated back pain affects almost 1/7 individual. The current treatments are palliative, mainly due to poor understanding of the molecular mechanisms of disc growth and differentiation, and of the changes associated with of its degeneration. Our approach has been to identify signaling pathways involved in the normal postnatal growth and differentiation of the disc and to see if the abrogation of these signals causes premature disc degeneration, using the mouse as a model. Our previous studies have shown that Shh is synthesized postnatally by the nucleus pulposus (NP) cells, derived from the embryonic notochord, and is a key regulator of postnatal disc growth and differentiation. Shh expression decreases with age. Here we test the hypothesis that Shh signaling continues to be essential for maintenance of the IVD during adult life, by generating a conditional mouse model using an NP Cre driver line to conditionally target Shh in almost a year old mouse disc following tamoxifen treatment. There were dramatic histological and molecular changes in the lower lumbar discs of Shh targeted mice compared to littermate controls three months onwards, including the appearance of chondrocyte-like cells in the disc space. These discs had an appearance similar to those of 2-year-old mice, and to human degenerated discs. In contrast, upper lumbar discs examined seemed unaffected. The reasons for these different effects are not yet clear. In another set of experiment, we conditionally activated the hedgehog pathway in the NP cells by using the SmoM2 allele and observed dramatic changes in the structure and molecular markers in the sacral discs at 12 -14 weeks of age, which were otherwise were collapsed to form the sacrum in the littermate controls. The results suggest that Shh signaling is critical for maintenance of the lower lumbar and sacral mouse discs, and our mouse models can provide insights into etiology and progression of disc degeneration.

**16. Functions of CLIC Proteins in Heat Stress in *C. elegans***

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Chloride intracellular channel proteins (CLIC) are multifunctional proteins. Cellular stress molecules induce endogenous CLIC4 to translocate from the cytoplasm into the nucleus. The physiological functions of CLIC in whole animals level are not well understood, in particular how the genes regulate thermotolerance is largely unknown. To address these, we took advantage of viable CLIC mutants in *C. elegans* and characterized its functions in heat stress. There are two CLIC homologs in *C. elegans*: EXL-1 and EXC-4. *exc-4* mutants develop cysts in the excretory canal, while abnormal phenotypes of *exl-1* mutants have not been identified. We analyzed integrated EXL-1::GFP transgenic lines in wild type background and observed strong fluorescence in intestinal cells, which is consistent with reported study. Interestingly, we subjected those animals under heat shock, and EXL-1::GFP indeed is translocated into the nucleus. However, we did not observe any nuclear translocation of EXC-4 upon heat shock. Supporting functional importance of this, *exl-1* loss-of-function mutants are thermo-sensitive, in compare with wild type animals. We also performed sequence alignment of all human and nematode CLICs, the data show that EXL-1, not EXC-4, bears a non-classic Nuclear Localization Signal (NLS). This may explain why *exl-1* translocates into the nucleus upon heat stress, while *exc-4* still remains in the cytoplasm. Our phylogenetic tree showed that CLICs are highly conserved across species and duplication of CLICs occurs independently in vertebrate and invertebrate. Mammalian Schnurri-2 is required for CLIC4 nuclear translocation in response to Transforming Growth Factor – b (TGF-b). Schnurri-2, a transcription cofactor, is homologous to *C. elegans* SMA-9 which functions in the DBL-1/TGF-b pathway. We conducted preliminary study of genetic interactions among *exl-1, sma-9,* and the DBL-1 pathway components. However further investigation is required to make a comprehensive conclusion.

**17. Retinoic Acid Receptor Isoforms Reciprocally Control Proliferation of Cytokeratin 5-Positive Salivary Epithelial Cells**

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Controlled expansion and differentiation of progenitor cell populations is essential for organogenesis and maintenance of homeostasis in adult organs. In the developing submandibular salivary gland, cytokeratin 5-positive ($K5^+$) epithelial cells have been described to be a progenitor population that can give rise to different epithelial cell populations, and yet how the $K5^+$ cell population is controlled is incompletely understood. In other epithelia, retinoic acid regulates the expansion and differentiation of progenitor cells, through binding to retinoic acid receptor isoforms (RARs). Although previous work indicates that retinoic acid signaling is necessary for salivary gland development and impacts the $K5^+$ population, the impact of isoform-specific retinoic acid receptor signaling on the $K5^+$ cells has yet to be elucidated. Through investigation of isoform-specific signaling utilizing specific RARα and RARγ agonists and antagonists in organ explants, we found that RARα negatively regulates $K5^+$ cell proliferation, but activation of RARα does not promote ductal differentiation. Instead, RARα activation stimulated ectopic lumenization, as detected by expression of Prom-1. Interestingly, we found that RARγ positively regulates the $K5^+$ population through positive regulation of cell cycle, promoting the maintenance of the $K5^+$ population and reducing their expression of lumenization marker Prom-1. In epithelial explants lacking mesenchyme and innervation, K5 levels were similarly regulated by RAR signaling, consistent with direct regulation of $K5^+$ epithelial cells through endogenous receptors independent of innervation. We conclude that RARα and RARγ reciprocally contribute to the maintenance of the $K5^+$ progenitor cell population in the developing salivary gland epithelium and their differentiation into a lumenized cell.

**18. Canonical Wnt Signaling Requires Dkk2 activity to specify neural crest cells**

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Neural crest cells are generated through a sequence of events orchestrated by the modulation of several signaling pathways and the activation of a complex network of transcription factors. A large body of evidence indicates that attenuation of BMP signaling in conjunction with activation of canonical Wnt pathway is critical to specify the neural crest. Interfering with any components of the canonical Wnt pathway blocks neural crest formation in the embryo. The Dickkopf family of secreted glycoproteins acts as modulators of Wnt signaling. In vertebrates, Dkk1 prevents neural crest formation in the anterior-most region of the embryo. The role of Dkk2 is not as well established. There is evidence that Dkk2 can either positively or negatively regulate Wnt signaling, depending on cellular context.Here we show that Dkk2 is required to specify the neural crest in Xenopus embryos. Dkk2 is broadly expressed in the embryo, and knockdown of Dkk2 using morpholino antisense oligonucleotides interfering with Dkk2 mRNA translation or splicing, caused a specific loss of Snail2 and Sox10 expression at the neural plate border, associated with an expansion of the neural plate. Conversely, Dkk2 over-expression increased the neural crest progenitors pool, reminiscent of Wnt8 or b-catenin gain-of-function phenotype. We show that this activity of Dkk2 depends directly on LRP6 and b-catenin function. In neuralized animal cap explants Dkk2 is required for the neural crest inducing activity of Wnt8. Importantly, in this assay Dkk2 knockdown did not affect FGF, BMP or TGF-b signaling indicating that Dkk2 is specifically acting in canonical Wnt pathway. Dkk2’s function is not limited to neural crest specification, it is also required for dorsal axis duplication by Wnt8. Altogether, these results reveal an unexpected role for Dkk2 in neural crest specification. We propose that Dkk2 is an essential component of the signaling pathway that mediates canonical Wnt response.

**19. Smooth muscle differentiation during spiral valve formation in the little skate, Leucoraja erinacea**

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Vertebrates require an extensive intestinal surface area in order to absorb sufficient nutrients to sustain growth and development. Most vertebrates contain a long looping, vilified intestine. Villi formation is partially dependent on a constrictive force provided by differentiated circular smooth muscles encasing the proliferating luminal endoderm, causing longitudinal ridges to form. Formation of longitudinal muscles later in development results in further compression such that a luminal zigzag pattern arises, leading to formation of individual villi. In contrast, the little skate, *L. erinacea* has a relatively short intestine that is internally spiraled to compensate for the loss of surface area that accompanies the loss of length. The internal spiral is covered with villi to further increase absorptive surface area. To determine if the internal spiral of the skate intestine forms from similar constrictive forces, we characterized smooth muscle differentiation in the developing spiral intestine. As in the chick, circular muscles differentiate first in the outer submucosa of the intestine. After initiation of spiral folds, helical muscle differentiation occurs within the inward-rotating folds. Helical muscle differentiation proceeds from the lateral edge and moves inward toward the spiral center. In the chick, villus formation is predominantly dependent on constrictive smooth muscle causing tissue buckling. As circular muscle in the outer submucosa appears first in both chick and skate, we are exploring if similar constrictive buckling by muscle differentiation contributes to spiral formation in the skate intestine

**20. Newborn Death in Uroplakin 2-/- Mice**

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The apical surface of the urothelium is covered by two-dimensional crystals of uroplakin (UP) particles that include Upk1a, Upk1b, Upk2, and Upk3. *Upk2*$^{-/-}$ mice display several urinary tract defects and have renal failure as adults. For unclear reasons, some Upk2$^{-/-}$ mice die as newborns, possibly due to renal failure based on high levels of blood urea nitrogen. To understand the basis of the kidney failure, kidney weights were obtained, uroplakin 1a, 1b and 2 expression was examined, and kidney histology was assessed in mutant and wildtype pups. There was no significant difference in kidney weight or in overall kidney histology between mutant and wildtype pups. Immunofluorescent studies revealed the expression of UPK2 protein within collecting ducts in the inner medulla. Because UPK1a forms heterodimers with UPK2, its expression was assessed during several stages of kidney development. UPKIB partners with UPK3, and it was also examined to determine if its expression was altered in the absence of UPK2. UPK1A and 1B expression was observed at embryonic day (E) 18 in epithelial cells within the renal pelvis, the papilla, and the collecting duct. At postnatal day (P) 1, their expression was restricted to the renal pelvic epithelium. Specific nephron segment markers for the proximal tubule (Aquaporin-1), the thick ascending limb (Uromodulin), and the collecting duct (Aquaporin-2) were examined by immunofluorescent staining and no differences in expression were noted between wild type and *Upk2*$^{-/-}$ mice. We hypothesized that the loss of *Upk2* might affect the differentiation of intercalated cells within the collecting duct that are important for acidification of urine. Indeed, some *Upk2*$^{-/-}$ litters had a higher urine pH, this was observed in the offspring from 2/4 breeding pairs. However, survival of offspring from these breeding pairs suggests that a disturbance in body pH is not the explanation for the postnatal lethality observed in some newborn pups.

**21. Investigating the roles of Plexin A1 and Plexin A2 receptors during early eye development in zebrafish.**

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During early eye development, retinal precursor cells (RPCs) bud from the diencephalon and coalesce to form optic vesicles. It is crucial that RPCs must correctly bilaterally migrate and proliferate to ensure the foundation for proper eye development. Little is known about the mechanisms that control the proliferation and migration of RPCs. However, using zebrafish as a model organism, we have begun to uncover novel early roles for the Plexin A (PlxnA) family of transmembrane receptors in regulating these processes. Plexins are a large family of transmembrane receptors, which are activated by semaphorin (sema) ligands. There are 4 members of the PlxnA family, PlxnA1-A4, each of which show unique expression patterns and have different roles throughout development, but can be bound and activated by overlapping members of the Semaphorin family of ligands. Using *in situ* hybridization, we have examined the expression of all 4 family members and have shown that *plxnA1* and *plxnA2* are expressed in the optic vesicles at very early stages of eye development, in ovelapping regions with *sema6A*. Interestingly, *plxnA1* and *A2* morphants share common phenotypes of decreased proliferation and cohesion of RPCs in optic vesicles. Furthermore, we have shown that *plxnA1* and *A2* double knockdowns show compounding phenotypes and single morphants show reciprocal increases in expression when the other is knocked down. Future work will investigate if they have compensatory roles in this system. Investigation into potential transcriptional targets downstream of Sema6a/PlxnA2 signaling using microarray analysis has uncovered a set of 58 genes that are differentially regulated by PlxnA2 and Sema6A that are involved in proliferation and migration, and have functionally validated two genes, *rasl11b and shtn-1* to have important roles in these processes respectively.

**22. The microtubule plus-end-tracking protein TACC3 promotes persistent axon outgrowth and mediates axon guidance signals during development**

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Formation of precise neuronal connections requires proper axon guidance. Microtubules (MTs) of the growth cone provide a critical driving force during navigation of the growing ends of axons. Pioneer MTs and their plus-end tracking proteins (+TIPs) are thought to play integrative roles during this navigation. TACC3 is a +TIP that we have previously implicated in regulating MT behavior within axons. However, the role of TACC3 in axon guidance has not been previously explored. Here, we show that TACC3 is required to promote persistent axon outgrowth and prevent spontaneous axon retractions in embryonic *Xenopus laevis* neurons. We also show that overexpressing TACC3 can counteract the depolymerizing effect of low doses of nocodazole, and that TACC3 interacts with MT polymerase XMAP215 to promote axon outgrowth. Moreover, we demonstrate that manipulation of TACC3 levels interferes with the growth cone response to the axon guidance cue Slit2 *ex vivo*, and that ablation of TACC3 causes pathfinding defects in axons of developing spinal neurons *in vivo*. Together, our results suggest that by mediating MT behavior, the +TIP TACC3 is involved in axon outgrowth and pathfinding decisions of neurons during embryonic development.

**23. Regulation of nucleus-nucleus interactions during myonuclear movement**

Eric Folker, Mary Ann Collins, Torrey Mandigo, Jaclyn Camuglia
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The positioning of nuclei within the skeletal muscle syncytium provides a striking image. Up to thousands of nuclei are at the periphery of the cell with the distance between adjacent nuclei maximized. The importance of this distribution of nuclei is highlighted by the high correlation between mispositioned nuclei and muscle disease. Although the mechanisms are not fully known, myonuclear movement is known to depend on the microtubule cytoskeleton, proteins localized to the nuclear envelope, and the interaction between these two sets of proteins. The mechanisms that drive similar nuclear movements in mononucleated model cell types are more well understood, but it is difficult to translate these mechanisms because the impact that multiple nuclei sharing a common cytoplasm has on nuclear movement has not been investigated. Using Drosophila as a model organism we have determined that nuclei in the developing skeletal muscle experience both attractive and repulsive interactions with other nuclei. Disruption of nuclear envelope genes which have been linked to Emery-Dreifuss Muscular Dystrophy increases the attractive interactions between nuclei. This indicates that the nuclear envelope actively participates in the separation of nuclei from other nuclei. Conversely, disruption of membrane shaping genes linked to Centronuclear Myopathy decreased the attractive interactions between nuclei and resulted in premature dispersion of nuclei. This suggests that Centronuclear Myopathy-linked genes play an active role in maintaining the associations between nuclei. Importantly, the disruption of either process results in generally mispositioned nuclei. Together these data demonstrate that myonuclear position requires a balance of forces and that these forces are differentially affected by distinct muscle diseases. Therefore, although aberrant nuclear position is a phenotype common to many muscle disorders, the mechanisms that underlie this phenotype are disease-specific.

**24. Role of the microtubule cytoskeleton in NMJ development**

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Neuromuscular junctions (**NMJs**) are highly plastic synapses that form between motor neurons and skeletal muscles. These two tissues communicate during development to assure the proper growth of the junction. On the presynaptic site, NMJ contains boutons, oval-shaped structures that harbor synapses where neurotransmitters are released. On the muscle site, specialized postsynaptic structures expressing neurotransmitter receptors appose the boutons. The mechanisms that regulate the different stages of bouton formation, development and maintenance are not fully understood. Because of the fundamental role of microtubules in many cellular processes, our goal is to understand the functional relevance of the muscle microtubule cytoskeleton on NMJ development. The Drosophila larval NMJ is a relatively simple system to investigate this question. Our approach is to evaluate changes in the NMJ structure in larval muscles that have been depleted of cytoskeletal proteins associated with microtubules. Using the Gal4-UAS system, we knocked down specifically in the muscle the kinesin heavy chain (KHC), dynein heavy chain (DHC) and several of their partners. NMJ development is affected in all mutants, where nerve branches are less complex and with fewer boutons than controls. Surprisingly, we found that the microtubule associated protein ensconsin inhibits bouton formation, and it is also the only protein in our screen required to form an appropriate branch angle. Thus, perturbing the muscle cytoskeleton has a surprising effect on the morphology of the innervating neurons. Moreover, we found the distance of a subset of myonuclei to the NMJ changes in all the analyzed muscles, suggesting their possible instructive role in NMJ development. In conclusion, we show that the motor neuron branch growth and maturation responds non-autonomously to changes in the muscle microtubules network.

