Program Abstract #1
Skin-resident immune cells actively coordinate their distribution with epithelial stem cells during homeostasis
Valentina Greco
Yale University, USA
Our organs consist of multiple cell types that ensure proper architecture and function. How different cell types coexist and interact to maintain their homeostasis in vivo remain elusive. The skin epidermis comprises mostly epithelial cells, but also harbors Langerhans cells (LCs) and Dendritic Epidermal T cells (DETCs). In response to injury or infection, these epidermal immune cells become activated and play critical immunological roles. During homeostasis, they coexist with epithelial stem cells in the basal layer of the epidermis. Whether, and how, epidermal immune cell distribution is regulated during homeostasis is unclear. Here, we addressed this question by tracking the same LCs, DETCs and epithelial stem cells over time within the skin of live adult mice. We show that epidermal immune cells gradually adjust their position as neighboring epithelial stem cells continuously self-renew and differentiate. Moreover, LCs and DETCs rapidly and maximally explore epithelial stem cell junctions through their dendritic movements. Intriguingly, we found that altering the epithelial cell density triggers corresponding changes in the immune cell density, but not vice versa, suggesting that epithelial cells determine immune tissue composition in the epidermis. Moreover, epidermal immune cells are organized in a tiling pattern that is actively maintained: when LCs are ectopically removed, neighboring epidermal LCs move into the emptied spaces and re-establish the pattern. Finally, we showed that the GTPase Rac1 is required in LCs to maintain their dendritic morphology, limited mobility and tiling pattern. Overall, we discovered that the spatial distribution of immune cells is highly regulated in homeostatic epidermis, at least in part by the epithelial stem cells. We propose that these cellular mechanisms provide the epidermis with an optimal response to environmental insults.

Program Abstract #2
Breaking the Culture Bias in Science
Mary Alice Scott
New Mexico State University, USA
In this talk, I argue that although it is sometimes difficult to identify “culture” in science because the two concepts are frequently opposed, science does have culture(s). When unexamined, scientific culture can limit the potential of scientific endeavors and exclude the diverse ideas that could propel the field in new and productive directions. I base these arguments on anthropological work in science and technology studies as well as my own ethnographic research on cultures of medicine and medical education that I have conducted in collaboration with physicians and other social scientists over the last five years. In closing, I offer some practical tools for beginning to identify and assess cultural values in scientific practice.

Program Abstract #3
A Carrier Bag Theory of Non-Fiction: Removing COWDUNG and Prick Tales from our Biological Narratives
Scott Gilbert
Swarthmore College, USA
Humans are the story-telling species. While language can transmit information between contemporaries and from generation to generation, stories provide the framework for understanding, appreciating, and using this information. Scientific stories are those narratives that integrate data into coherent testable frameworks. These
stories are expected to be "universal"; however, they are constructed by people who live within specific religious, ethnic, and other social narratives. Ursula K. Le Guin (1986) famously wrote of the masculinist construction of fictional narratives in "The Carrier Bag Theory of Fiction." Such stories of heroic competition and annihilation are called "killer stories" by Le Guin and "prick tales" by Donna Haraway (a social theorist who was trained as a developmental biologist). Here, this analysis is extended to non-fictional hero myths, such as those seen in our accounts of fertilization and microevolution. Indeed, biological science has crafted alternative stories that, I will claim, fit the data better, and which open our science to an appreciation of the abundance of cooperation and reciprocity. Fertilization is a tale of two immature cells activating each other into maturity; and microevolution requires the drama of co-developing with numerous symbionts. Culturally biased narratives, reflecting and reproducing the dominant social view (what embryologist C. H. Waddington called "COWDUNG—the conventional wisdom of the dominant group"), are damaging not only to individuals who are not part of that culture, but they are damaging to science, itself. Having multiple stories and biologically more appropriate stories is one of the reasons for the inclusion of marginalized people into science. As Haraway said, "It matters what stories tell stories." Funded by a Swarthmore Collee Faculty Research Grant

Program Abstract #4
Patterning the microtubule cytoskeleton during development
Ariana Sanchez, Lauren Cote, Tess Branon, Alice Ting, Jessica Feldman
Stanford University, USA
Microtubules are organized by cellular sites called microtubule organizing centers (MTOCs). The vast majority of research on MTOCs has focused on the centrosome, an organelle used by animal cells to organize microtubules during mitosis. In differentiated cells, however, MTOC function is often reassigned to non-centrosomal sites, and these non-centrosomal MTOCs (ncMTOCs) are critical for cell function. Despite the ubiquity of ncMTOCs across cell types and organisms, ncMTOC components remain largely unidentified. To address this gap in knowledge, we developed biotin-based proximity labeling in living C. elegans with TurboID, a fast-acting biotin ligase mutant that labels proximal proteins for subsequent identification. By expressing a transgene of TurboID fused to the ncMTOC component PTRN-1 in differentiated intestinal cells, we spatially enriched biotinylation to ncMTOCs at the apical membranes. We identified 69 proteins proximal to PTRN-1 and focused on two conserved proteins: VAB-10, a spectraplakin whose orthologs have non-centrosomal microtubule functions, and WDR-62, a protein we identify as a homolog of vertebrate WDR62, mutations of which are associated with primary microcephaly. Using endogenous localization and depletion studies, we found that VAB-10B and WDR-62 appear to independently regulate the growth and localization of non-centrosomal microtubule arrays; depletion of VAB-10B results in disorganized microtubules and delayed localization of the microtubule nucleation complex g-TuRC while loss of WDR-62 decreases microtubule numbers and abolishes g-TuRC localization. These changes in ncMTOC formation occur downstream of cell polarity and suggest a division of labor model where microtubule anchorage and nucleation are regulated by distinct complexes. Moreover, as this is the first report for non-centrosomal roles of WDR62, our study expands the basic cell biological role of this important disease protein. Funding: NIH DP2GM119136-01, 2T32GM007276