**25. Formation of the olfactory bulbs and connection of the olfactory/vomeronasal neurons to the brain are not needed for GnRH-1 neuronal migration from the nose to the hypothalamus.**

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Gonadotroping Releasing Hormone I (GnRH-1) neurons play a pivotal role in controlling the reproductive axis of vertebrates. During embryonic development, GnRH-1ns migrate from the developing olfactory pit (OP) into the hypothalamus. Migration ofgonadotropin releasing hormone-1 (GnRH-1) neurons (GnRH-1ns) is required for sexual development.Disturbances in the migration of GnRH-1ns or in GnRH-1 signaling lead to various degrees of hypogonadotropic hypogonadism (HH). Around 50-60% of patients affected by HH have difficulty perceiving odors (hyposmia) or entirely lack of the ability to smell (anosmia). HH associated with congenital olfactory defects is clinically defined as Kallmann Syndrome (KS). The association of anosmia with HH in KS suggested potential direct relationship between defective olfactory, vomeronasal development and defective GnRH-1 neuronal migration into the brain. Though frequent genetic correlation between olfactory defects and defective GnRH-1 migration exist, it has never been experimentally proven that olfactory bulbs (OBs) development and correct axonal connections of the olfactory and vomeronasal neurons to their functional targets are necessary events for the migration GnRH-1 neurons to the hypothalamus.

*Loss*-of-function of the homeobox gene Arx-1 impairs interneuron progenitors proliferation and entry into the OBs. Lack of function for Arx-1 in mice leads to defective OBs development.

We exploited the Arx-1 null mouse line to investigate the role of correct development of the olfactory system in controlling GnRH-1 migration to the brain. Our data proves that correct development of the OBs and axonal connection of the olfactory and vomeronasal sensory neurons to the forebrain are not needed for GnRH-1 neuronal migration. Using an array of genetic tools we prove that the terminal nerve, which forms the GnRH-1 migratory scaffold follows different guidance cues and has a distinct genetic identity from olfactory and vomeronasal neurons.

**26. Defining gene expression during formation of the spiral valve intestine *in Leucoraja erinacea***

Samantha Frye, Alexis Wotjowicz, Nicole Theodosiou
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The digestive tracts of vertebrates is a complex system of organs essential for the digestion of food and absorption of nutrients. In chick and most other vertebrates, the intestines are long and coiled to increase surface area for difficult to absorb diets. This contrasts with the shortened spiral valve intestine of the little skate, *Leucoraja erinacea*, a member of the Elasmobranch Family. The unique structure of the spiral intestine allows for a large absorptive surface area within a short length of intestine. In chick, tissue patterning occurs by differential gene expression along the anterior-posterior axis of the linear embryonic gut tube. To ask how patterning of organs occurs along a 3-dimensional spiral, we focused on the expression of two key developmental homeotic genes, *Hoxd12* and *Cdx2*. We first isolated and cloned these genes using PCR, amplifying from skate embryonic cDNA. Protein sequence alignment showed 60% and 52% identities between skate and chick *Hoxd12* and *Cdx2*, respectively. To detect expression in skate embryos, we created antisense RNA probes and performed RNA whole mount in situ hybridization and the expression patterns were compared in skate and chicken embryos. *Cdx2* expression is found in the developing skate endoderm, similar to that of chicken. While *Hoxd12* is found in the pelvic fins of the skate, it similarly is expressed in the developing limb buds of the chick embryos. These results show that although the skate is separated from the chicken through thousands of years of evolution, the function of these key developmental genes has remained conserved and thus must be important in early stages of development.

**27. Targeting claudins to modify tight junction barrier properties**

Enrique Gamero-Estevez, Amanda Baumholtz, Jasmine el Andalousi, Bertrand Jean-Claude, Makoto Nagano, Indra Gupta, Aimee Ryan
*Mcgill, CA*

Claudins are essential for tight junction (TJ) formation and function. The combination of claudins within an epithelial cell layer determines its paracellular barrier properties. Nutraceuticals like quercetin can modify claudin expression, while molecules such as the non-toxic C-terminal domain of *C. perfringens* enterotoxin (C-CPE) can bind to and remove a subset of claudins from TJs. To alter the specificity of C-CPE, we replaced its claudin binding domain with the amino acid sequence of the second extracellular loop of individual claudins. Currently, we are testing the ability of these substances to transiently modify the claudins and TJ barriers. We showed that all three approaches modify claudin expression, localization or postranslational modifications affecting the TJ barrier properties in different ways. Our goal is to develop tools that can be used to modify the TJ composition and barrier properties to extend the use of these reagents to treat disease and improve health.

**28. The Regulatory Landscape of Whole-Body Regeneration**

Andrew Gehrke, Mansi Srivastava
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The ability to regenerate nearly any missing body part (whole-body regeneration) is a phenomenon that is widely spread across animal phyla. While the rise of high throughput transcriptomics has vastly improved our understanding of the genes responsible for whole-body regeneration, how the epigenome responds to wounding is unknown. In order to understand the dynamics of genome structure during regeneration, we have performed an assay to identify changing chromatin conformation (ATAC-seq) on regenerating fragments of the acoel worm *Hofstenia miamia*. The three-banded panther worm is an ideal organism to investigate at the chromatin level due to the animals' high capacity for whole-body generation and the availability of a quality genome with sufficiently large scaffolds to interrogate gene regulation. We find hundreds of non-coding sequences that change from "closed" to "open" (and vice-versa), suggesting highly dynamic activation and suppression of regulatory elements during regeneration. Furthermore, we find the resolution of ATAC-seq allows us to identify transcription factor (TF) binding footprints inside of dynamic chromatin peaks. By combining TF binding site predictions with functional studies via RNA interference, we are able to construct direct gene regulatory networks for early regeneration response genes. Taken together, ATAC-seq in the highly regenerative worm *Hofstenia miamia* has revealed epigenomic and transcriptional regulatory networks that underlie whole-body regeneration.

**29. FGF signaling is required for neuromesodermal progenitor formation**

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The vertebrate body plan is established though two major developmental phases: gastrulation, which results in anterior tissue patterning, and posterior growth, which leads to the elongation and formation of the posterior body. During posterior growth, neuromesodermal progenitors (NMPs), which reside in the caudal end of the embryo known as tailbud, undergo differentiation to form the posterior body. NMPs make a basic germ layer decision to form the posterior spinal cord and somites. Although recent studies have revealed the signaling cues that regulate the differentiation and movement of NMPs during the posterior growth phase, the signaling networks which induce NMPs during early development (gastrulation) are unknown. Previous studies have revealed that fibroblast growth factor (FGF) signaling induces both posterior neural and mesodermal tissues. We hypothesize that FGF signaling, rather than separately inducing mesoderm and neural tissue, instead specifies the NMP population that later becomes posterior neural or mesoderm based on the local signaling environment. Through cell transplantation and *in situ* hybridization techniques, we find that FGF inhibition leads to the loss of posterior neuronal and somitic tissue without significantly affecting other tissues. Furthermore, FGF inhibition of NMP precursors during gastrulation leads to changes in cell positioning and productive membrane protrusion formation as visualized by *in vivo* time lapse movies. Together, our results show that NMP formation is dependent on the presence of FGF signaling during gastrulation.

**30. Defining the cis-regulatory logic that drives a cell invasion program**

R Antonio Herrera, David Q Matus

*Stony Brook University, USA*

Tissue invasion is a cellular behavior critical for development and disease. Development of the nematode *C. elegans* egg laying apparatus begins with the anchor cell (AC) breaching two basement membranes and contacting the adjacent epithelium. Several genes with direct roles in AC invasion exhibit patterns of gene expression that are either enriched or exclusively expressed in the AC. We and others have identified multiple ACels within the upstream genomic region of several genes, including *nhr-67*/Tlx and *lin-3*/EGF. Using a combination of single cell visual analyses and bioinformatics approaches we are examining ACels activity to determine if there is a cis-regulatory logic driving anchor cell expression. To see *in vivo* ACel activity and quantify expression dynamics we use ACel-driven fluorescent reporters integrated with CRISPR/Cas9 at single copy. The nuclear-localized fluorescent reporters are destabilized with a PEST tag in order to avoid over-accumulation and visualize gene expression output. To determine if there are regulatory sequences which are over represented in the ACels, we are identifying the minimal regions of DNA required to drive expression in the AC. Next, we will determine if there are known motifs for transcription factors to bind which are present within the minimal ACels. The goal of this analysis is to define both the cis-regulatory code and trans-activating factors that together mediate acquisition of an invasive phenotype.

**31. Ex vivo culture of pre-attachment chorion and allantois reveals that TMED2 is required in both the chorion and the allantois for normal chorioallantoic attachment.**

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*Tmed2*, a transmembrane emp24 domain (TMED) protein, is required for normal transport of secretory cargoes between the ER and Golgi. *Tmed2* is expressed in the allantois and the chorion, and null mutation in *Tmed2* results in abnormal chorioallantoic attachment and failure of placental labyrinth formation. Expression of genes associated with spongiotrophoblast, *Tpbp*α, and syncytiotrophoblast, *Gcm1,* differentiation was reduced in *Tmed2* null placenta. We aimed to investigate the tissue-specific requirement for *Tmed2* using previously established *ex vivo* allantoic and chorionic explants. To track allantoic and chorionic tissue, RFP and GFP were crossed into the *Tmed2* mutant mouse line, respectively. To determine if *Tmed2* was required in the chorion or the allantois, *Tmed2* null decidua/ectoplacental cone/chorions (chorions) were co-cultured with wildtype allantoises, or *Tmed2* null allantoises were co-cultured with wild type chorions. Control samples using *ex vivo* explants of wild type chorions and allantoises underwent chorioallantoic attachment, had allantoic and chorionic cell mixing (mixing), maintained expression of *Tpbp*α, and *Gcm1*, and increased mitotic index in the allantoic region. Explants with *Tmed2* null chorion and wild type allantois showed abnormal chorioallantoic attachment and no mixing, reduced expression of *Tpbp*α and *Gcm1;* however, mitotic index in the allantois was comparable to that found in wild type explants. Explants of wild type chorion and *Tmed2* null allantois showed mixing, reduced expression of *Tpbp*α and *Gcm1,* and reduced proliferation in the allantois. Our data indicates that *Tmed2* is required in both the chorion and the allantois during placental development. *Tmed2* is required in the chorion for mixing of allantoic and chorionic cells, and in the allantois for its normal proliferation. Intriguingly, wild type expression of *Tmed2* in both chorion and allantois is required for normal expression of *Gcm1* and *Tpbpa*.

**32. Pitx1 regulates cement gland development in *Xenopus laevis* through activation of transcriptional targets and inhibition of BMP signaling**

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Embryos of the African clawed frog *Xenopus laevis* are widely used for the study of early vertebrate development. The cement gland is one of the first fully functional tissues to differentiate during *Xenopus* embryogenesis. It secretes mucus to help the tadpole attach to solid supports and thus live in relative safety. The gland develops from the outer layer of anterior ectoderm situated between the neural plate and non-neural ventral ectoderm. It has been shown that intermediate level of BMP signaling is essential for cement gland formation. In addition, several transcription factors have been linked to cement gland development. One of these, the homeodomain-containing protein Pitx1, can induce ectopic cement gland formation; however, the mechanisms underlying Pitx1-mediated cement gland induction and its relationship with BMP signaling remain obscure. We report here, for the first time, a requirement for Pitx proteins in cement gland formation, in vivo: knockdown of both Pitx1 and the closely related Pitx2 inhibits endogenous cement gland formation. Pitx1 transcriptionally activates cement gland differentiation genes through both direct and indirect mechanisms, and functions as a transcriptional activator to inhibit BMP signaling. This inhibition is partially mediated by an increase in Pitx1-dependent *follistatin* expression. Rescue of Pitx1-mediated inhibition of BMP signaling represses the expression of *pitx2*, but does not affect other cement gland markers. Suppression of BMP signaling inhibits induction of cement gland markers by Pitx1; furthermore, we find that Pitx1 physically interacts with Smad1, an intracellular transducer of BMP signaling. We propose a model of cement gland formation in which Pitx1 recruits Smad1 to activate cement gland genes, and limits local BMP signaling within the cement gland primordium.

**33. Characterizing Polycyclic Aromatic Hydrocarbon Induced Teratogenesis of the Pharyngeal System**

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Polycyclic aromatic hydrocarbons (PAH's) such as Naphthalene (naph), are hazardous compounds which could be found at high concentrations in crude oil released during the Deepwater Horizon Disaster. By using zebrafish as a vertebrate model system for PAH induced teratogenesis, we have investigated the developmental mechanisms by which naph impairs craniofacial development. The craniofacial skeleton is derived in part from cranial neural crest cells (NCCs) which migrate through transient structures known as the pharyngeal pouches and arches. We postulated that naph could be targeting its effect on three possible cell types: the cranial NCCs, cells of the developing pharyngeal pouches, or epibranchial placodal cells that interact with pouch/arch growth. Using antibody and transgenic reporters for both arches and pouches, we demonstrate that naph causes specific malformations in the posterior most region of the pharyngeal system.Using *in situ* hybridization on embryos treated at 10hpf with a 200 mM naph dose, we characterized the expression of *dlx2a*, *hoxa2b*, *hoxb2a*, and *jag1b at* 30, 36, and 42hpf. Although naphthalene treated embryos labeled for *hoxb2a* and *jag1b* showed no difference in expression from the control embryos at any of the time-points, *dlx2a* and *hoxa2b* expressions were reduced at all time-points. *dlx2a* is known to promote the formation of arches and NCCs, while *hoxa2b* is thought to assist in the formation of the pouches. The specific molecular mechanisms that naph is operating upon are still unknown, but we are continuing to narrow in on the involvement of these specific cell types most affected by naph. We are currently assessing the involvement of placodal cells and the dynamics of the interactions between the arches, pouches and placodes following naph treatment. It is our prediction that naph operates through the Aryl hydrocarbon receptor pathway to influence the molecular regulation of posterior pouch migration.

**34. Does a role in dorsoventral axis specification make the BMP signaling pathway nervous? Investigating BMP function in an annelid**

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Bone Morphogenetic Protein (BMP) signaling plays an important role in establishing the dorsoventral (DV) axis and inducing neural fate in *Xenopus laevis* and *Drosophila melanogaster.* Studies in other organisms suggest that BMP signaling may have played an ancestral role in DV axis specification in the last common ancestor of Bilateria. It has also been inferred that BMPs had an ancestral function in neural induction; however, recent studies in hemichordates, cnidarians, and annelids suggest that BMPs are not similarly involved in neural induction. This challenges the idea that BMPs also played a role in neural induction in the early bilaterian ancestor and is evolutionarily conserved, but more studies in diverse animal groups are necessary to better understand this. The third major clade of Bilateria, Spiralia, is understudied and could help answer this question. Here, we start to assess the role of BMP signaling in the spiralian annelid *Capitella teleta,* which has a clear DV axis and a centralized nervous system consisting of a ventral nerve cord and a dorsoanterior brain. Components of the BMP signaling pathway including BMPs and certain antagonists were identified in the *C. teleta* genome and cloned, and gene expression was analyzed throughout developmental stages. Early cleavage stage embryos were incubated in the drug dorsomorphin dihydrochloride, which has been shown to block BMP signaling. Resultant phenotypes were assessed by immunohistochemistry and in-situ hybridization. Inhibition of BMP signaling affected multiple tissues, including muscle, foregut, and neural tissue. However, the head and the brain remained largely unaffected. Preliminary data suggests that BMPs may play a role in DV axis specification in *C. teleta*, but whether it is involved in neural induction and to what extent, remains unclear.