Program Abstract #5
How to build a necessary wall: progenitors apically polarize WNT inhibitors to orchestrate tissue development
Irina Matos
The Rockefeller University, USA
To spatially co-exist and differentially specify fates within developing tissues, morphogenetic cues must be correctly positioned and interpreted. Here, we investigate mouse hair follicle development to understand how morphogens operate within closely spaced, fate-diverging progenitors. Coupling transcriptomics with genetics, we show that emerging hair progenitors produce both WNTs and WNT inhibitors. Surprisingly, however, instead of generating a negative feedback loop, the signals oppositely polarize, establishing sharp boundaries and consequently a short-range morphogen gradient that we show is essential for three-dimensional pattern formation. By establishing a morphogen gradient at the cellular level, signals become constrained. The progenitor preserves its WNT signaling identity and maintains WNT signaling with underlying mesenchymal neighbors, while
its overlying epithelial cells become WNT-restricted. The outcome guarantees emergence of adjacent distinct cell types to pattern the tissue. The author was recipient of a postdoctoral fellowship from Women & Science at The Rockefeller University

Program Abstract #6
An adhesion code ensures robust pattern formation during tissue morphogenesis
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An outstanding question in embryo development is how spatial patterns are formed robustly, despite large scale cellular re-arrangement required during tissue morphogenesis. In the zebrafish spinal cord, thirteen distinct cell types are specified along the ventral-to-dorsal axis to form stripe-like patterns, under the instruction of opposing gradients of Shh and BMP/Wnt. The Shh signal is noisy, resulting in cells specified in a mixed pattern initially. In addition, concurrent morphogenetic movement could further disrupt patterns. Nevertheless, the stereotypic stripe-like patterns still form reproducibly. To understand how adhesion-based mechanism may assist in patterning robustness, we developed two cell-based mechanical assays based on micropipette aspiration to measure adhesion forces and preferences among three types of spinal cord neural progenitors (p3, pMN, and p0 cells). Interestingly, each cell type exhibited preference to stabilize homotypic contact and adhered more strongly to cells of the same type. Subsequent genetic analyses revealed three adhesion molecules (N-cadherin, Cadherin 11, and Protocadherin 19) that are differentially expressed among the three cell types, forming a three-molecule adhesion code. When the adhesion code is perturbed, the adhesion preference to cells of the same type is lost, and the neural progenitor pattern in the spinal cord is disrupted in vivo. Both the cell fate and adhesion code are co-regulated by the common upstream morphogen signal Shh. We propose that robust patterning in tissues undergoing morphogenesis results from a previously unappreciated interplay between morphogen gradient-based patterning and adhesion-based self-organization. Our findings connect adhesion specificity at the molecular level to the sorting behavior at the cellular level that explains mechanisms of tissue-scale pattern formation. This work is funded by NIGMS, NICHD, Damon Runyon Fellowship, Burroughs Wellcome Fund, and The Company of Biologists.

Program Abstract #7
Making heads or tails of an embryo: differential foxd3 regulation in the cranial neural crest and tailbud neuromesodermal progenitors
Martyna Lukoseviciute, Tatjana Sauka-Spengler
The MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

Foxd3 transcription factor acts either as a chromatin activator or repressor to mediate neural crest (NC) specification and differentiation. Here, we investigated auto-regulation of the foxd3 locus during early embryonic patterning and uncovered two foxd3 cis-regulatory elements, one active in the cranial and the other in the caudal embryo region. The cranial enhancer is activated while the caudal enhancer is repressed by foxd3 in NC cells. By exploring new transgenic lines reporting both enhancer activities, we have discovered that unlike cranial foxd3 enhancer active in the NC, the caudal foxd3 enhancer labelled bipotent neuromesodermal progenitors (NMps). In the NMps, foxd3 is expressed at minimal levels, where its expression is maintained by a different set of enhancers in comparison to NC cells. By integrating single-cell transcriptional and epigenomic profiles we are reconstructing the global gene regulatory network underlying NMp specification in amniotes, where NMp contribution to posterior axis extension was previously debatable. We show, in vivo and ex vivo, that zebrafish embryos maintain a bipotent tailbud NMp pool after gastrulation, capable of depositing mesodermal and neural progenitors in the developing tail. Furthermore, we have also compared single-cell transcriptomes of foxd3-control and foxd3 knockout cells, which has revealed that loss of foxd3 leads to the expansion of NMps at the expense of NC cells. Interestingly, foxd3-mutant NMps have some NMp-mesodermal genes down-regulated and some NMp-neural genes up-regulated hinting towards foxd3 importance for the bipotent NMp differentiation trajectory split.
Strikingly, we also identify an intermediate cluster between the NC and NMp cells, suggesting shared features and possibly common origin of trunk NC and NMp programmes in vivo. Funding: Wellcome Trust Senior Research Fellowship (215615/Z/19/Z) to T.S.S.; RDM Scholarship and Oxford-MRC Doctoral Training Partnership Supplementary Funding to M.L.