**35. Dual control of PCNS expression and function by ADAM13/33 via AP2alpha and Arid3a.**

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ADAM13/33 is a cell surface metalloprotease critical for cranial neural crest (CNC) cell migration in *Xenopus laevis*. It can cleave multiple substrates including fibronectin and cadherin-11 at specific sites. Cleavage of cadherin-11 produces an extracellular fragment that promotes CNC migration. In addition ADAM13 also cleaves itself releasing a large portion of its extracellular domain including the active metalloprotease while its cytoplasmic domain is cleaved by gamma secretase, translocates in the nucleus and regulate multiple genes. Here we show that ADAM13 regulation of gene expression depends on the transcription factor AP2α, which in turns activate multiple genes including the proteocadherin PCNS. ADAM13 physically interact with another transcription factor Arid3a/Dril1 to control AP2α expression. In addition to this transcriptional control of PCNS, ADAM13 cleaves PCNS generating an extracellular fragment that can also regulate cell migration. Loss of ADAM13 can be partially rescued by the expression of PCNS, the extracellular fragment of PCNS or AP2α.

**36. A Classroom-Based RNAi Screen Reveals a Requirement for Nonsense-Mediated mRNA Decay in Planarian Regeneration**

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From middle school science fairs to biomedical research labs, the dramatic regenerative abilities of planarian flatworms have long captivated scientists at all stages of development. Recently, tools like RNAi have rendered freshwater species such as *Schmidtea mediterranea* amenable to molecular genetic analysis. We exploited this experimental tractability to design a semester-long, discovery-based lab for an undergraduate developmental biology course. Each of the 16 students in the class identifies and clones at least one uncharacterized *S. mediterranea* gene and uses RNAi to screen for regeneration phenotypes. We have identified three new genes to date required for formation of the blastema, the stem cell-derived mass of tissue that forms at sites of amputation to replace lost body parts. These include a homolog of the RNA helicase *UPF1*, a core factor in the nonsense-mediated mRNA decay (NMD) pathway. While NMD was originally identified as a quality control mechanism that eliminates aberrant mRNAs with premature termination codons, recent studies indicate it also degrades many wild-type transcripts. Strikingly, the *UPF1(RNAi)* phenotype points to at least some regeneration-specific functions for NMD in planarians – failed blastema formation is a hallmark of stem cell loss, yet *UPF1(RNAi)* animals do not exhibit the tissue homeostasis defects observed when stem cells are ablated. We verified maintenance of normal stem cell number and fate in uncut animals by immunostaining and in situ hybridization. RNAi knockdown of another NMD factor, *UPF2*, led to a nearly identical, regeneration-specific phenotype. Taken together, these results suggest NMD orchestrates post-transcriptional changes in gene expression necessary for stem cells to mount an effective response to injury. We are currently addressing this hypothesis through further phenotypic characterization and will report results at the meeting.

**37. Analyzing the Mechanism of Wnt Mediated Cell Fate Decisions in the Zebrafish Tailbud**

Brian Kinney, Richard Row, Benjamin Martin
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The zebrafish tailbud contains a population of neuromesodermal progenitors (NMPs) that serve as the primary source of posterior spinal cord and somites. A balance between Wnt and *sox2* maintains the NMP population. In these progenitors an increase in canonical Wnt signaling leads to mesodermal cell fate by inducing an epithelial to mesenchymal transition (EMT), while a loss of Wnt prevents EMT and results in a neural fate. The mechanism of how Wnt signaling controls morphogenetic and cell fate decisions is unknown. We have identified a Wnt responsive gene, the classical type II cadherin *cdh6* that is involved in EMT in other systems and is expressed in the tailbud NMPs. Using CRISPR mutants and heat shock inducible transgenic lines, we aim to provide a mechanistic understanding of the role *cdh6* plays downstream of Wnt signaling during cell fate decisions in the tailbud.

**38. A fluorescence based cell cycle state biosensor in *C. elegans* and its use in characterizing cell cycle state during vulval morphogenesis.**

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During organismal development, differential regulation of the cell cycle is critical to many cell biological processes, including differentiation and morphogenesis. While the complete cell division lineage of *C. elegans* is known, how the control of cell cycle is linked to fate specification and morphogenesis remains poorly understood due to our inability to directly visualize cell cycle state. In order to visualize cell cycle state live, we have adapted a CDK2 biosensor for use in *C. elegans*. Our biosensor uses the dynamic nuclear/cytoplasmic localization of a portion of Human DNA Helicase B (DHB) linked to GFP to assess cell cycle state. The dynamic localization is the result of phosphorylation of the biosensor by CDKs. We have modified this sensor to allow for algorithmic assessment of cell cycle state. Similar to reported results from cell culture, we are using this biosensor to quantify lineage specific differences between cycling cells, quiescence and differentiation, provide new biological insights into the control and timing of the cell cycle in specification of uterine cells and the morphogenesis of the *C. elegans* vulva.

**39. ROCK signaling controls ductal progenitor populations and ductal differentiation in submandibular salivary gland epithelium**

Matthew Koslow, Deirdre Nelson, Melinda Larsen
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In the developing submandibular salivary gland, epithelial progenitor cells sense signals from the mesenchyme through the surrounding ECM. Rho-associated protein kinase (ROCK) is crucial for SMG development as its inhibition results in decreased branching, disrupted basement membrane assembly, and altered polarity. However, the tissue specific role of ROCK in either epithelial or mesenchymal cells has on the cell progenitor populations and their subsequent differentiation has not been examined in the salivary gland. In this study, the effect of ROCK signaling has on the epithelial differentiation of E16 primary epithelial cells was examined in the presence of 10 μM of the ROCK inhibitor, Y27632, in either simple media or mammary epithelial media. ROCK inhibition, as well as growth in mammary media prevented epithelial-mesenchymal transition (EMT) and myofibroblastic conversion. Interestingly, the K5+ basal ductal cell population increased significantly with ROCK inhibition in both media types. The number of cells for ductal lineage marker K19 were increased after ROCK inhibition. However, the total expression of these markers on per cell basis was decreased. These results were repeated in the mature ductal marker, K7. These studies indicate that ROCK signaling expands the K5+ basal ductal cell population and disrupt subsequent ductal differentiation.

**40. The Expression of Claudins during Branching Morphogenesis in Chick Lung**

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Claudins are a family of proteins that are located in the tight junctions of epithelial cells. More than twenty members are found in vertebrates and each has unique characteristics, which affect the barrier permeability properties of the tight junction. Claudins are also widely expressed throughout development, including during branching morphogenesis of several organs. Previous data from my lab showed that removing claudins from the tight junctions of embryonic mouse kidneys decreased branching morphogenesis. From these results, we hypothesize that claudins have a common role in branching morphogenesis of the kidneys, the lungs and the submandibular glands. To study claudin function in lung morphogenesis, characterization of claudin expression in the embryonic lung is necessary. Previous work from our lab showed that *Cldn1*, *Cldn3* and *Cldn10* are expressed in the lung beginning at embryonic day (E) 5. Using whole mount *in situ* hybridization, I found that *Cldn1*, *Cldn3* and *Cldn10* are expressed in the bronchi and the buds of the chick developing lung between E4 and E6 and that *Cldn10* expression begins at E5. I also found that *Cldn5* is expressed in the buds of embryonic chick lungs at E5 and that *Cldn4* and *Cldn8* are not expressed in the lungs between E4 and E6. Next, I optimized the conditions to culture chick lungs explants on a membrane to permit explants to grow well for up to 72 hours. The optimal media was DMEM/F12 and contained 5% fetal bovine serum and 5% chick serum. I will determine if the removal of specific claudins affects branching morphogenesis in the lungs. For these studies, I will treat chick lung explants with a truncated nontoxic version of the *Clostridium perfringens* enterotoxin that removes a subset of claudins, including Claudin-3 which is expressed in the embryonic lungs. The phenotypes of treated lungs and controls will be compared to determine effects on branching morphogenesis.

**41. Characterization of Four Craniofacial Mutants from an ENU Mutagenesis Screen**

Kristi Lamonica, Saba Abuzaid, Debbie Yang, Greg McCrary, Stephen Morawski, Dianne Girard, Mary Witkowski

*The Sage Colleges, USA*

The craniofacial skeleton is derived from cranial neural crest cells (cncc) that are induced at the border between the neural and the non-neural ectoderm, and subsequently migrate into the pharyngeal arches and interact with the surrounding environment to form the craniofacial skeleton. To identify novel genes involved in craniofacial development, we are in the process of characterizing several mutants from an ENU zebrafish mutagenesis screen performed at UC Denver – Anschutz Medical Campus with the Artinger and Appel labs. Here we are focusing on phenotypically characterizing four mutants through Alcian blue staining at 6 days post fertilization (CO15.13, CO82.1, CO161.3, and CO275.5). All four mutants are lacking posterior craniofacial structures, and some have other varying phenotypes. Further studies will focus on positional cloning for CO15.13, CO82.1, and CO161.3 (but not 275.5 since sequencing suggests the mutation is in CTP synthase) along with gene expression analysis for all four mutants.

**42. Embryonic Alcohol Exposure Negatively Impacts Specification of Mossy Fiber Precerebellar Neurons**

Patience Cournoo, Curtis Schutz, Travis Townsend, Rebecca Landsberg
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Exposure to alcohol during gestation can lead to fetal alcohol syndrome (FAS), a disease characterized by a range of cognitive and physical disabilities including mental retardation and impaired gross motor control. Studies using mouse models of FAS investigating the loss of motor coordination have traditionally focused on the cerebellum and in particular, the effects of alcohol on the granule and Purkinje cells. Subsequent analysis of brainstem structures from these neonates, such as those of the precerebellar nuclei (pcn), that function to relay inputs from the cortex and spinal cord to the cerebellum, also reveal large numbers of apoptotic cells. Unclear from these studies, is whether the apoptosis of pcn is due to a primary effect of alcohol on pcn development or due to a secondary failure of these neurons to properly establish synapses with cells in the cerebellum. Recent studies focusing on examining the direct effects of alcohol on the development of the pcn conducted by our lab has shown that chronic alcohol exposure results in abnormalities in the mossy fiber projecting pcn that function to relay inputs to cerebellar granule cells. The pcn arise from dorsally situated progenitors in the lower rhombic lip (LRL) of the embryonic hindbrain and migrate ventrally to the pons and medulla of the brainstem. To understand if embryonic alcohol exposure affects specification events in the LRL or post-mitotic migration, alcohol was administered to pregnant females during distinct developmental windows correlating with times when specification or migration of these neurons occur. Analysis of neonate brains suggest mossy fiber neuron abnormalities occur when alcohol is administered during a developmental window when specification occurs but not during the time when migration takes place. This finding is supported by analysis of gene expression in the progenitors of the LRL which show that embryonic alcohol exposure leads to changes in normal gene expression patterns.

**44. Using *Xenopus laevis* as a model for studying genes associated with intellectual disabilities.**

Micaela Lasser

*Boston College, USA*

Intellectual disabilities are complex neurodevelopmental disorders that affect a significant portion of the general population and can be an immense health issue. Genetic mutations caused by rare copy number variants (CNVs) have been shown to play a role in intellectual disabilities or severe developmental delays. It has been established that one such CNV results in a deletion within chromosome 16 in a subset of children clinically diagnosed with an intellectual disability. Individuals with this deletion, located at 16p12.1, display severe features of intellectual disabilities, cardiac defects, seizures, growth retardation, and craniofacial abnormalities. This deletion encompasses six genes, many of which have not yet been studied in relation to brain development. In order to elucidate the role of these six genes during development, we have created antisense morpholino oligonucleotides to knock down gene function and observe phenotypic differences in *Xenopus laevis*. Importantly, each gene is highly conserved between humans and *Xenopus laevis*, making it an ideal model organism to understand the developmental mechanisms of this genetic deletion. Here, we show phenotypic differences of the 16p12.1 deletion by quantifying changes in craniofacial features, brain morphology, and axon outgrowth patterns of each individual gene knock down.

**45. Identification of Claudin Sequence Variants in Children with Chronic Kidney Disease**

Maria Laverde, Amanda Baumholtz, Jasmine El Andalousi, Indra Gupta, Aimee Ryan
*McGill University, CA*

To understand how congenital kidney malformations arise in children, it is important to define the molecular and cellular events that are critical for kidney formation. Kidney development begins with an aggregate of mesenchymal cells that epithelialize to form the nephric duct. The nephric duct elongates caudally and an outgrowth known as the ureteric bud develops at the level of the hind limb. The ureteric bud gives rise to the collecting duct system through a process known as branching morphogenesis, which is dependent on reciprocal signaling between the adjacent mesenchymal cells and the ureteric bud epithelium. We are focusing on a family of integral tight junction transmembrane proteins, the claudins that are expressed during kidney development. Claudins regulate the intercellular barrier and pore properties of epithelia, and are required for the formation of tight junctions. Previously we showed that removal of a subset of claudins from tight junctions results in branching defects in the mouse embryonic kidney and disrupts nephric duct elongation in the chick embryo. Through *in situ* hybridization, we observed that *Claudin-1* and *-3* are expressed in the nephric duct in the chick embryo at Hamburger Hamilton stages 10 through 20. In the mouse embryo, we have shown that *Claudin-3*, *-4*, *-6*, *-7*, and *-8* are expressed in the ureteric bud at embryonic day 9.5 and 10.5, and in the ureteric bud tips and trunk at E13.6 and E16.5. Based on their expression patterns, we hypothesize that CLDN sequence variants will disrupt nephric duct elongation and/or ureteric bud branching resulting in congenital kidney malformations in children. We sequenced 24 claudins in 96 patients with congenital renal malformations from the NIH-sponsored CKiD (Chronic Kidney Disease) study. We identified nine rare and one novel non-synonymous protein altering variant in eight different claudins. The variants are now undergoing functional assessment.

**46. Influence of Arg overexpression and knockdown on microtubule dynamics**

Kyle Lawrence
*Boston College, United States*

Arg (Abl2) is a tyrosine kinase that has been shown to regulate the cytoskeleton by phosphorylating various substrates and by interacting with cortactin to stabilize actin filaments. Arg also contains a microtubule binding domain and has been shown to crosslink microtubules (MTs) with actin in vitro. Beyond this however, little is known about the effects Arg has on MT behavior. This study, in collaboration with the Koleske Lab of Yale University, aims to explore the effect of over-expression and endogenous Arg knockdown on microtubule dynamics in vivo. Using time lapse imaging and microtubule plus-end tracking software, we have quantified MT growth length, lifetime, and velocity in the growth cones of developing Xenopus laevis neurons after injection with various Arg mRNA constructs, as well as with an Arg-specific morpholino. Future studies will be required to investigate both the mechanism by which Arg regulates MT dynamics in vivo and the interaction Arg may have with other MT associated molecules in vivo.

**47. TACC3 mitigates Nocodazole-induced growth cone effects and is required for proper embryonic neural development and morphology**

Eric Lee, Burcu Erdogan
*Boston College, USA*

Proper navigation of the developing axon necessitates the complex interactions between the microtubules (MTs) of the growth cone, associated proteins, and extracellular cues. Specifically, plus-end tracking proteins (+TIPs), proteins that reside at the plus-end of microtubules, play a crucial role in the development of embryonic neurites. We have previously demonstrated that TACC3 functions as one of these +TIPs and binds plus-ends of MTs in Xenopus laevis embryonic growth cones. Here, using quantitative analysis of high-resolution live imaging, we also show that TACC3 mitigates reduction in MT dynamics parameters -growth speed, length and lifetime- in response to Nocodazole exposure at low concentrations, and that TACC3 also limits neurite retraction under low concentrations of Slit2. Furthermore, we have observed several neural morphological differences between the telencephalic hemispheres in Xenopus laevis embryos that have had TACC3 expression levels site-specifically manipulated. In conclusion, our results illustrate that TACC3 may play a role in altering a developing axon's response to external guidance cues and in facilitating proper neural morphological development.

**50. Genetic characterization of the planarian poles identifies nr4A as a muscle specific transcription factor that maintains patterning in the head and tail**

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For proper regeneration to occur, newly generated cells must adopt the correct identities and positions to replace exactly what is missing. This process, referred to as patterning, is likely orchestrated by precisely timed and coordinated instructive cues. How cell-cell signaling consistently and faithfully organizes new tissue into a replica of the old remains incompletely understood. Given their remarkable regenerative ability and simple body plan, planarians serve as ideal organisms to study patterning. Here we examine patterning along the anterior-posterior (AP) axis of the planarian *Schmidtea mediterranea.* We focus on clusters of cells at the tip of the head and tail, respectively termed anterior and posterior poles, that are defined by the expression of Wnt signaling genes (e.g. *notum* in the anterior pole and *wnt1* in the posterior pole) and genes encoding transcription factors (e.g. *foxD* in the anterior pole and *pitx* in the posterior pole). Using bulk and single-cell RNA sequencing of head and tail fragments containing the anterior and posterior poles, we identified many new pole genes encoding secreted proteins, transmembrane proteins, and transcription factors. Among these was *nuclear receptor subfamily 4A* (*nr4A*), which is expressed specifically in planarian muscle cells. RNAi of *nr4A* in intact worms resulted in posterior expansion of head tissue identities (e.g. ectopic posterior eyes) and anterior expansion of tail tissue identities (anteriorly shifted *wnt1* domain). RNA sequencing and histological studies of RNAi worms indicated that *nr4A* regulates head and tail patterning by regulating the expression of many muscle-specific genes and maintaining muscle fiber integrity of the planarian head tips. To our knowledge, this is the first gene of its kind with expression gradients at both ends of the animal and with patterning functions in both the head and the tail.

**51. A novel signaling molecular for vertebrate synovial joint development-discovering a new role of hyaluronan**

Yingcui Li

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Hyaluronan, a very large linear glycosaminoglycan, is a major component in the extracellular matrix of cartilage cells, where it is synthesized by primary hyaluronan synthase *Has2*. Due to its high water entrapping capacity it has been known as a major structural molecule in cartilage matrix through binding to other molecules to form large proteoglycan aggregates so as to stabilize and organize the matrix. We have found previously the up-regulation of *Has2* at the distal posterior subridge mesoderm right underneath the apical ectodermal ridge AER during limb outgrowth and down-regulated at the interdigital mesoderm. Up-regulation of *Has2* through retrovirus vector or conditional knock out *Has2* through Prx1Cre promoter in limb mesenchymal cells would affect the outgrowth of limb and its skeletal patterning. Most recently, we have discovered the *Has2* synthesized hyaluronan working with Tgf family factors at joint interzone. It demonstrated *Has2* working as a signaling molecule during vertebrate synovial joint development.

**52. The Transcription Factor AP2e is a Key Component of the Gene Regulatory Network Controlling Terminal Differentiation of Vomeronasal Neurons**

Jennifer Lin

*University at Albany, USA*

The generation of cellular diversity relies on a series of spatially and temporally controlled differentiation steps that can be influenced by both intrinsic and extrinsic signals. This is best exemplified in the nervous system where a multiple neuronal cell types must synchronize through complex but precise networks. Identifying and understanding how this level of cellular diversity is produced and maintained is important for understanding the basis of neurodegenerative diseases. The vomeronasal organ (VNO), a pheromone detecting organ, contains two non-overlapping apical and basal populations of vomeronasal sensory neurons (VSNs), project to distinct locations in the brain, and mediate well characterized innate behaviors. In this project, we begin to unravel key components of the gene regulatory network (GRN) controlling the dichotomy of vomeronasal neuron populations. Here we show the transcription factor Tfap2e (AP2e) is a key component of the GRN controlling the successful terminal differentiation of basal VSNs. By utilizing an AP2eCre knock-in/knock-out mouse line, we have characterized the expression, cell lineage, and function of AP2e within the VNO. We have found that AP2e is selectively expressed in basal VSNs, and is strongly expressed in actively differentiating neurons. Further characterization of the AP2e $^{Null}$ showed the loss of functional AP2e results in the loss of the defined basal identity, and acquisition of apical specific markers. Our data strongly suggests that AP2e is actively selecting for the basal VSN program, and the cells that have been induced, but incapable of expressing a functional copy of AP2e, are unable to fully differentiate, causing an imbalance in the composition of the VSN population. We propose that AP2e is a crucial component of the gene regulatory network, necessary for the successful terminal differentiation of cells selected for the basal cell fate.

**53. Feedback and robustness in the Nodal-Lefty patterning system**

Nathan Lord

*Harvard University, USA*

Developing embryos must accurately coordinate the activities of their constituent cells in space and time. The signaling pathways that convey instructions to cells often drive the production of their own inhibitors. This negative feedback is commonly invoked to explain spatial or temporal restriction of signaling activity. Here, we examine the role of the feedback inhibition in early embryonic patterning by the morphogen Nodal. Mutants lacking the Nodal inhibitor Lefty exhibit overactive signaling, excess mesendoderm and embryonic lethality. Surprisingly, these defects can be rescued with without feedback: treatment of lefty mutants with low doses of a drug that inhibits Nodal signaling leads to normal patterning and fertile adults. Lefty-mediated feedback is thus not required for normal mesendodermal patterning. However, drug-rescued mutant embryos are more sensitive than WT embryos to signaling perturbations. Injecting low doses of lefty mRNA or reducing Nodal gene dosage compromises patterning in drug-rescued lefty mutants, but not in WT animals. We therefore propose that inhibitory feedback helps to recognize and correct unexpected perturbations to normal signaling.

**54. BMP Signaling Regulates C. elegans Body Size Via Transcriptional Regulation of Collagen Genes**

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Body size is a tightly regulated phenotype during development in metazoans, and it is dependent on both intrinsic and extrinsic factors. While signaling pathways such as insulin, Hippo, and myostatin are known to control organ and body size, the downstream effectors that mediate their effects are still poorly understood. In the nematode *C. elegans*, a Bone Morphogenetic Protein (BMP)-related signaling pathway is the major regulator of growth and body size, which is an essential developmental process. DBL-1, the BMP-related ligand, is secreted by neurons and body wall muscle, and acts as a dose-dependent regulator of body size. We investigated the transcriptional network through which the DBL-1/BMP pathway regulates body size and identified cuticle collagen genes as major effectors of growth control. We have demonstrated that cuticle collagen genes can act as positive regulators (*col-41*), dose-sensitive regulators (*rol-6*), and negative regulators (*col-141, col-142*) of body size. Moreover, we have shown a requirement of DBL-1/BMP signaling for stage-specific expression of cuticle collagen genes. We used chromatin immunoprecipitation followed by high throughput sequencing (ChIP-Seq) and electrophoretic mobility shift assays to show that the Smad signal transducers directly associate with conserved Smad binding elements in regulatory regions of *col-141* and *col-142*, but not of *col-41.* Hence, cuticle collagen genes are directly and indirectly regulated via the DBL-1/BMP pathway. Based on presented evidence, we propose that cuticle collagens act as effectors of body size regulation via the DBL-1/BMP pathway. This work thus provides the first mechanistic link between BMP signaling and effectors of body size regulation. Since mutations and misregulation of collagen genes can lead to numerous diseases and disorders, studying regulation of collagens can provide valuable insights for human health.

**55. Genome-wide allele-specific analysis reveals novel imprinted genes expressed during mouse gastrulation**

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Appropriate epigenetic regulation of gene expression during lineage allocation and tissue differentiation is required for normal development. One example of epigenetic regulation is genomic imprinting. Defined as parent-of-origin, mono-allelic gene expression, imprinting is established largely due to epigenetic differences arriving in the zygote from sperm and egg haploid genomes. In the mouse, there are approximately 150 known imprinted genes, many of which are coordinately regulated in imprinted gene clusters. Differential methylation is the primary epigenetic mark regulating mono-allelic expression for known imprinted clusters. In previous work, we have shown that differential methylation at the known imprinted cluster *Igf2r/Airn* undergoes changes in imprinted expression and epigenetic modifications during specific stages of mouse gastrulation, indicating that these epigenetic marks can be dynamic during development. In order to determine if other imprinted genes have similarly dynamic imprinted expression patterns and to identify novel imprinted genes, we performed allele-specific RNA sequencing during mouse gastrulation. Additionally, we performed whole genome bisulfite sequencing (WGBS) to assay global DNA methylation changes and identify epigenetic regulation corresponding with imprinted expression. These data have led to the identification of several novel imprinted loci that have transient allele-specific expression during gastrulation. This suggests a novel epigenetic mechanism separate from gametic methylation regulating imprinted expression at these sites. Taken together with global and locus-specific methylation, these data help to define the epigenetic dynamics during mammalian gastrulation.

**56. Regulation of stomodeum size: coordinating brain and jaw evolution.**

Rachel Master, Jennifer Fish

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Variation in jaw size is observed in both evolutionary and disease processes, but mechanisms generating this variation are still not completely understood. Avians are good models for studying jaw size because of the great diversity in beak morphology in this clade. In particular, duck and quail exhibit large differences in jaw size, which make them a suitable system to study how development regulates jaw size. We have previously shown that differences in jaw size occur very early in development. In fact, duck and quail jaw primordia are distinct from the very earliest formation of the first pharyngeal arch. This initial difference in jaw size is associated with differences in patterning of the brain and anterior head. Comparison of *Otx2*, *Fgf8*, *Shh*, and *Krox20* expression in duck and quail HH10 embryos highlights differences in fore- and midbrain size and shape. Differences in brain regionalization affect the morphology of the stomodeum (primitive mouth), around which the first pharyngeal arch forms. Thus, brain regionalization may influence the initial size of the jaw progenitor population. To test this hypothesis, we conducted a series of experiments to inhibit *Wnt* signaling, which is involved in early head patterning. We have attempted to alter fore- and midbrain size by expanding Dkk1 levels anterior to the node in HH5-6 chick embryos. Application of Dkk1 soaked beads to HH5-6 embryos can shift *Otx2* expression in the anterior neural tube of HH10 embryos. Furthermore, the length of the stomodeum increases with reduction to the *Otx2* domain. These data suggest that early developmental alterations to brain patterning also have implications for the regulation of jaw size.

**57. The Role of Negative Regulators in Modulating Fgf Signaling in the Developing Zebrafish**

Jennifer Maurer, Charles Sagerstrom
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The Fgf signaling pathway plays important roles in the development and patterning of the early embryo. For example, in the zebrafish, Fgf signaling works cooperatively with Wnt signaling in the tailbud to promote trunk elongation. Fgf signals from the developing retina encourage the differentiation of lens fiber cells. In the hindbrain, Fgf signaling is responsible for patterning the forming rhombomeres and defines their boundaries. Various studies have blocked or inactivated the Fgf signaling pathway, either by pharmacological inhibitors or over-expression of negative regulators, and demonstrated that tight control over this signaling pathway is necessary for proper development. To better understand how this regulation plays a role in early development, we asked what effect mis-regulation of the Fgf signaling pathway could have. Using CRISPRs, we have created knock-out zebrafish lines for two dual-specific phosphatases responsible for deactivating ERK downstream of the Fgf signaling pathway – *dusp2* and *dusp6*. *dusp2*$^{-/-}$*dusp6*$^{-/-}$ mutant embryos have a lower survival rate through gastrulation. Many, but not all, mutant embryos are unable to complete the early cleavage stages, remain stalled for several hours, and die by 8-10 hpf. The surviving mutant embryos appear to exhibit higher variability in expression of early dorsal-ventral patterning markers. Despite this, the surviving embryos show no hindbrain phenotype, specifically in the patterning of pERK or genes downstream of the Fgf signaling pathway. As we continue to investigate the cause and mechanism of the early embryonic death seen in the double mutants, we plan to determine if affected embryos were fertilized or able to be fertilized as oocytes. There are known roles for *dusp* proteins and ERK signaling in the maturation of oocytes, so it may be that some oocytes did not develop properly. Using these mutant fish lines, we aim to determine what role these regulators play embryonic and oocyte development.

**58. Thyroid Hormone Coordinates Craniofacial Osteogenesis**

Catherine May, Sarah McMenamin
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Thyroid hormone plays a vital role in skeletogenesis during post-embryonic development. Changes in the timing and coordination of ossification can lead to severe birth defects. Cranial bones in larval zebrafish undergo substantial growth and remodeling during the post-embryonic metamorphosis from larva to juvenile. Using a transgenic thyroid ablation system, we found that thyroid hormone plays a crucial role in coordinating the ossification sequence of the bones in the skull. Hypothyroid fish show a lack of ossification in many cranial bones even at late stages of development. We are investigating in greater detail the roles of thyroid hormone in regulating the timing and coordination of skeletal changes during the larval-to-juvenile transition. Comparing two developmental stages, larval and metamorphic transitional, we characterize the changes in skull shape and ossification that are controlled by thyroid hormone. A morphometric analysis of developing intramembranous and endochondral bones elucidates the roles of this critical endocrine factor on cranial osteogenesis.

**59. Uncoiling the role of modified histone arginine residues in Drosophila oocytes**

Alicia McCarthy
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As oogenesis is an energetically costly developmental process, many species have evolved methods to respond to nutritional cues to abort oogenesis when resources are sparse. We hypothesize that the developing egg may sense environmental signals using post-translational modifications deposited on histone tails. We propose that histones with dimethylated arginine residues on histone H3 (H3R17me2), deposited by Arginine methyltransferase 4 (dArt4), serve as an environmental sensor during oogenesis. We observe that H3R17me2-modified histones pool, unassociated with DNA. Furthermore, these modified histones are spatially and temporally dynamic in the developing oocyte, localizing to the oocyte nuclear lamina and then forming a punctum proximal to the oocyte nucleus. This dynamic change occurs prior to vitellogenic stages of oogenesis, which can undergo cell death due to environmental conditions such as starvation. Surprisingly, we have shown that Coilin, a Cajal Body (CB) protein, is required for this relocalization of H3R17me2-modified histones from the oocyte nuclear lamina to a punctum in the CB. Although the function of the CB is not well understood, Survival of Motor Neurons (SMN) protein localizes to this membraneless organelle. SMN proteins shuttle between the cytoplasm, CB, and nucleolus in order to assemble spliceosomal machinery. We show that SMN is required in the germline, and loss of SMN leads to aborted egg chambers just prior to vitellogenesis. And, remarkably, we observe that not only is SMN required for CB formation but is also needed for H3R17me2-modified histone relocalization. It has been shown that dArt4-deposited H3R17me2 can coordinate nutrition-induced gene expression to promote apoptosis. As we observe a dynamic relocalization of H3R17me2-modified histones just prior to a nutrition checkpoint during oogenesis, we propose that this histone pool serves as a nutrient sensor for the developing egg.