Program Abstract #8
Reading a gradient twice: the cis regulatory logic of a multi-enhancer system
Peter Whitney
New York University, USA
Metazoan transcription is controlled by non-coding elements called enhancers. Enhancer activity is determined by the transcription factor binding motifs found in their sequence, allowing combinations of transcription factors to drive precise gene expression in space and time. Many developmentally regulated enhancers work together, often acting on the same gene simultaneously. These so-called “shadow enhancers” are thought to confer developmental robustness in response to environmental or genetic insult. Shadow enhancers encode similar transcription factor binding sites and drive broadly overlapping regions of expression. However, little is known about how systems with multiple enhancers coordinate their activity mechanistically. Here we present the first steps for understanding how a pair of shadow enhancers work together to drive the expression pattern seen in the developing Drosophila embryo. Using CRISPR-Cas9, we created multiple enhancer deletions for the gene short gastrulation (sog). Differences in total transcriptional output of the sog locus in each enhancer deletion line result in changes in downstream tissue patterning through altered BMP signalling and lower embryo viability. Using a novel internally controlled in situ expression assay, we quantify the perturbations in expression caused by enhancer deletions at the single cell level across the Dorsal morphogen gradient. Through high-resolution microscopy, we demonstrate that deletion of each enhancer alters the transcription factor microenvironment surrounding the site of transcription, indicating that shadow enhancers work together to drive local transcription factor accumulation. This differential transcription factor accumulation observed in our enhancer mutation lines is correlated with differences in transcriptional output, providing further evidence that the transcription factor microenvironment is an important component of transcriptional activation. T32 NIH Training Grant, Developmental Genetics.

Program Abstract #9
Tension heterogeneity instructs morphogenesis and fate specification during heart development
Rashmi Priya, Srinivas Allanki, Alessandra Gentile, Shivani Mansingh, Hans-Martin Maischein, Didier Stainier
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A central question in developmental biology is how morphogenetic patterns and cell fate emerge stochastically during organogenesis. During cardiac development, the myocardial wall transforms from a monolayer to an intricate topological structure consisting of two distinct types of cardiomyocytes (CMs): outer compact and inner trabecular layer CMs. This process of cardiac trabeculation is crucial for cardiac function. Yet, the mechanisms underlying the emergence and specification of trabecular CMs remain unknown. Using the zebrafish heart in combination with high-resolution quantitative microscopy, in vivo measurements of tension/subcellular dynamics and genetic mosaic tools, we now report that contractility couples morphogenesis and cell fate to ensure robust self-organization of CMs into compact versus trabecular layer. Proliferation induced crowding triggers symmetry breaking by generating local differences in cellular contractility. These effects lead to stochastic delamination of CMs from the outer compact layer to seed the inner trabecular layer. By manipulating contractility at the single cell-level, we show that reducing contractility abrogates delamination while inducing contractility augments delamination, and strikingly, inducing contractility is sufficient to drive delamination even in the absence of critical trabeculation signals like Nrg/ErbB2 or blood-flow. Further, using controlled perturbations to decouple mechanical cues from biochemical signaling, we find that mechanical cues drive CM fate specification. Inducing tension heterogeneity (and thereby CM delamination) by manipulation of cell density or contractility is sufficient to generate differential Notch activity as well as polarity. Overall, our study reveals how complex multicellular systems emerge from simple interactions between individual cells. Funding – Max Planck Society, EMBO, Humboldt Foundation
Program Abstract #10

Hemichordate anteroposterior patterning in contrasting life history strategies.

Paul Bump, Paul Gonzalez, Christopher Lowe

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Hemichordate are a deuterostome phylum, sister group to echinoderms and closely related to chordates. They are an important phylogenetic position for understanding the evolutionary origins of vertebrate developmental mechanisms. On a morphological level, it is challenging to make direct comparisons with chordates, but our studies on early development have revealed that despite this morphological disparity, early embryonic patterning shows really remarkable similarities with vertebrates, especially during early anteroposterior patterning. Like most invertebrate phyla, hemichordates exhibit a wide range of early developmental life history strategies. In one group of enteropneust worms, the embryo gives rise to a larval body plan that exhibits a striking morphological contrast to the adult that it will give rise to much later in development, after an extended period of feeding and development in the plankton. In the other group, like chordates, the embryo gives rise directly to the adult without an intervening larval stage. I will review the early anteroposterior patterning program of the direct-developing hemichordate *Saccoglossus kowalevskii* to demonstrate the close patterning similarities with chordates. I will then present anteroposterior patterning data in *Schizocardium californicum*, an indirect-developing species, that reveals significant differences in early posterior patterning with *S. kowalevskii*. I will discuss the significance of these differences and the importance of considering life history strategies in broad body plan evolutionary comparisons of bilaterian developmental mechanisms. This work was supported by NASA Exobiology, and Chan Zuckerberg BioHub.

Program Abstract #11

Medaka gli3 mutants reveal deep conservation of fin/limb developmental programs

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One important question in evolutionary biology is to try to understand how appendages (fins) first emerged and then changed during vertebrate evolution (limbs). Despite it was previously suggested by other research groups, we recently demonstrated with functional experiments that tetrapod paired limbs evolved from fish paired fins. By using the CRISPR/Cas9 technology in medaka fish (*Oryzias latipes*) we deleted the cis-regulatory elements responsible for the expression of the *shh* gene in posterior developing paired fins. This mutant fish (ZRS+sZRS) lack *shh* expression in early fin buds and show complete truncation of the paired appendages, mimicking the phenotype observed in mouse limbs lacking *Shh* activity. The *Gli3* gene is also expressed in vertebrate appendages during embryonic development but, in contrast to *Shh*, it is restricted to the anterior region of the developing bud. Limbs from a mouse mutant for *Gli3* show digit polydactyly in a *Shh*-independent process, as appendages lacking both *Gli3* and *Shh*, mimic the polydactyly phenotype found in single *Gli3* mutants. In order to reveal homologies between fish fin and tetrapod limb skeletal elements, we transiently knocked-down *gli3* in medaka wild-type and ZRS+sZRS mutants using CRISPR/Cas9. In these experiments, we found that *gli3* removal in the ZRS+sZRS mutant background completely rescue paired fins similar to what observed in the *Shh/Gli3* mouse double mutant. In addition, we found that *gli3* medaka mutants show extra proximal radial bones in pectoral fins, a phenotype strikingly similar to the polydactyly defect observed in mouse limbs lacking *Gli3* activity. These functional experiments strongly suggest that fin and limb formation rely mainly on deep evolutionary conserved developmental programs that, with some modifications but maintaining the same toolkit of genes, can generate a diverse array of appendages form. Funding: FONDECYT GRANT #11180727 to JL, ERC GRANT #740041 to JLGS

Program Abstract #12

Morphogenesis is stressful – Elastic properties of folding cell sheets

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Events of cell sheet folding are essential during development, examples including gastrulation, neurulation, and
organogenesis. Due to the complexity of morphogenesis in traditional model organisms we still cannot predict how local cellular changes translate into shape changes at the tissue level. The micro-algal family Volvocales is uniquely suited for studies on epithelial morphogenesis from the subcellular and cellular to the tissue and organism scales. Unlike higher organisms, embryos within this order achieve cell sheet folding without cell divisions, intercalation or migration, which facilitates both experimental and computational approaches. Volvocalean embryos consist of bowl-shaped or spherical cellular monolayers which invert their curvature in order to expose their flagella. *Volvox globator* exhibits one of the most striking processes of cell sheet folding: Through inwards folding at the equator of the initially spherical cell sheet it adopts a mushroom shape and eventually turns itself entirely inside-out through an anterior opening [1]. These global deformations are driven by several waves of active cell shape changes [2, 3]. A combination of advanced imaging and computational analyses is used to correlate cell shape changes, tissue contractility and the occurring tissue invagination and involution. The associated internal stresses are determined through laser ablation experiments which allow conclusions on the underlying forces as well as the elastic properties of dynamic cell sheets [manuscript in preparation]. This research is supported by a Wellcome Trust Investigator Award, 207510/Z/17/Z. [1] Höhn S and Hallmann A. *BMC Biology* 9, 89 (2011). [2] Höhn S, Honerkamp-Smith AR, Haas PA, Khuc Trong P, and Goldstein RE. *Physical Review Letters* 114, 178101 (2015). [3] Haas PA, Höhn S, Honerkamp-Smith AR, Kirkegaard JB, and Goldstein RE. *PLOS Biology* 16, e2005536 (2018).

### Program Abstract #13
**A Spatial Gradient of Cell Size Regulates Genome Activation and Vertebrate Early Development**

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In early embryogenesis, embryos divide without growth. After rapid reduction in cell volume, the embryos turn on nascent transcription in a process named zygotic genome activation (ZGA), in which development switches from maternal to zygotic control. ZGA is essential for subsequent gastrulation and germ-layer specification, but the pattern of ZGA and its implication on embryonic development is less studied. Through metabolically labeling of nascent transcripts, we have identified a spatial and temporal pattern of ZGA in *Xenopus* embryos that occurs initially in small cells at the animal pole and is delayed by two hours in cells of the vegetal pole. This spatiotemporal pattern tightly correlates with the gradient of cell sizes present in the blastula embryo, which induced by the asymmetric cell division and the difference in division timings. To study the developmental importance of this evolutionally conserved cell size gradient and the resulting ZGA pattern, we developed an embryo temperature controller to eliminate or even reverse the pattern. It works by slowing down the division rate of the cells in the animal pole and speeding up the division of the vegetal cells. By generating *Xenopus* blastula embryos with reversed cell size gradients, we found that the spatial pattern of ZGA is also reversed; genome activation occurs first in the vegetal pole and is drastically delayed in the animal pole. Intriguingly, the ‘reversed’ embryos still have a similar threshold of cell size for genome activation onset, regardless of which the temperature gradient they have experienced. Those ‘reversed’ embryos tend to arrest in gastrulation as they fail to close the blastopore before neurulation. This work suggests that regulated spatial patterns of ZGA are important for coordinated embryonic development, particularly in blastula embryos that contain gradients of cell sizes. We are grateful to the funding source of the National Institute of General Medical Sciences (R35GM12874802).

### Program Abstract #14
**Insect wings and body wall evolved from ancient leg segments**

Heather Bruce

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The origin of insect wings has long been debated. Central to this debate is whether wings are a novel structure resulting from gene co-option, or evolved from an exite (outgrowth, e.g., a gill) on the leg of an ancestral crustacean. Here we report the phenotypes for the knockout of five leg patterning genes in the crustacean,
Parhyale hawaiensis, and compare these to their previously published phenotypes in Drosophila and other insects. This leads to an alignment of insect and crustacean legs that suggests that two leg segments that were present in the common ancestor of insects and crustaceans were incorporated into the insect body wall, moving the proximal exit of the leg dorsally, up onto the back to later form insect wings. Our results suggest that insect wings are not novel structures, but instead evolved from existing, ancestral structures. This work is supported by the National Science Foundation IOS to NHP and Graduate Research Fellowship to HSB.

Program Abstract #15
Axis specification in zebrafish is robust to cell mixing and reveals feedback from morphogenesis to pattern formation
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A fundamental question in developmental biology is how the early embryo breaks initial symmetry to establish the spatial coordinate system later important for the organisation of the embryonic body plan. In zebrafish, this is thought to depend on the inheritance of maternal mRNAs, cortical rotation to generate a dorsal pole of beta-catenin activity, and the release of Nodal signals from the yolk syncytial layer. To test whether aggregates can break symmetry in the absence of the geometrical constraints of the yolk, embryonic cells were explanted at the 256 cell stage and observed to elongate after 5 hours of culture. Extensive cell mixing prior to elongation of the explant demonstrates that any pre-existing asymmetry present is lost prior to the breaking morphological symmetry. This reveals that that the maternal pre-pattern is not strictly required for early embryo patterning but a nodal pole is required for elongation. This elongation has been shown to be the driving force which separates the anterior BMP from the posterior Wnt/TCF activity domain. Blocking of PCP-dependent convergence and extension disrupts this separation of the opposing domains and therefore prevents proper anterior posterior patterning of neural tissues. These results together suggest that morphogenesis plays a causal role in the establishment of morphogen gradients and pattern formation during zebrafish gastrulation. Funded by Royal Society/Wellcome Trust Sir Henry Dale Fellowship, Cambridge Trust Vice Chancellor’s Scholarship and EMBL Barcelona.