**60. Primordial Germ Cell Survival Depends on Contact with the Somatic Gonad in the *C. elegans* Embryo**

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We are studying the formation of the germline stem cell niche in the *C. elegans* gonad, and are investigating how niche cell contact promotes stem cell survival. Niche-derived signals often control stem cell quiescence, proliferation and differentiation. As such, formation of the niche is a critical event in development. In *C elegans*, the gonad primordium is a simple, easily observed organ consisting of just four cells – two primordial germ cells (PGCs), and two somatic niche cells (SGPs). The primordium forms via a fascinating morphogenetic process in which the two SGPs migrate posteriorly, recognize the PGCs, and surround the PGC cell body. Using genetics, fluorescent imaging and embryological manipulations we are testing the hypothesis that either signaling or adhesion between SGPs and PGCs is needed for germ cells to survive. Previously, it was reported that ablation of the SGPs resulted in a loss of the primordial germ cells. However, no mechanism has been reported for this phenomenon. We began by repeating this experiment using fluorescent reporters to follow cells in the primordium. We too found the PGCs are frequently lost in these animals. Surprisingly, we also observed that unprotected PGCs were engulfed by neighboring endodermal cells. We next repeated these ablations in mutants lacking endoderm. PGCs survived in these mutants. Previously, our lab showed that, the PGCs produce lobes that are pinched off and digested by endodermal cells. This process requires the coordinated activity of CED-10, LST-4 and DYN-1. We have shown that this pathway is also needed for death of PGCs following SGP ablation, which suggests SGP contact (rather than signaling) protects the PGC cell body, ensuring only the lobe is eaten by the endoderm. We are testing if SGP wrapping protects the PGCs by blocking the cytoskeletal re-arrangements needed for wrapping in the SGPs. Thus we can test if SGPs that contact, but do not wrap, the PGCs can still protect the germ cells.

**61. The gene regulatory network underlying invasive differentiation in *C. elegans***

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Invasion of cells through basement membranes (BMs) is a highly conserved behavior that is relevant to gastrulation, trophoblast implantation, leukocyte trafficking, and cancer metastasis. *C. elegans* vulval development provides a tractable *in vivo* model to study invasive differentiation. During this process, the anchor cell (AC), a specialized uterine cell, breaches the BM to connect the uterus with the underlying vulval epithelium. Prior research has identified five pro-invasive transcription factors (TFs) in *C. elegans*. These include the basic helix-loop-helix protein and E/Daughterless homolog *hlh-2*, the AP-1 proto-oncogenic subunit and sole Fos family homolog *fos-1*, and the nuclear hormone receptor and NR2E1/Tailless homolog *nhr-67*. In addition, two zinc-finger TFs, the EVI1/MEL1 homolog *egl-43* and Krüppel-like protein *mep-1*, are known to mediate AC invasion as well. Notably, the first four of the aforementioned TFs share homology with mammalian counterparts associated with invasive behavior and oncogenesis, and the latter three have been shown to regulate proliferation of the AC. In order to map out the gene regulatory network that programs invasive differentiation, a series of molecular epistatic experiments were performed using GFP reporters paired with RNAi-induced gene silencing. The data acquired provide the first characterization of the regulatory relationships between these pro-invasive TFs.

**62. A Non-Cell-Autonomous Role of BEC-1/Beclin1 in Coordinating Cell-Cycle Progression and Stem Cell Proliferation during Germline Development**

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Autophagy is a conserved cellular recycling process crucial for cellular homeostasis. In a multistep process, cellular material destined for degradation is enclosed in an organelle with a double-membrane, the autophagosome, which in turn fuses with the lysosome. BEC-1/Beclin1, a haploinsufficient tumor suppressor protein is crucial for the initial nucleation step of autophagosome formation. In previous studies we showed that BEC-1/Beclin serves important functions during development and longevity of multicellular organisms. In addition, we demonstrated a role for BEC-1/Beclin in endocytosis, including in retromer transport, from endosome to Golgi, and lipid homeostasis. We now describe a novel role for BEC-1/Beclin 1/BECN1 and autophagy in germ line stem cell homeostasis. The decision of a stem cell to proliferate or differentiate is finely controlled. We found that *bec-1,* and several other autophagy genes are required for germline proliferation. Interestingly, BEC-1/Beclin 1, ATG-18/WIPI and ATG-16.2/ATG16L act independently of the GLP-1/Notch or DAF-7/TGFβ pathways, but upstream of the DAF-2/insulin IGF-1 receptor (IIR) signaling pathway, to promote germline stem cell proliferation during development. In contrast, ATG-7 functions together with the DAF-7/TGFβ pathway, to promote germline proliferation, and is not required for cell cycle progression. Interestingly, BEC-1/Beclin 1 functions non-cell autonomously to facilitate cell cycle progression and stem cell proliferation. Thus, our findings demonstrate a novel, non-autonomous role for BEC-1/Beclin 1 in the control of stem cell proliferation, and cell cycle progression. Taken together, our findings may hint at a functional connection between germline development, lipid homeostasis and longevity, as autophagy is required for all three.

**63. Investigating defective BMP signaling as the cause of Nager Syndrome**

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Nager syndrome is a member of a class of disorders called the acrofacial dysostoses, which are characterized by structural malformations of the face and limbs. Recent genetic analyses suggest that most cases of Nager syndrome are caused by haploinsufficiency of SF3B4, a component of the U2 pre-mRNA spliceosomal complex. SF3B4 has also been shown to inhibit BMP-mediated osteogenic and chondrogenic differentiation. Therefore, skeletal anomalies associated with Nager syndrome may be due to either defects in splicing and/or defects in BMP signaling. To test the role of SF3B4 in both of these processes, we have manipulated *Sf3b4* levels both *in vivo* (chick embryos) and *in vitro* (mouse osteoblasts) models. We have found *Sf3b4* expression in the developing chick head and osteoblasts, similar to previous reports in other model systems. To elucidate how *Sf3b4* mediates variation in osteogenesis, we are examining single cell gene expression of genes in the BMP pathway in WT and *Sf3b4* over-expressing MC3T3 cells Additionally, we are using the avian retrovirus RCAS (A) to mediate RNA interference against *Sf3b4* in the developing chicken embryo.

**64. Expression of Craniofacial Morphology defects of Wolf-Hirschhorn Syndrome related genes NELFA and WHSC1 in *Xenopus Laevis***

Alexandra Mills
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Wolf-Hirschhorn Sydrome is a human neurodevelopmental disorder caused by a deletion or mutation on the short arm of human chromosome 4. Deletions or microduplications of this critical region result in craniofacial malformation, heart and skeletal defects, and mental retardation. This region, located on chromosome 4 in humans and chromosome 6 in Xenopus Laevis, consists of five genes; TACC3, NELFA, WHSC1, LETM1, and FGFR3. We have previously hypothesized that this disorder may be due to disrupted neural crest cell migration. Our lab has previously characterized TACC3 as a microtubule plus TIP which promotes axon elongation and regulates microtubule dynamics. Unpublished work from our lab suggests that TACC3 may function during neural crest cell migration, as well. To further understand the role that these genes play in cell migration, specifically in neural crest cell development, we examined their expression patterns in Xenopus embryos. This was done through in situ hybridization of Xenopus embryos at various developmental stages. We additionally explored the effect of knocking down these genes on craniofacial morphology of Xenopus embryos. Future work will explore the effect of combinatorial gene knockdown on craniofacial development. The characterization of gene expression and knockdown craniofacial morphology will allow for greater exploration of how cranial neural crest cell migration is controlled in Wolf-Hirschhorn Syndrome.

**65. A germline RNA is translationally suppressed to promote germline stem cell differentiation**

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During oogenesis, maternal deposition of mRNAs to the developing egg is critical to establish the future generation's developmental program. These maternally deposited RNAs are under strict translational regulation, mediated by a myriad of translational repressors, to ensure proper spatio-temporal expression. We asked how maternal RNAs are continually repressed, using *Drosophila* germline as a model system. *polar granule component* (*pgc*), a maternal RNA, is translationally repressed throughout oogenesis, except for transient expression in the stem cell daughter. First, we tested is *pgc's* 5' and 3'UTR contributes towards its regulation. Interestingly, we observed that *pgc* 5'UTR is required but not sufficient for suppressing Pgc expression in the germline stem cells (GSCs). On the other hand, *pgc* 3'UTR is required and sufficient to suppress its translation throughout oogenesis. To determine cis and trans-acting factors that control *pgc* translation, we carried out a phylogenetic analysis of its 3'UTR and identified conserved binding sites for translational repressor Pumilio (Pum). We found that Pum binds to the 3'UTR of *pgc* and together with its binding partners Nanos and Twin (CCR4 de-adenylase), represses *pgc* translation only in GSCs. Additionally, we also observed that the NOT complex, recruited by CCR4, also regulates Pgc expression. Intriguingly, previous research has shown that *me31b* interacts with the CCR4-NOT complex in the 3' end and cap proteins in the 5' end to suppress translation. We asked if me31b is also regulating Pgc expression in the GSCs via this complex. Interestingly, we found that loss of *me31b* in the germline upregulates Pgc expression in the GSCs. Altogether our data elucidates that an intricate protein complex in the GSCs bridges *pgc's* 5' and 3'UTR ends, masking it from translational machinery. We are currently identifying if there is a network of germline mRNAs that could be similarly regulated during *Drosophila* development.

**66. The wound response of the acoel Hofstenia miamia**

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Whole-body regeneration is a remarkable phenomenon where an adult animal can regenerate virtually any missing tissue, which is a process that is usually limited to a developing embryo. Strikingly, there are many organisms that are capable of whole-body regeneration but little is known about how the molecular mechanisms compare across animal phyla. Studies of *Schimidtea mediterranea*, a planarian flatworm that is an established model organism, have uncovered multiple genes required for regeneration. However, it is unknown whether the mechanisms required for regeneration in planarians represent conserved or unique biological pathways. To compare regenerative mechanisms between animal phyla, we are using a new acoel model organism, *Hofstenia miamia*, in addition to *Schimidtea*. Acoels represent the earliest lineage of bilaterally symmetric animals (bilaterians), which last shared a common ancestor with planarians 550mya. The aim of this project is to discover the earliest processes of regeneration in *Hofstenia* and then to compare these to the planarian wound response. RNAseq data collected from *Hofstenia* at various time points post-amputation, allowed us to identify a number of wound-induced genes that were verified via Fluorescent In Situ Hybridization (FISH). RNA interference (RNAi) has revealed some of these wound-induced genes to be required for regeneration in *Hofstenia*. We then utilized high-throughput qPCR and FISH analyses to determine how early wound-induced genes regulate each other. Finally, we compared *Hofstenia* wound-induced genes to known regulators in the *Schmidtea* wound response, and identified orthologous genes to operate with similar dynamics in both acoels and planarians. Our results suggest that despite 550 million years of independent evolution, some wound-induced networks have been conserved across bilaterians.

**67. Toddler signaling regulates mesodermal cell migration downstream of Nodal signaling**

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Toddler/Apela/Elabela is a conserved secreted peptide that regulates mesendoderm development during zebrafish gastrulation. Two non-exclusive models have been proposed to explain Toddler function. The "specification model" postulates that Toddler signaling enhances Nodal signaling to properly specify endoderm, whereas the "migration model" posits that Toddler signaling regulates mesendodermal cell migration downstream of Nodal signaling. Here, we test key predictions of each model. We find that in *toddler* mutants Nodal signaling is initially normal and engineering an increase in endoderm specification does not rescue mesendodermal cell migration. Mesodermal cell migration defects in *toddler* mutants are independent of endoderm, but endoderm migration defects are dependent on a Cxcr4a-regulated tether to mesoderm. These results suggest that Toddler signaling regulates mesodermal cell migration downstream of Nodal signaling and indirectly affects endodermal cell migration via Cxcr4a-signaling.

**68. Serial section immunogold electron microscopy of phosphorylated connexin 43 in ovarian granulosa cells**

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Gap junctions comprised of Connexin43 (Cx43) connect the granulosa cells of ovarian follicles in mammals. In response to luteinizing hormone, which triggers the resumption of meiosis, there are marked changes in Cx43 phosphorylation and an increase in internalized gap junctions (or connexosomes) is detected by electron microscopy. Results from Western blotting and immunofluorescence studies indicate that Cx43 phosphorylation is associated with gap junction internalization. To determine the precise localization of specifically phosphorylated Cx43, electron microscopy (EM) provides the best possible resolution. Further, serial sections of electron micrographs are needed to determine the whole structure of a gap junction, since invaginating gap junctions may appear to be fully internalized gap junctions in a single section. Here, we adapted a method used for 3D electron microscopy to work with immunogold labeling. In this manner, ultrathin tissue sections are collected on tape with an automatic tape collecting ultramicrotome (ATUM). The sections on tape are attached to a silicon wafer, then imaged by scanning EM. With this technique we can discern the full structure of a gap junction, an invaginating gap junction or connexosome. We labeled serial sections of preovulatory ovarian follicles with antibodies against either total Cx43, pS262 Cx43, or pS368 Cx43. We found that Cx43 is phosphorylated on S368 (a protein kinase C site) in both gap junctions and connexosomes. In contrast, Cx43 is phosphorylated on S262 (a MAP kinase site) only in some connexosomes. Our results suggest that MAP kinase phosphorylation of Cx43 may play a specific role in gap junction internalization or in connexosome processing in ovarian granulosa cells.

**69. Landmarks in Existing Tissue at Wounds Are Utilized to Generate Pattern in Regenerating Tissue**

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Regeneration in many organisms involves the formation of a blastema, which differentiates and organizes into the appropriate missing tissues. How blastema pattern is generated and integrated with pre-existing tissues is a central question in the field of regeneration. Planarians are free-living flatworms capable of rapidly regenerating from small body fragments. A cell cluster at the anterior tip of planarian head blastemas (the anterior pole) is required for anterior-posterior (AP) and medial-lateral (ML) blastema patterning. Transplantation of the head tip into tails induced host tissues to grow patterned head-like outgrowths containing a midline. Given the important patterning role of the anterior pole, understanding how it becomes localized during regeneration would help explain how wounds establish pattern in new tissue. Anterior pole progenitors were specified at the pre-existing midline of regenerating fragments, even when this location deviated from the ML median plane of the wound face. Anterior pole progenitors were specified broadly on the dorsal-ventral (DV) axis and subsequently formed a cluster at the DV boundary of the animal. We propose that three landmarks of pre-existing tissue at wounds set the location of anterior pole formation: a polarized AP axis, the pre-existing midline, and the dorsal-ventral median plane. Subsequently, blastema pattern is organized around the anterior pole. This process, utilizing positional information in existing tissue at unpredictably shaped wounds, can influence the patterning of new tissue in a manner that facilitates integration with pre-existing tissue in regeneration.

**70. Analyzing the 3D structure of the spiral valve intestine of a cartilaginous fish**

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The digestive tract of vertebrates with high protein diets requires a large surface area in order to absorb nutrients. While most vertebrates accomplish this by having long intestines covered in vili, the cartilaginous fish have a unique spiral valve intestine. The spiral structure allows for increased surface area in an enclosed, short length of intestine. Spiral intestines are present in all cartilaginous fish and the number of turns within the spiral is species specific. Here we define the progressive turns and pitch of the spiral during development in little skate, *L. erinacea*, embryos. The spiral begins as a single fold and turns increase in number during development until there are 8 turns in the skate intestine. Using microCT-scanned images of developing embryos and the software programs CTan and CTvol, we analyzed the dorsal and ventral pitches of the spiral from stages 25 to 32 of skate development. Pitch, the distance between the turns in the spiral, decreases along the anterior to posterior axis of the intestine. To determine the extent to which the spiral folding varies throughout the gut tube, we measured the angles of the spiral folds. Similar to pitch, the angles of the spiral folds decrease along the length of the intestine. The 3D analysis program Cloudcompare was used to reshape and edit the internal spiral from different developmental stages to better visualize the morphology of the spiral. 3D models of the volumetric structure of the skate intestines were printed with resin and propenoic acid/acrylate with a Stratasys (Connex 500). Understanding and characterizing the features of the spiral will help us understand the forces involved in generating the turns that lead to the spiral formation in the skate intestine.

**71. Translational regulation of a homeobox gene by a transcriptional repressor during *Drosophila melanogaster* embryogenesis**

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ncRNAs are required for gene regulation, from transcription to translation. The highly-conserved 7SK ncRNA is a scaffold that forms an snRNP that globally represses transcription. One member of the snRNP, MePCE, adds a methyl cap to stabilize 7SK. We previously found that the *Drosophila melanogaster* ortholog of MePCE, Bin3, interacts directly with Bicoid, a translational repressor of *caudal* mRNA in the anterior of the early *Drosophila* embryo. Unexpectedly, we have found that Bin3 is required for the efficacy of *caudal* translational repression via Bicoid during early embryogenesis. Loss of *bin3* results in anteroposterior patterning defects in the embryo, consistent with its role in translational repression of *caudal*. This translational regulation is not mediated a poly(A) tail-dependent mechanism. We also found that Bin3 is required for the accumulation of 7SK in the *Drosophila* embryo, and that 7SK also interacts directly with Bicoid. Our results suggest that 7SK forms a snRNP with Bicoid and Bin3, which facilitates the translational repression of *caudal* during early embryogenesis. We are using CRISPR to epitope-tag the native *bin3* gene, as well as region-specific translating ribosome affinity purification to identify other mRNAs that Bin3 interacts with, and to determine whether Bin3 plays a role in their translational regulation.

**72. Determining the role of secondary structure in translational control**

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The central dogma of cellular biology asserts that DNA is transcribed to mRNA, which in turn is translated to a protein. This simplistic conduit of gene expression is heavily regulated at multiple levels to ensure proper temporal and spatial expression of genes. One such mode of regulation occurs at the juncture at which RNAs are translated to proteins, known as translational regulation. mRNAs can be translationally regulated by trans-acting factors, such as RNA binding proteins (RBPs), and/or cis-acting factors. Regulation via these two modalities can depend on structure as well as RNA sequence. Secondary structures, such as stem loops, are unique motifs that can be recognized by RBPs. To study the influence of structure on translational regulation, we used the *polar granule component* (*pgc*) of *Drosophila melanogaster* as a model mRNA. It has been shown that the 3'untranslated region (UTR) of *pgc* is sufficient to temporally and spatially regulate the mRNA throughout development. Secondary structure analysis via RNA footprinting showed that *pgc* forms a unique and stable secondary structure. By utilizing a GFP reporter that is expressed under the control of a *pgc* 3'UTR, we sequentially explored the influence these structures had on *pgc* translation. We found that a stem loop, SL3, when deleted resulted in a loss of somatic translational control during embryogenesis. To elucidate the mechanism by which SL3 influences translational regulation of *pgc* we established an *in vitro* dimerization assay, which showed that cis-acting interactions are possible. This form of interaction could suggest that two or more *pgc* mRNAs interact to potentially occlude the mRNA from active translation. Further in vivo and in vitro studies are needed to identify the role that SL3 plays in translation control, with it being either a dimerization motif, or a putative binding site for RBPs.

**73. Somitic mesoderm plays a novel role in de novo blood vessel formation in developing zebrafish embryos**

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The formation of the vasculature in a developing embryo is a critical process that requires delicate timing and precise cellular movement. In chordates, vasculature is formed *de novo* from a pool of progenitor cells called angioblasts. These angioblasts migrate from the lateral mesoderm to a midline structure known as the vascular plexus, and coalesce to form the earliest blood vessels. I have obtained preliminary data suggesting that somitic mesoderm is required for proper angioblast migration to the vascular plexus in the zebrafish embryo. Disruption of this mesoderm prevents proper localization of angioblasts at the midline and results in improper formation of blood vessels in the larvae. This study presents evidence that somites provide an essential component in angioblast migration and vascularization.

**74. Characterizing the morphological changes and molecular mechanisms involved in the self-correction of craniofacial defects in pre-metamorphic *Xenopus laevis* tadpoles**

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Craniofacial birth defects, such as cleft palate, fetal alcohol syndrome, and microcephaly occur in more than one in every six hundred births. Infants with these developmental defects are challenged with severe physical, mental, and social difficulties. Despite the prevalence and seriousness of craniofacial abnormalities, there are limited medical treatments available to the people affected by these birth defects. To promote the advancement of treatment options that correct craniofacial abnormalities in humans, we must first understand how craniofacial morphology is established and maintained in vertebrate model organisms. For the past decade, research has primarily focused on discovering the causes of craniofacial abnormalities in vertebrates. Our alternative approach takes advantage of results obtained from previous studies to investigate the possibility of reversing abnormal craniofacial morphology. We know that craniofacial defects can be resolved because of a study carried out by our collaborators in the Levin lab at Tufts University in 2012; they demonstrated that *Xenopus laevis* tadpoles can normalize abnormal craniofacial morphology prior to metamorphosis. Therefore, we now aim to characterize the limitations of this remodeling response as well as elucidate the underlying mechanisms that regulate this self-correction in Xenopus tadpoles. We have established that pre-metamorphic *X. laevis* tadpoles can self-correct some, but not all, malformed craniofacial features resulting from a range of mechanical, genetic, or chemical perturbations. Our current focus is to identify the molecular mechanisms that regulate the remodeling of malformed craniofacial tissues in these tadpoles. Ultimately, by fully understanding how pre-metamorphic *X. laevis* tadpoles detect, and subsequently correct, abnormal craniofacial morphology, we will gain valuable knowledge of the importance of tissue remodeling as a mechanism for maintaining normal craniofacial morphology in vertebrates.

**75. The effects of Shroom2 knockdown on axonal transport of mitochondria**

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Dynamic distribution of mitochondria in axons is crucial for neuronal function. The mechanisms of axonal transport are complex and highly regulated in order to achieve the optimal distribution. Defects in axonal transport of mitochondria have been linked with neurodegenerative diseases. Mitochondrial movement along axons is dynamic. Mitochondria display bidirectionality, travelling in both retrograde and anterograde directions. Rates vary and are inconsistent, with mitochondria often stopping, starting, and changing speed and direction. Distinct mechanisms of transport have also been identified, with transport able to be driven by kinesin and dynein motors along microtubules as well as by myosin along F-actin. Much remains to be elucidated about these complex mechanisms of axonal transport of mitochondria. Shroom2 is a member of the Shroom family of proteins, which regulates both actin and microtubule cytoskeletons. Here we show that Shroom2 knockdown leads to faster mitochondrial transport speeds in both retrograde and anterograde directions in embryonic *Xenopus laevis* neurons, with both fast-moving and slow-moving transport rates displaying a significant increase. The results demonstrate that Shroom2 plays an important role in modulating axonal mitochondrial transport.

**76. Autophagy Regulation due to DBL-1 Signaling in the model C. elegans**

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Autophagy, literally meaning “self-eating”, is a ubiquitous cellular process essential for maintaining homeostasis, and functions by the delivery of cytoplasmic material to the acidic lysosome for degradation. In the nematode *C. elegans*, autophagy is required for dauer development and lifespan extension induced by multiple pathways. One autophagy-regulating signal is DAF-7, a member of the transforming growth factor- beta (TGF-β) family of cell signals, an evolutionarily conserved class of secreted growth factors. We asked whether another member of the TGF-β family, the BMP-related ligand DBL-1, also plays a role in autophagy regulation. When autophagy is induced, the *C. elegans* protein LGG-1 (ortholog of mammalian L3) changes from its diffused cytoplasmic cellular localization to form punctate structures that label the preautophagosomal and autophagosomal membranes. By analyzing multi-planar images of various *C. elegans* strains that have been genetically crossed with the molecular marker GFP::LGG-1 we are able to quantify the number of punctate structures in *dbl-1* mutants and compare them to wildtype. Under basal conditions, there is no significant difference in the quantity of punctate structures in the *dbl-1* mutants compared with that of wildtype. However, animals with induced autophagy levels through the depletion of *daf-2* (encoding the *C. elegans* Insulin/IGF-1-like Receptor) by RNA interference (RNAi), showed that loss of DBL-1 activity significantly reduces the elevated levels of autophagy observed in *daf-2* depleted animals. An increase in DBL-1 activity, mediated by *lon-2* loss, enhances the level of autophagy present in *daf-2* mutants, indicating that DBL-1 signaling may regulate autophagy in a dose-dependent manner.

**77. Assessing the role of sonic hedgehog signaling in jaw size**

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Sonic Hedgehog (SHH) plays a critical role in jaw development, where it affects proliferation and outgrowth. In humans, abnormal expression of SHH can cause defects in jaw size, including micrognathia, cleft lip and/or palate. Yet it is unknown how differences in SHH signaling may contribute to species-specific differences in jaw size in normal development. To test the hypothesis that species-specific differences in SHH signaling are associated with differences in jaw size during development, we took advantage of three avian species, chicken, duck, and quail, which vary in relative jaw size. We examine the regulation of the SHH pathway through several critical genes including Patched-1, a negative regulator of SHH signaling, and Gli-1, an SHH signaling effector. We first compared protein and mRNA levels in chicken, duck, and quail mandibles at HH21, HH24, and HH27. To test if chicken, duck, and quail mandibular mesenchyme respond to SHH signaling in a species-specific manner, we isolated mandibles from HH21 embryos and cultured them in media containing differing concentrations of exogenous SHH protein. After 24 hours of culture, we evaluated the response to SHH signaling by measuring Gli-1 levels via RT-qPCR. Our data indicates that avian have species-specific patterns of SHH pathway regulation in jaw development, with differences in Patched-1 especially prominent. Future experiments will test the functional role of Pathced-1 in jaw size by manipulating its expression in jaw development.

**78. Biochemical characterization of the phospho-dependent interaction of Dcbld1 and 2 with the adaptor protein CrkL: Implications for a novel signaling pathway governing the development of the neural retina.**

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Development of the vertebrate nervous system involves complex molecular mechanisms to direct proper neuronal positioning, much of which remains unknown. Discoidin, CUB, and LCCL Domain-containing (Dcbld) 1 and 2 form a novel class of transmembrane orphan receptors that possess similar domain structure to neuropilins, critical co-receptors for neuronal guidance cues. We have previously identified tyrosine (Y) phosphorylation sites in YxxP motifs on the Dcbld2 intracellular domain that are essential for phosphorylation-dependent binding of the signaling adaptors Crk/CrkL (CT10 regulator of kinase/Crk-Like), important effectors in neural development. Here, we demonstrate phosphotyrosine-dependent binding of Dcbld1 to the CrkL-SH2 domain and investigate the effect of Fyn, a representative SFK, and Abl activity on phosphorylation of Dcbld1/2 intracellular YxxP motifs, and subsequent binding of the CrkL-SH2 domain. Although Fyn and Abl each demonstrated the ability to phosphorylate tyrosine residues of Dcbld1/2 and induce CrkL-SH2 binding, Fyn activity had a greater effect on CrkL interaction with Dcbld2, as did Abl for Dcbld1. To determine SFK- and Abl-directed sites on Dcbld2, stable isotope-containing peptide standards were spiked into tryptic digests of immunopurified Dcbld2 expressed in HEK293 cells under kinase active/inhibited conditions. This work characterizes the phospho-dependent interaction of Dcbld1 and 2 with Crk/CrkL and suggests that Crk/CrkL, Fyn and Abl participate in the recently discovered roles of Dcbld1 and 2 in the development of the zebrafish neural retina.

**79. Developmental mechanisms underlying phenotypic variation in Satb2-mediated craniofacial disease**

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Special AT-rich Sequence Binding Protein 2 (Satb2) is a transcription factor that, next to a role in differentiation of upper layer neurons, plays an important role in jaw development and osteogenesis. Individuals with mutations in *Satb2* exhibit a variety of malformations, most of which are associated with the jaw. Malformations of the SATB2-associated syndrome (SAS) include long and occasionally asymmetric faces with small mouths, cleft palates, micrognathia, maxillary hypoplasia, and tooth overcrowding. However, individuals with similar mutations often exhibit phenotypes that show significant variation in severity. Similarly, mice heterozygous for a *Satb2* mutation show a high degree of variability in their jaw lengths and also display jaw asymmetries. Phenotypic penetrance as in this case is thought to have a genetic basis, however, it has been proposed that additional roles may exist for stochastic developmental variation. In particular, mutations may increase variation in gene expression and cell behavior. Here we are proposing several ways to test how varying *Satb2* expression levels lead to differences in osteogenesis and osteoblast survival. RNA FISH and immunohistochemistry in a mouse osteoblast precursor cell line (MC3T3) suggest that *Satb2* gene product expression varies highly between single cells and is possibly linked to the cell cycle. However, during osteogenic differentiation, *Satb2* gene and protein expression becomes more uniform. To further elucidate how *Satb2* mediates variation in osteogenesis, we are examining single cell gene expression in WT and *Satb2*+/- MC3T3 cells, to test the differences on the *Satb2* transcriptional network and its influence on osteogenesis in an otherwise isogenic background.

**80. Assessing Hepatoblast Potency During Murine Liver Development Using Genetic Inducible Fate Mapping**

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The mature liver is composed of a variety of cell types with stereotypical roles and organization, however, the developing liver lacks these cell types and instead is composed of progenitors that produce functionally mature cell types. Many of the signaling pathways, transcriptional regulators, and tissue interactions that direct the differentiation process have been characterized, furthermore, extensive fate mapping has been performed to unveil various lineage decisions within the developing liver. A crucial oversight lies in the assumption that individual hepatoblasts are bipotential produce both hepatocytes and cholangiocytes. To formally test this hypothesis, genetic inducible fate mapping (GIFM) mediated by a tamoxifen inducible tissue specific CreER ($FoxA2^{mcmCre}$) in conjunction with the $Rosa26^{LacZ}$ reporter to establish the threshold of tamoxifen required to recombine and label a single hepatoblast progenitor *in vivo*. Because the label generated by the *LacZ* allele is inherited by all daughter cells, subsequent analysis allows us to assess the fate of individually labeled hepatoblasts. To perform this, embryos collected at particular milestones in development were subjected to a retrospective lineage analysis in the embryonic liver using both hepatocyte markers and biliary markers on X-Gal labeled livers. Additionally, valuable information about the cell cycle rate of the developing liver as well as any oriented growth patterns of labeled clones was recorded.