Program Abstract #16
A self-generated Toddler gradient directs mesodermal cell migration during zebrafish gastrulation
Jessica Stock¹, Edouard Hannezo², Andrea Pauli³
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Directed cell migration is one of the underlying mechanisms that coordinate essential tissue arrangements during embryogenesis. The majority of these arrangements occur during gastrulation, during which complex global cell movements form the three germ layers. In gastrulating zebrafish embryos, mesoderm is specified at the margin and then undergoes directed migration to the animal pole. However, the molecular mechanisms underlying mesoderm guidance have remained a mystery. Traditionally, directed cell migration is guided by a chemokine gradient that arises from a localized source. In contrast to this idea, we discovered that mesoderm migration is guided by a self-generated gradient of the small protein Toddler/Apela/ELABELA, and that gradient formation is coordinated by its own receptor, the Apelin receptor (Aplnr), which is expressed in the responding mesodermal cells. Our studies show that Toddler acts as a chemoattractant for Aplnr-expressing cells, yet rescue of mesoderm migration defects in toddler mutants is independent of the site of Toddler expression. To reconcile these contradicting results, we combined computational modeling and experimental approaches to show that (i) the location of the sink, not the source, determines direction of mesoderm migration, (ii) a collective of Aplnr-expressing cells, but not an individual cell, can undergo directed migration in a uniform Toddler environment and (iii) Aplnr takes on a dual role by providing a sink for Toddler at the margin and sensing the self-generated gradient to drive mesoderm migration. Taken together, our work provides a compelling explanation for the long-standing question of how mesodermal cells are directed to the animal pole during zebrafish gastrulation. This model of a self-generated gradient, in which generation and reading of the guidance cue are mediated by a single receptor, provides a simple yet robust mechanism for mesodermal cells to steer their own directional migration.
Program Abstract #17
Cellular and structural orchestrations that sustain skin regeneration captured by live imaging
Shuangshuang Du, Valentina Greco
Yale University, USA

Organ regeneration is sustained by the orchestration of various cells/structures. Within the skin, fibroblasts, epidermal cells and collagen exist in close proximity. While a role for fibroblasts and their secreted collagen in epithelial stem cell proliferation has been established in vitro, whether and how these key cells and structures interact in vivo to sustain regeneration is unknown. Thus, I leveraged our two-photon imaging to track fibroblasts, epidermal cells and collagen simultaneously in intact live mice. I found that on average one fibroblast can extend its membranes upwards to directly contact 2 epithelial stem cells, although its footprint spans ~25 epithelial cells, which may implicate selectivity for intracellular communication. To test whether fibroblasts act as a stem cell niche, I utilized fibroblast genetic ablation, which resulted in defects in both the adjacent epithelial stem cell layer and the overall epidermal architecture. First, the resulting stem cell layer showed planar polarity compared to random cell geometry in the control. Second, the epidermis was thicker due to increased stem cell differentiation. Next, I leveraged an established Rac-deficient model, where fibroblast membrane activities and coverage are reduced, to further dissect the consequence of altered fibroblast-epithelial interactions. Strikingly, the epidermis in the Rac deficiency phenocopies the ablation model, suggesting that fibroblast membrane coverage rather than density sustains epithelial regeneration. Intriguingly, collagen in the Rac1-deficient model aligned with the orientation of the polarity in the epithelial stem cell layer. This indicates a possible functional correlation of epithelial stem cells and the neighboring collagen. I will further pursue the analysis of aging with mosaic fibroblast loss and experiment with collagen perturbation to determine the regulation of skin regeneration in live mice. Funding: CSC-Yale Program, NIH DP1 and HHMI Scholar Awards.

Program Abstract #18
Repeated evolution of eye loss in Astyanax mexicanus
Itzel Sifuentes-Romero, Estephany Ferrufino, Sunishka Thakur, Michael Solomon, Johanna E. Kowalko
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Evolution under similar environmental conditions often drives different species to converge on similar anatomical and behavioral traits. A classic example of convergent evolution is the suite of traits that characterize cave organisms, which includes the loss of pigmentation and eyes. While these traits have evolved many times, whether they have evolved repeatedly through the same molecular and developmental mechanisms is still unresolved. We are using the blind Mexican cavefish, Astyanax mexicanus, to study the genetic and developmental mechanisms of repeated evolution of cave traits. A. mexicanus exists as two forms, a sighted, surface-dwelling form and at least 29 populations of a blind, cave-dwelling form that initially develops eyes that subsequently degenerate. Here, we compare eye development in surface fish and two independently evolved cavefish populations, Pachón and Molino. We find that many of the same molecular and morphological alterations characterize early eye development and eye degeneration in these two cave populations. However, for many of these traits, the Molino cavefish presents an intermediate phenotype between surface fish and Pachón cavefish. Further, cave-cave hybrid fish have larger eyes and lenses during early development compared to fish from either parental population, suggesting that at least some different genetic changes underlie eye loss in these two populations. Together, these data support the hypothesis that these two cavefish populations evolved eye loss independently, utilizing many of the same genetic pathways and developmental processes. This work was supported by NSF awards DEB1754231 to JEK, IOS1933428 to JEK and NSF EDGE award 1923372 to JEK.

Program Abstract #19
Adult chondrogenesis and spontaneous cartilage repair in the skate, Leucoraja erinacea
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Mammalian articular cartilage is an avascular tissue with poor capacity for spontaneous repair. We have shown that embryonic development of cartilage in the skate (Leucoraja erinacea) mirrors that of mammals, with developing chondrocytes co-expressing genes encoding the transcription factors Sox5, Sox6 and Sox9. However, in
skate, transcriptional features of developing cartilage persist into adulthood, both in peripheral chondrocytes and in cells of the fibrous perichondrium that ensheaths the skeleton. Using pulse-chase label retention experiments and multiplexed in situ hybridization, we identify a population of cycling Sox5/6/9+ perichondral progenitor cells that generate new cartilage during juvenile and adult growth, and we show that persistence of chondrogenesis in adult skates correlates with ability to spontaneously repair cartilage injuries. Skates therefore offer a unique model for adult chondrogenesis and cartilage repair and may serve as inspiration for novel cell-based therapies for skeletal pathologies, such as osteoarthritis. This work was funded by Wellcome (PhD studentship 102175/Z/13/Z), the Royal Society (University Research Fellowships UF130182 and URF/R/191007 and Research Fellows Enhancement Award RGF\EA\180087), the Isaac Newton Trust (award 14.23z) and by a research grant from the Fisheries Society of the British Isles.

Program Abstract #20

Rare and transient somatic cell states are induced by injury and required for whole-body regeneration
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Wound-healing and tissue regeneration requires the functional coordination of stem cells, their progeny, and differentiated cells. Past studies have focused on the regulation of stem cell identity and proliferation, but less is known about the contributions of post-mitotic cells. The planarian *Schmidtea mediterranea* provides a unique opportunity to study regenerative signaling across an entire organism due to its remarkable ability to repair or regrow any organ system from a tiny tissue fragment. Here, we present a single-cell reconstruction of regeneration and identify rare, transient, somatic cell states induced by injury and required for regeneration. After determining that planarian regeneration requires a healthy tissue fragment 1.00mm in diameter (~10,000 cells), we captured 299,998 single-cell transcriptomes over two weeks of regeneration taken from tissue fragments competent and incompetent to regenerate. Amputation-induced cell states were non-uniformly distributed across tissues, and particularly enriched in the epidermis (ectoderm), muscle (mesoderm), and intestine (endoderm). These states were also very rare and transiently induced after injury in only a subset of cells in each tissue. RNAi-mediated knockdown of genes enriched in amputation-induced states produced complex homeostatic and regeneration defects that drastically reduced regenerative capacity. Altogether, these results characterize the cellular components required for regeneration at unprecedented molecular resolution, facilitating the discovery of rare cell states and novel molecules required for regeneration. Moreover, these data indicate regenerative capacity in general may be linked to the activation of transcriptional plasticity in a rare subset of differentiated cells.

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Program Abstract #21

Rapid remodeling of the translatome underlies wound closure and regeneration.
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Stanford University, USA

An outstanding mystery in biology is why some species, like the axolotl, can scarlessly heal and regenerate tissues while most mammals cannot. Recently, selective translation of mRNA has emerged as an important layer of regulation of gene expression. Because it is inherently faster than transcription, regulation of translation offers the possibility of rapid ‘on-demand’ remodeling of gene networks that may be particularly important in the context of injury. However, it is unknown whether this mode of regulation is deployed during rapid wound healing characteristic of regenerative species. Here, we demonstrate that rapid activation of protein synthesis is a unique, and previously uncharacterized feature of limb regeneration in the axolotl. By applying translational profiling, we identify hundreds of pre-existing transcripts, including annexins, S100-family proteins and components of the ribosome, which do not change in their overall mRNA abundance but are selectively activated at the level of translation in response to amputation. In contrast, we show that translation is not activated in response to injury in non-regenerative species like the mouse. Further, we identify a key upstream signal that mediates the...
regenerative translation response in the axolotl and demonstrate that inhibition of translation is sufficient to prevent wound closure and disrupt regeneration in this species. Together, these findings highlight the unanticipated impact of the translatome on orchestrating the early steps of wound healing in highly regenerative species and provide a missing link in our understanding of vertebrate regenerative potential. This work was funded by postdoctoral fellowships from the Helen Hay Whitney Foundation and Canadian Institutes of Health Research and by a K99/R00 - The Pathway to Independence Award to O.Z. and the The Stanford Medicine Translational and Clinical Innovation Fund to M.B.

Program Abstract #22
Injuries Induce an Oral-Specifying Wnt Signaling Cascade in Hydra
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Animals capable of whole-body regeneration (WBR) can fully restore any tissue lost due to injury. Because different injuries may require the regeneration of different structures, transcription during WBR must dynamically adapt to meet the requirements specific to any given injury. The regulatory mechanisms that ensure wounded tissues mount the appropriate transcriptional response are poorly understood at the molecular level. Therefore, we used the highly regenerative cnidarian, *Hydra vulgaris*, to better understand how transcription in wounded tissue responds to its surrounding tissue environment to ensure the restoration of the original body plan. We found that canonical Wnt signaling, which specifies oral tissue in cnidarians, was initially activated by injury regardless of how the injury was ultimately resolved. This activation was part of a broader early injury response that was independent of tissue environment and appeared to be driven by the upregulation of the Wnt ligands *wnt9/10c* and *wnt3* via injury-responsive bZIP transcription factors. Following wound closure, Wnt signaling activity became dependent on the surrounding tissue environment, with Wnt signaling only persisting in tissue undergoing oral regeneration at 8 hours post-amputation. Inhibition of injury-induced Wnt signaling was dependent on pre-existing organizers, as removing tissue at the poles of the oral-aboral axis induced non-amputation injuries to undergo ectopic head regeneration. Finally, we found that inhibiting TCF, the downstream transcriptional effector of canonical Wnt signaling, delayed the transcriptional specialization of both oral and aboral regeneration, suggesting that Wnt signaling is necessary for oral and aboral specification during regeneration. Our study builds on the pre-molecular models from previous *Hydra* research to provide an updated molecular framework for understanding the initiation and regulation of patterning during WBR. Funding provided by NIH R35 GM133689