**81. XMAP215 affects the spatio-temporal guidance of MT in *Xenopus laevis* growth cone**

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During neuronal development, neurons migrate towards their final destination and elongate their axons to form new connections. The structure in charge of the navigation, that senses and interprets cues in the embryonic terrain, is the growth cone. The growth cone is a dynamic structure located at the tip of the axon and is rich in cytoskeletal components that underlie its shape. The axonal growth cone responds to guidance cues by coordinating the cross-talk between microtubules (MTs) and actin filaments (F-actin), that is required for generating an axonal morphological response. The family of plus-end tracking proteins (+TIPs) binds and regulates MT dynamics. Our lab has demonstrated that the +TIP, XMAP215, participates in axon elongation, MT translocation rates and MT trajectory collinearity, features that are dependent on F-actin regulation. Our goal is to determine the XMAP215 contribution to growth cone morphology and to MT/F-actin coupling. For that purpose, we use *Xenopus laevis* spinal cord explants after XMAP215 knockdown (KD). We analyzed the growth cone morphology using spinning disc confocal microscopy. We found that the XMAP215 KD growth cones are larger and have longer filopodia, while the number of filopodia is not altered. By analyzing MT/F-actin coupling using super resolution microscopy, we found that the number of MTs that are coupled to F-actin bundles in the peripheral zone of the XMAP215 KD growth cone is not altered, but the MTs showed an increase in the number and directions of exploring MTs that are not coupled to the F-actin bundles. Additionally, XMAP215 KD MTs are more spread throughout the entire growth cone than in the control. These results show that XMAP215 could have a role in the regulation of spatio-temporal guidance of MTs, a function that is attributed to the actin cytoskeleton. Thus, XMAP215 could be a participant in the MT/F-actin coupling.

**82. Investigating the role of the SWI/SNF chromatin remodeling complex in the differentiation of the invasive phenotype**

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The success of many metazoan developmental programs relies on the ability of specialized cells to transgress basement membranes (BMs). Cancer progression also relies on cellular invasion. Though the developmental and clinical importance of cell invasion is evident, studying its dynamics *in vivo* has proven to be challenging. Using high-resolution microscopy, as well as genetic and cell biological techniques we study the process of anchor cell (AC) invasion during *C. elegans* development. We have recently demonstrated that the conserved nuclear hormone receptor transcription factor, NHR-67, is required to maintain the AC in G1/G0 cell cycle arrest, a requirement for invasive behavior. Independent of cell cycle arrest, the AC utilizes the histone deacetylase, HDA-1, for the generation of invadopodia, and the expression of pro-invasive genes. These results suggest that invasion is a differentiated cellular behavior requiring cell cycle arrest and epigenetic modification of the genome. To identify additional chromatin modifiers that mediate invasion, we are conducting a tissue-specific RNAi screen. To date, we have identified several new pro-invasive genes which encode subunits of the SWI/SNF chromatin remodeling complex. The complex exhibits pleiotropy, and in *C. elegans* it contributes to cell fate specification in the somatic gonad, and cell-cycle exit and differentiation of muscle precursor cells. Here, we show a conserved role for the SWI/SNF complex in coordinating cell cycle arrest, as loss of *swsn-1* results in a mitotic AC. Specifically, we are examining the role of the SWI/SNF complex in maintenance of the post-mitotic state and the regulation of pro-invasive gene expression, through potential interactions with NHR-67, HDA-1, other chromatin modifiers and the cell cycle machinery. Together, these results will provide new insight into the role of the SWI/SNF complex in orchestrating invasive activity.

**83. The Biochemistry of PlexinA Signaling in Zebrafish Eye Development**

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The precise wiring of neuronal processes during development is essential for every task of the nervous system, from movement and sensation to emotion and cognitive functioning. In order to make accurate connections and develop a healthy and functional nervous system, migrating neurons must respond appropriately to extracellular cues. Using the zebrafish as a model, we have shown that one such cue, Semaphorin6A, and its PlexinA receptors are critical for vertebrate eye development. However, the mechanisms underlying this pathway are not yet fully understood. Semaphorin6A is a transmembrane guidance molecule that regulates neuronal migration upon binding to PlexinA receptors. We and others have shown that PlexinA2 and the close family member PlexinA1 interact with, and are phosphorylated by the Src-family tyrosine kinase Fyn. We hypothesize that Fyn-dependent phosphorylation of PlexinA receptors is essential to its downstream signaling and its roles in zebrafish eye development. However, the mechanisms of these interactions are not well characterized. The objective of this study is to determine the functionally-important Fyn-induced phosphorylation events on both PlexinA1 and PlexinA2. Mass spectrometry and biochemical analysis identified the major Fyn-dependent tyrosine phosphorylation sites on PlexinA1 and PlexinA2. We also show preliminary work using CRISPR/Cas9-disrupted *PlexinA2* zebrafish to determine the functional relevance of these phosphorylation events in the development of the vertebrate visual system.

**84. The TALE Factors in Transcription Complex Assembly and hox-Mediated Transcription Activation**

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The TALE factors are a set of transcription factors that comprise the Pbx and Prep/Meis gene families. In zebrafish Pbx4 and Prep1 are maternally deposited factors that are essential for expression of *hoxb1a*. Although Pbx4 and Prep1 can recruit RNA polymerase II and positive transcription elongation factor b (P-TEFb) to *hoxb1a* transcription does not begin until Hoxb1b binds the complex. How Hoxb1b releases the paused complex and where else in the genome this complex may also act remain unclear. To address the former question our lab used JQ1 to inhibit Brd4, which activates P-TEFb. JQ1 specifically reduced expression of the mammalian *hoxb1a* ortholog *hoxb1*, suggesting that Brd4 plays a role in its activation. To address the latter question our lab performed ChIP-seq experiments for Pbx4, Prep1, and Hoxb1b in zebrafish embryos at 12 hours post-fertilization (hpf). The results showed that Pbx4 and Prep1 co-bound more than 26,000 genes but only 299 of those genes also bound Hoxb1b. The genes bound by all three factors primarily play roles in transcription and central nervous system development. Interestingly the TALE factors frequently bind near the well-characterized CCAAT and GC boxes. Nuclear factor Y (NF-Y), a trimeric factor that drives transcription of genes implicated in a wide range of functions, commonly binds the CCAAT box. NF-Y knockout mice die early in development suggesting that it is crucial early on but its role in this regard is poorly understood. Co-IP experiments show that Pbx4 and Prep1 interact with NF-Y, suggesting that the factors may cooperate. Finally, ChIP-seq for Prep1 at 3.5 hpf shows that the CCAAT box is more significantly associated with the TALE factors early in development while Hox binding sites are more enriched at 12 hpf. This suggests that the TALE factors form distinct early complexes with NF-Y and then later form complexes with Hox.

**85. The gang's all here- Investigating role of the zebrafish dmrt gene family in gonadal and sexual development**

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The primary signal for sex determination is highly variable in animals. Hence, it is remarkable that the *dmrt* (*doublesex and mab-3 related transcription factor*) family of genes is frequently involved in sex determination and differentiation across metazoans. Specifically, *dmrt1* has been found to play a critical role in male sexual development in numerous species, including zebrafish. In domesticated zebrafish populations, sex determination is polygenic, and thus the underlying mechanisms have historically been challenging to characterize. In zebrafish, *dmrt1* is integral for normal male specification and development. However, a small number of *dmrt1* mutants adopt male sexual fate, indicating that *dmrt1*-indepedent mechanisms for male sex determination exist. We hypothesize that other *dmrt* genes function in sexual and gonadal development. Zebrafish have five *dmrt* genes: *dmrt1*, *dmrt2a, dmrt2b*, *dmrt3*, and *dmrt5*. All *dmrt* genes are expressed in the gonad, suggesting that they may have roles in sex differentiation or determination. We are generating mutations in individual *dmrt* genes using CRISPR/Cas9 to investigate their potential roles in sexual fate determination. Double mutants with *dmrt1* are also being generated to look for exacerbation of the *dmrt1* mutant phenotype. Homozygous mutants for *dmrt5* are larval lethal, and we found that homozygous mutants for *dmrt2a* are larval lethal as well. We are investigating if these mutants survive to the age of gonadal sex differentiation. Characterization of these genes may lead to new knowledge regarding the players and mechanism of sexual and gonadal development in zebrafish.

**86. Dcbld2 is Essential for the Development of the Zebrafish Retina and Optic Tract**

Helaina Stergas
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**Background:** We previously found the neuropilin-like receptor DCBLD2 capable of scaffolding intracellular signaling molecules involved in neuronal positioning. Neuropilins are best characterized as co-receptors that importantly govern migratory cells of the developing nervous system and vasculature. While Dcbld2 has been shown in mouse and zebrafish to modulate development of the vasculature, the potential role of Dcbld2 in the nervous system hasn't been explored. Here, the role of Dcbld2 was examined during the formation of the highly-ordered neuronal tissue of the zebrafish retina.

**Results:** *In situ* hybridization of developing zebrafish revealed ubiquitous *dcbld2* expression, with strong expression in the developing eye and nervous system. In addition to displaying gross defects in retinal development, *dcbld2* morphant zebrafish exhibited specific disruption of retinal ganglion cell (RGC) differentiation and optic tract formation. We confirmed previous results showing *dcbld2* morphants had defects in the developing vasculature, specifically in the intersegmental vessels of the tail. Expression of human *DCBLD2* mRNA during development rescued the *dcbld2* morphant retinal and vasculature phenotypes.

**Conclusions:** We have uncovered an essential role for Dcbld2 in the developing zebrafish retina, specifically in the differentiation of RGC's and their innervation of the optic tectum. Together these data suggest a novel function in neuronal development and organization for this poorly-characterized, but highly-conserved scaffolding receptor.

**88. The conserved role of Wnt signaling in bilaterian posterior patterning**

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The signaling pathways that control the formation of body plans across the metazoa are highly conserved. One such pathway, Wnt signaling, plays a key role in the formation of the anterior-posterior (AP) axis across metazoan species. During development in many species, the earliest distinguishing feature of posterior identity is active Wnt signaling and an initiation of gene expression changes mediated by the transcriptional co-activator β-catenin. While the importance of Wnts in metazoan posterior patterning is well established, whether the early transcriptional program driven by β-catenin during this process is conserved, is not fully understood. In order to answer this question within the bilateria, we turned to two species, the planarian flatworm *Schmidtea mediterranea* and the basal bilaterian *Hofstenia miamia*. Both species perform whole body regeneration and maintain all tissues through constant cellular turnover during homeostasis. We identified a conserved set of genes that are down regulated early after β-catenin inhibition in both species as well as species-specific β-catenin targets along the anterior-posterior axis. Given the diversity of form across the metazoa, we hope to better understand how conserved transcriptional programs driven by key developmental pathways guide the formation of tissues across evolutionarily distant species.

**89. Wnt signaling regulates head regeneration in the Starlet Sea Anemone *Nematostella vectensis***

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The Starlet Sea Anemone, *Nematostella vectensis*, is an excellent model system for investigating anthozoan cnidarian embryonic development and regeneration, as well as addressing questions about the evolution of developmental and regenerative mechanisms. Like many cnidarians, Nematostella is highly regenerative and can renew any amputated portion of its body, but knowledge of the cellular and molecular mechanisms which regulate regeneration in Nematostella are only beginning to emerge. We are investigating how an amputated aboral fragment of an adult polyp (the physa) is able to regenerate an entire polyp, and how bisected polyp pieces regenerate missing structres with proper oral or aboral polarity and tissue identities. We are surveying the expression and function of key developmental signaling pathways for roles in these regenerative processes using the physa as the model, and we have described a morphological staging system for oral (mouth tentacle) and body regeneration as a starting point for molecular and cellular analysis. In the present study we have investigated the action of Wnt signaling in oral regeneration. The Nematostella genome encodes a full set of Wnts that are orthologous to bilaterian Wnts, and we find that in the amputated physa the full complement of Nematostella *wnt* genes are expressed in a temporal sequence during head and body regeneration. This sequence of *wnt* gene activation is initiated in response to wound-induced MAPK signaling, and the early wnt signals trigger cell proliferation at the regenerating oral pole of the physa. Blocking wnt signaling inhibits oral and body regeneration, demonstrating that wnt signaling is essential for oral regeneration. In the complementary, aboral cut surface of the amputated polyp body, MAPK signaling is also triggered by amputation, but wnt genes are not activated as the physa regrows on the cut aboral end of the polyp. Our findings demonstrate that the wnt signaling pathway is essential for regeneration of oral and body structures from the amputated physa, but that this wnt response is highly polarized and not used for aboral physa regeneration. In Hydra wnt genes are expression and wnt signaling is required for head regeneration, suggesting that head regeneration mechanisms among Hydrozoan and Anthozoan cnidarans are conserved

**90. Differing Roles for Calcineurin Regulatory Subunits in Zebrafish Brain Development**

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Calcineurin (CN), a serine/threonine phosphatase that is integral for immune function, has been a target for immunosuppressant drugs for decades. Use of CN inhibition drugs during pregnancy is of particular concern since little is known about the role of CN during brain development. Additionally, recent research in the field suggests that CN may have a role in Down Syndrome, as the Down Syndrome critical region of chromosome 21 contains a gene that is known to inhibit CN activity. We have previously shown that exposing zebrafish embryos to cyclosporine, a CN inhibitor, has led to decreased brain size in larval zebrafish. The current studies have examined the specific role of CN in the developing zebrafish brain. Zebrafish embryos were injected with varying levels of morpholinos (MOs) against one of the two regulatory subunits of CN (ppp3r1a or ppp3r1b). Brain sizes were assessed at 3 days post fertilization (dpf) and behavioral tests were performed at 5 dpf. At 'medium' injection levels the ppp3r1a MO showed a decrease in all measured brain regions, the ppp3r1b MO injected embryos showed only a decrease in the midbrain size. In addition, we found a significant decrease in zebrafish brain size when embryos were injected with 'low' levels of both MOs together, but separate injections of each MO showed no difference. 'Low' injection embryos underwent behavioral testing and both exhibited abnormal social behaviors. Additionally, ppp3r1b injected larvae were hypoactive compared to control and ppp3r1a injected, while the ppp3r1a exhibited less thigmotaxis behavior compared to the controls and the ppp3r1b injected. Preliminary experiments have shown an increase in apoptosis, in ppp3r1a/r1b morphants when compared with control injected. These results suggest an integral role of CN during early brain development in zebrafish, potentially by regulating apoptosis. The results also suggest that the CN regulatory subunits have different roles in brain and behavioral development.

**91. Developmental defects in the extracellular matrix (ECM) composition of the urinary tract and their link to vesicoureteral reflux (VUR) and bladder diverticulum (BD)**

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VUR is the retrograde flow of urine from bladder toward the kidney due to developmental defects in the junction of ureter with bladder—the ureterovesical junction (UVJ). BD is the pouch formation within the bladder wall due to developmental defects in its musculature. The ability of the UVJ to close during voiding and that of the bladder to withstand rupture during urine collection and expulsion, are dictated by the biomechanical forces exerted by the ECM. Indeed, genetic ECM syndromes such as Cutis Laxa, Marfan, Ehlers Danlos (EDS) and Williams are characterized by the presence of VUR and BD. Using three mouse lines we investigate the effect of developmental defects in the ECM on the UVJ and the bladder. The C57Bl/6J (B6) mice do not exhibit VUR and are used as controls. The C3H/HeJ (C3H) mice exhibit VUR. The *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$ mice on the B6 background are used as models of EDS. We developed a bladder compliance assay to evaluate for risk of rupture and/or formation of BD in the newborn stage (P1). The bladders of *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$ micerupture at lower filling pressures compared to B6 mice, while C3H mice do not rupture. Histological analysis of the bladder and ureter at embryonic day (E) 15, P1 and adult stages shows that the composition of matrix changes with age. At E15 collagen fibres are sparsely detected in the bladder and ureter, while at birth, the ureter and bladder is collagen-rich. Starting at P1, the relative amount of collagens and α-SMA in the ureter and the bladder of C3H mice is significantly higher than in B6 mice. The increase translates into a stiffer UVJ prone to VUR and a stiffer bladder resistant to rupture. The bladder and ureter of newborn *Tnxb*$^{+/-}$ and *Tnxb*$^{-/-}$micehas significantly less collagen, causing a reduction in tensile strength in the bladder leading to its rupture. The results suggest that the ECM develops over time and is critical for maintaining competency of the UVJ and compliance of the bladder.