Program Abstract #23
The 3D regulatory landscape of whole-body regeneration
Andrew Gehrke
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When injured, the majority of animals are capable of healing wounds or replacing select tissue. A minority possess the incredible ability to regrow their entire bodies from even a small fragment of the original host, in a process called whole-body regeneration. While the transcriptomes of animals undergoing whole-body regeneration have been well studied, how the genome itself responds to orchestrate this complex process is less understood. Here, we use the highly regenerative acel worm *Hofstenia miamia* to investigate the regulatory logic of whole-body regeneration from the level of transcription factor binding site to 3D genome organization. Using a chromosome-level genome assembly and a combination of epigenomic and transcriptomic profiling, we identified a regulatory network for early regeneration that is controlled by a master pioneer factor. Following the initiation of this network, we find that the transcription factor CTCF, which is known to impact gene expression by partitioning vertebrate genomes into specific self-interacting units, is upregulated upon wounding and becomes significantly more bound at target sites during regeneration. To understand the role of CTCF in regulating genome organization during regeneration, we performed chromosome conformation capture experiments in *Hofstenia*. We find that the acel genome is organized into small compartment-like units that are defined by a combination of open chromatin, gene expression, and methylation state. *Hofstenia* CTCF does not define compartment boundaries and instead plays a role as a traditional transcription factor during
regeneration, suggesting that the genome organization role of CTCF is a derived feature of vertebrates. Taken together, we provide a multi-layered view of regulatory landscapes during regeneration, laying the groundwork for a regulatory-focused comparison of regeneration and development. Funding: Simeon J. Fortin Foundation (A.R.G), Milton Foundation and NSF (M.S.)

Program Abstract #24

Shh is expressed early after skelatal injury and is required for large-scale bone regeneration
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The mammalian skeleton has a limited ability to regenerate large bone-defects beyond simple fractures. Fractures can also fail to heal resulting in persistent non-unions which can greatly impact quality of life. Recently, our laboratory discovered that murine ribs are capable of regenerating extraordinarily large skeletal segments. Using this novel model, we identified a rare Sox9+ skeletal progenitor population that migrates into the injury site and coordinates large-scale repair. We have observed that loss of the Smoothened receptor in this Sox9-lineage drastically impedes healing, demonstrating that receptor-mediated Hh signaling is critically important for repair. Both Shh and Ihh are expressed during early repair stages with Shh expressed strongly and within the first few days. To determine if Shh is required, we used a conditional strategy to genetically remove Shh at different time points of repair. Animals induced at early but not later stages of repair exhibited a consistent and substantial reduction in callus size and a considerable delay in bone repair when compared to uninduced controls. Shh signaling is typically studied in the context of embryonic limb development, however our results suggest that Shh acts as a critical growth factor during bone repair in adults. One possibility is that in the context of injury, the embryonic program is reactivated. As little is known about the signaling molecules that initiate bone repair, our studies are the first to identify Shh as a required initiator during large-scale rib repair. Furthermore, our studies investigating the time when Hh signaling is critical (likely in the first few days post injury) informs the development of future therapeutic treatments to target this pathway. This study was supported by an R01 grant from NIH, NIAMS to F. V. M.

Program Abstract #25

Waves and flows: physical principles of organization of embryogenesis and regeneration
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Embryonic development and tissue regeneration both require a high degree of spatiotemporal coordination. I will describe my lab efforts to understand the synchronization of the cell cycle in early Drosophila embryos and the coordination of tissue growth in zebrafish bone regeneration. I will show how synchronization of the cell cycle in Drosophila embryo is linked to precise nuclear positioning and how biochemical and cytoskeletal signals self-organize to ensure such accuracy. I will also show that excitable waves of growth factor dynamics drive cell and tissue growth in the osteoblast population controlling zebrafish scale regeneration. This work was supported by NIH (R01-GM122936 R01-AR076342) and the Shipley Foundation, Inc.

Program Abstract #26

Multiscale spatial heterogeneity enhances particle clearance in airway ciliary arrays
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Flow generation is essential for the development and physiology of organisms across the tree of life. Flows required for processes that range from swimming in single cell organisms to mucus clearance in humans are generated by arrays of motile cilia. To generate large-scale fluid flows, motile cilia must coordinate their activity across entire tissues. The mechanisms of fluid transport have been studied extensively at the level of the individual cilium and collectively moving metachronal waves. However, the connection between local cilia arrangement and the patterns of flow they generate remains largely unexplored. Here, we image the mouse airway from the sub-cellular (nm) to the organ scales (mm), characterizing quantitatively its ciliary arrangement and the generated flows. Locally we measure heterogeneity in both cilia organization and flow structure, but
across the trachea fluid transport is coherent. To examine this result we developed a hydrodynamic model to explore systematically the types of flows generated by different ciliary architectures. Surprisingly, we find that disorder in multiciliated cell arrangement or ciliary misalignment, gives rise to coherent flows with enhanced particle clearance. Altogether our results establish a quantitative link between the topology of the flow generated by a ciliary array and its underlying spatial organization. Furthermore, our work provides a framework to understand the origin of defects in fluid transport, which are the underlying cause of human airway pathologies. This work was supported by funding from the National Science Foundation Center for Cellular Construction (NSF grant DBI-1548297).