**92. A novel component of the Sema-PlxnD1 signaling modulates angiogenic patterning**

Jesús Torres-Vázquez
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Our prior work has demonstrated that Semaphorin-PlexinD1 signaling determines fundamental aspects of the vascular pattern, such as the positioning, abundance, and pathfinding of angiogenic sprouts.

In this talk, I will present unpublished data incorporating zebrafish studies and both structural and biochemical approaches to describe the discovery and functional characterization of a new intracellular component of the Sema-PlxnD1 pathway that limits signaling output.

**93. A Somatic Temporal Switch In The Mode Of Wnt Signaling orchestrates Germline Development In Drosophila**

Maitreyi Upadhyay[1], Michael Kuna[1], Sara Tudor[2]
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Germline stem cells (GSCs), both self-renew and differentiate into gametes that pass genetic information to the next generation. Self-renewal and differentiation are regulated by both intrinsic factors in the germ line and by extrinsic factors such as structural support and signaling from the somatic niche. While *decapentaplegic* (DPP, a TGF-β homolog) signaling in the somatic niche promotes GSC self-renewal, little is known about how the somatic niche promotes differentiation. We previously discovered that expression of a Wnt ligand, dWnt4, in the escort cells is critical for GSC differentiation. Wnts are known to signal through either the canonical or the non-canonical pathway to affect processes such as cardiogenesis and wing development. We have previously demonstrated that dWnt4 promotes GSC differentiation via the canonical, β-catenin dependent transcriptional regulation of *innexin-2*, a gap junction protein also required for adhesion. Surprisingly, we observed that loss of non-canonical PCP pathway components in the escort cells also leads to loss of differentiation. Additionally, these components genetically interact with *dWnt4,* suggesting that dWnt4 regulates differentiation through the non-canonical pathway. We find that the non-canonical pathway functions earlier in development, at the larval stages, to regulate differentiation. Intriguingly, we observed that the Wnt canonical reporter, Frizzled 3 is expressed at a lower level in the larval gonad as compared to the adult germaria, while the levels of Wnt non-canonical components, Rho, Rac and cdc42 are higher in the larval gonad as compared to the adult germaria. We hypothesize that unlike the current dogma, which states that Wnts use either the canonical or the non-canonical pathway, dWnt4 employs a novel strategy in the escort cells switching from non-canonical to a canonical arm during development to facilitate proper differentiation of the GSCs.

**94. Effect of Lead on NGF Regulated Embryonic Axonal Development: Novel understanding from chicken embryonic Dorsal Root Ganglion Cultures**

Kristia Vasiloff, Yingcui Li
*University of Hartford, College of Arts and Sciences, United States of America*

Lead consumption continues to be a severe problem, even in developed countries, despite the fact that its effects, including cognitive impairments, mobility issues, and organ damage, are common knowledge. This problem is especially pronounced when lead is present during fetal development, however the effects of lead on fetal neuronal development are still understudied. Nerve growth factor is essential for regulating neuronal growth, proliferating and differentiating axons, and sensory nerve survival; it is also incredibly vulnerable as it is holistically affected by lead toxicity. In this study, we investigated the growth and differentiation of dorsal root ganglion (DRG) cells, cultured from 8-10 day old chicken embryos and exposed to nerve growth factor (NGF) (200ng/ml). Four primary cultures (NGF only, DRG only, Lead only, and Lead & NGF; 12 DRG cells per culture) were observed and compared for six days, using ZEN and QCapture to document axonal growth daily. Repeated experiments and different concentrations of lead served as further levels of treatment. Preliminary results suggested growth and differentiation differences in axonal development. Overall, DRG cells were less dense and have less overall neurite outgrowth when treated with lead, with or without the presence of NGF. Digital phase contrast live images of these cultures were analyzed by Image J and continued analysis by the Sholl method along with immunostaining, using neuron markers with nucleus florescent counterstaining. Our findings showed stunted axonal growth when DRG developed in growth medium concentrated with Lead Acetate Trihydrate comparing to those that have not been exposed to lead during their developmental process.

Support for this work was provided through the Dorothy Goodwin Scholarship to KV by The Women's Advancement Initiative, advancing each woman's potential in the HCW tradition at the University of Hartford, and the Dean's research fund to YL from University of Hartford.

**95. The effects of exogenous nutrient signaling in patterning the amphibian limb regenerate**

Warren Vieira, Stephanie Souza, Kaylee Wells, Catherine McCusker
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Nutrient signaling plays a fundamental role in the patterning and differentiation of cells during embryogenesis and regeneration. Retinoic acid (RA) signaling is one such pathway, critical for the establishment of positional fields in developing embryos and patterning of regenerating limb structures in amphibians. Exogenous RA is, furthermore, able to reprogram positional information within blastema tissue. Vitamin D signaling shares several features with RA signaling, including the use of the same co-receptor to regulate gene transcription. Based on the limited data available, vitamin D signaling is involved in embryogenesis; however, the importance of this pathway in pattern formation is difficult to delineate from tissue differentiation effects. The involvement of vitamin D in patterning during regeneration is also understudied. As the *Ambystoma mexicanum* model system is ideal to study the effect of nutrient signaling pathways on patterning, we tested the effect of exogenous vitamin D on regeneration along both the anterior-posterior and proximal-distal limb axes. The treatment was found to effect patterning as well as differentiation. We hypothesize that exogenous vitamin D, like RA, is able to reprogram positional information in blastema tissue. Further studies are required to validate and determine the mechanisms involved in this process.

**96. Elucidation of Histidyl-tRNA Synthetase's role in zebrafish auditory and visual system development**

Ashley Waldron, Graham Wright, Christopher Francklyn, Alicia Ebert
*University of Vermont, USA*

Histidyl tRNA Synthetase (HARS) is a member of the vital family of enzymes responsible for attaching amino acids to tRNA molecules so that protein translation can occur. Recent observations suggest that there may be more to this enzyme than originally thought. One such observation, the association of HARS with a deafness-blindness disorder called Usher Syndrome, prompted us to look at the function of HARS in retinal and auditory tissues. Using the zebrafish as a model we have found that while HARS is expressed ubiquitously throughout zebrafish larvae, it appears to be most highly expressed in the early retina and the ear. We have also found that these tissues are particularly sensitive to misexpression of HARS during development. Specifically, we found that while much of an embryo's overall morphology is maintained, HARS knock-down results in smaller retinas, with fewer neurons and fewer neuromasts of the lateral line system, an external mechanosensory system frequently used as a proxy for the auditory system. Interestingly, HARS overexpression also appears to disrupt the development of these two systems. These results suggest precise regulation of HARS in these systems is required for proper development. Our observed phenotypes are reminiscent of phenotypes found when some cell cycle genes are disrupted. In support of a role for HARS in cell cycle regulation, other studies have found that HARS is required for cell cycle progression in stable cell lines via regulation of cyclin accumulation. With this in mind, we have begun exploring the whether HARS may be required for cell cycle progression in the developing retinal and auditory systems.

**97. cdk21 encodes a putative cell cycle regulator of germ cell mitosis in zebrafish**

Kaitlyn Webster, Jaclyn Grenier, Cristina Rivera, Kellee Siegfried
*University of Massachusetts Boston, USA*

Germ cell survival and differentiation relies on complex regulation of mitotic and meiotic progression. Cyclin-dependent kinases (CDKs) and their activating Cyclin partners are known to have highly specialized roles in stem-like populations across species, including germ cells, but these are only well characterized in mouse and human cancers. Very little is known about CDK/Cyclin function in zebrafish, or the regulation of germ cell maintenance and differentiation. Using a mutagenesis screen for gonadogenesis defects in zebrafish combined with exome sequencing, a mutation affecting *cdk21* (*cyclin-dependent kinase 21*) was identified. The *cdk21* gene is unique to fishes, though the encoded protein is highly similar to the D-cyclin partners, Cdk4 and Cdk6, which are known to be upregulated in human germ cell tumors. The *cdk21* mutation disrupted the kinase domain of the Cdk21 protein (*cdk21*$^{G204W}$*)* and was tightly linked to early gonad hypoplasia and dysgenesis, gradual germ cell depletion, and 100% male sex development in zebrafish. Interestingly, thisphenotype most closely resembles that of mouse *Cyclin A1* mutants (a partner of G2 CDKs 1 and 2). Histological evidence of aberrant cell division in developing *cdk21* mutants suggests that this gene is necessary for maintenance of mitotic germ cells in the testis, and ultimately the renewal of spermatogenic cells through cell-cycle regulation. Expression analysis revealed sexually dimorphic expression and indicates that *cdk21* expression is restricted to testicular germ cells. We propose that *cdk21* is a male sex-specific regulator of the cell cycle in zebrafish, which is necessary fortesticular germ cell mitosis and sustaining fertility.

**98. How is proportionality determined in regenerating Ambystoma mexicanum limbs?**

Kaylee Wells[1], Roni Milgrom[1], Larissa De Souza[1], Warren Vieira[1], Julian Sosnik[1,2], Catherine McCusker[1]

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The Mexican Axolotl (*Ambystoma mexicanum*) is one of the few tetrapod species capable of regenerating complete limbs. One significant, and understudied, aspect of limb regeneration is the mechanism by which the regenerated structure grows to the size that is proportionally appropriate to the size of the animal. In larger animals, it is apparent that growth of the limb regenerate occurs in two stages. The first stage includes the formation of the early regenerate (the blastema) and its growth, patterning, and differentiation into the missing limb structure. This initial limb regenerate is proportionally very small in size relative to an uninjured limb. During the second stage of growth, the "tiny limb" grows at a rapid rate relative to the rest of the animal, until it reaches the appropriate size. The mechanisms that maintain rapid growth on the tiny limb, and slow this growth once the regenerated limb has reached the appropriate size are not known. One potential factor is nerve signaling; if the nerve is continually deviated from the late stage blastema, it will regenerate a tiny limb that does not grow to reach the appropriate proportional size. Additionally, while the nerve is necessary for the growth during the first stage (blastema development), we have discovered that the growth factors associated with this step are decreased in expression during the "tiny limb", suggesting that different nerve-derived factors are essential for the second stage of growth. We are employing molecular, biochemical, and classical embryology techniques to explore the role of the nerve in signaling proportionality as well as other mechanisms of organ size regulation.

**99. Targeting candidate genes for a small-eye zebrafish mutant**

Nicole Winchester

*SUNY Geneseo, USA*

A chemical mutagenesis screen for eye defects in zebrafish revealed the *good effort* (*gef*) mutant. This mutant is characterized by normal morphology for the first two days post fertilization (dpf), followed by elevated cell death, most notably in neural, branchial arch, limb bud, and retinal cells. The gef mutants were found to have a 3-bp deletion in the *chromosome assembly factor 1b* (*chaf1b*) gene at the splice donor site of exon 3, resulting in severe protein truncation. Chaf1b is a subunit of the Chromatin Assembly Factor 1 complex which loads histones onto newly synthesized DNA during S phase, facilitating chromatin formation. Loss of Chaf1b function is believed to result in accumulation of DNA damage and triggering of tp53-mediated apoptosis. The recent development of the targeted genome-editing technique, CRISPR/Cas9, represents a method to knockout multiple genes. We developed a multiplex CRISPR DNA construct to determine the role Tp53 plays in structuring the *gef* phenotype. This five guide RNA vector will simultaneously target *chaf1b* to generate a gef phenocopy, *tp53*, *puma*, and *caspase-3a* to inhibit apoptosis, and *tyrosinase* to induce an albino phenotype as an indicator of knockout success.

**100. Planarian epidermal stem cells respond to positional cues to promote cell type diversity**

Omri Wurtzel[1,2,3], Isaac M. Oderberg[1,2,3], Peter W. Reddien[1,2,3]
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Successful regeneration requires that progenitors of different lineages form the appropriate missing cell types. However, simply generating lineages is insufficient. Cells produced by a particular lineage often acquire specialized functions depending on their position within the organism. How this process occurs and supports the dynamic body plan of regenerating organisms is largely unexplored. In planarian regeneration, new cells arise from a proliferative stem cell population (neoblasts), which generate a myriad of cell types. We used the planarian epidermal lineage to study how the cellular location of adult progenitor cells results in the acquisition of distinct functions in differentiated cells. Single-cell RNA sequencing of cells from throughout the epidermal lineage revealed the emergence of distinct epidermal spatial identities as early in the lineage as the epidermal stem cells, eight days before the cells complete their maturation. Establishment of dorsal-ventral epidermal functions required neoblasts, which responded to BMP signaling from a distinct muscle cell population. Our work identified positional signals that activate regionalized transcriptional programs in the stem cell population and subsequently promote cell type diversity in the epidermis.

**101. Expression of cypin in *Xenopus laevis***

Jingzong Yan, Jackson Bowers, Laurie Hayrapetian, Sangmook Lee, Laura Anne Lowery
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Cypin, a guanine deaminase, is critical to neuronal development and dendrite branching. Studies from another lab have shown that cypin is capable of binding to tubulin heterodimers and promote microtubule polymerization *in vitro*. Cypin has a CRMP homology domain that is critical in modulating its guanine deaminase activity and directly affects dendrite outgrowth. Others have shown that overexpression of cypin showed an increase in dendritic branching, both primary and secondary. However, no previous studies have examined the role of cypin during development. In our lab, we seek to gain a better understanding of cypin and its function during *Xenopus* embryonic development. We have begun by determining the localization of cypin expression at different developmental stages of *Xenopus laevis* embryos. RT-PCR data suggests that cypin is expressed in low amounts at younger developmental stages. Through *in situ* hybridization, we found that cypin expression is highly enriched in the otic vesicles, suggesting a novel function of cypin in the development of the auditory system.

**102. Effects of Prenatal Music Stimulation on Embryonic Development of *Gallus gallus***

Emma Strujo, Cliff Simon Vital and Poongodi Geetha-Loganathan

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It is a common belief that listening to classical music such as Beethoven or Mozart will promote fetal brain development. Although there has been no solid scientific evidence, earlier studies on chick embryos and rat pups have shown slight increase in size of neuronal cells when exposed to classical music such as Mozart during late developmental stages. However, there have not been studies on the effect of different genres of music on development as well as the effect of noise and music on early development. Here we investigate the effect of different genre music (classical/rock) on early embryonic development in chicken embryos as pregnant women are constantly exposed to music even during early pregnancy. We tested two different types and decibel levels of classical and rock music on embryo development. To provide music impulse an iPod with a playlist connected to speaker was set inside the incubator and the music played for every 15 min with a 45 min of recorded silence in between. Control eggs were incubated at the same condition but received no sound impulse. Following incubation the embryos were fixed at two different stages, day 9 and 16, to analyze the phenotypes causes by sound exposure. Also the morphological parameter such as height, weight, forelimb/hind limb length, beak size, and eye diameter were measured. We found that high decibel music (HDM) irrespective of the genre increased mortality rate in chicken embryos. Further HDM resulted in severe morphological defects due to delayed development. We presently investigate the effect of different levels of music on early embryo development and the time window during development at which the loud music is affecting morphogenesis. Results derived from our study can be applicable to other vertebrates including humans, will lead to better understanding on how the environment to which pregnant women expose themselves impact the development of the embryo.

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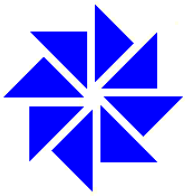

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