Program Abstract #27
Co-occurring differentiation and proliferation behaviors define epidermal regeneration.
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Maintenance of adult tissues depends on the differentiation of stem cell populations to replace functional cell types that are continuously being shed, but how this process is orchestrated in vivo remains poorly understood. In the mammalian skin epidermis, highly proliferative stem cells reside in an underlying basal layer and differentiate upwards to replenish the outermost protective barrier of the skin. Although basal cells that have initiated differentiation are thought to have lost their proliferative capacity, how the commitment to differentiation and loss of stem cell characteristics are temporally coordinated in individual cells is not known. Here we use 2-photon intravital imaging to elucidate the early steps of stem cell differentiation in the epidermis of living adult mice. Using a live reporter of differentiation status, together with single-cell sequencing to track global transcriptional changes, we find that cells commit to their differentiated fate several days before they begin to delaminate up and out of the basal layer. Surprisingly, we observe that a large proportion of these differentiation-committed cells can divide, producing daughter cells that later complete the differentiation trajectory and exit the stem cell layer. These divisions occur in response to neighboring delamination events, suggesting that differentiating cells proliferate to preserve cell density within the stem cell compartment and not necessarily as part of an obligate transit amplifying program. To test this, we block divisions within the differentiation-committed basal population and find that the tissue can adapt to maintain the size of the differentiating cell pool in their absence. Together, these results demonstrate that cell fate commitment and loss of proliferative capacity are temporally uncoupled during epidermal regeneration, with differentiating cells able to divide if needed to preserve cellularity in the stem cell compartment. Funding provided by CIHR.

Program Abstract #28
A unique population of Asomitic Mesodermal cells controls axolotl tail regeneration.
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Segmentation of the vertebrate primary body axis is dependent on the formation of somites. In axolotl this segmented pattern persists throughout life and is recapitulated during tail regeneration. Using morphological observations, Cre-LoxP lineage tracing, scRNAseq, and gene function analysis, we are investigating the mechanisms which underly segmentation of the axolotl tail during regeneration. The virgin tail contains segmented vertebrae and muscle, half a segment off-set from each other reflecting their shared somitic origin. Regeneration occurs in the absence of physical somites, and the relativeVertebrae – Muscle distribution is not accurately recapitulated, suggesting that the shared mechanism of somitogenesis is not presence during regeneration. Using lateral injuries, we show that muscle segmentation during regeneration is dependent on the presence of Myotendinous junctions (MTJ). Using Cre-loxP mediated lineage tracing, we show that the cells residing at the MTJ contribute extensively to the regenerating tail. Similar to the potential of the somitic compartment, the MTJ cells contribute to dermatome, sclerotome, and myotome during regeneration. Consistent with this observation, our single cell data indicates expression of the somitic mesoderm markers in the cells
residing at the MTJ. Taken together these data suggest that the axolotl tail contains a unique population of Asomitic Mesodermal (ASM) cells, which pattern and re-establish the regenerating tail. We are currently further characterizing the mechanisms by which the asomitic mesoderm controls segmentation of the tail during regeneration. Funding: Austrian Science Fund (M-2444 to W.M.)

Program Abstract #29
A single base pair change dramatically alters binding site affinity and enhancer activity
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Enhancers are genomic elements that control the precise tissue-specific gene expression patterns required for proper development. In the chordate Ciona intestinalis, many enhancers active in the developing heart have been found; however, we still do not understand how enhancer sequences encode heart-specific expression. This means we cannot identify heart enhancers from sequence alone, nor can we predict which sequence changes will alter heart enhancer activity and cause heart defects. Upon a Fgf/Ets signal, the Foxf enhancer activates expression of the gene Foxf in the developing heart, which is needed for proper cell migration. We find that the Foxf enhancer requires suboptimal affinity Ets transcription factor binding sites to encode heart-specific gene expression. Optimizing the affinity of all Ets sites in the enhancer leads to expression in many other tissues. Individually optimizing each of the Ets sites also leads to ectopic expression in non-heart cells. The position of the optimized Ets site within the enhancer affects the location and level of ectopic expression, hinting at the importance of grammar. Remarkably, a single point mutation dramatically increases the affinity of an Ets site and expression in non-heart cells. To establish a genotype-phenotype link, we are currently evaluating the impact of this optimizing point mutation on heart development using genome editing. Our results illustrate the importance of suboptimal affinity binding sites and enhancer grammar in restricting expression to the developing heart. Furthermore, our discovery that a single base pair change can optimize binding affinity and lead to ectopic expression provides a mechanistic framework to predict the types of mutations in enhancers that lead to patterning defects during development. GAJ acknowledges support from AHA grant 18POST34030077 (Award year 2018) and NIH grant T32HL007444. JJS is supported by NIH grant T32GM127235. All authors are supported by NIH grant DP2HG010013.

Program Abstract #30
A first look into whether psychological stress delays regeneration in spiny mice (Acomys cahirinus)
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It is well established that psychological stressors can delay development and the trajectory of repair and recovery after injury, but whether such phenomena extend to regeneration remains understudied. Capitalizing on the robust regenerative abilities and unique social nature of spiny mice (Acomys cahirinus), we took a first look into whether differential social stress in the home environment is linked to differential rates of regeneration following musculoskeletal injury. We hypothesized that Acomys exposed to higher levels of social stress associated with subordinate social status would regenerate slower than their dominant ranked cage-mates, regardless of sex. After objectively coding dominance behavior of dyads across three weeks and tracking individual ear-hole closure/regeneration following a 4mm biopsy punch, we found that subordinate ranked Acomys participating in higher levels of stress-related social behaviors indeed regenerate slower than their dominant cage-mates, regardless of sex, with an overall delayed time to wound closure. We also found that subordinate ranked male and female Acomys engage in high levels of social avoidance behavior (e.g. induced flee), and refrain from stereotypical freezing and supine posturing observed in common laboratory rodents. Given these findings we are further examining dominance rank related differences in the regulation of the endocrine stress and growth axes using qPCR and radioimmunoassays. Subsequent work will measure differences in tissue proliferation across multiple phases of Acomys regeneration. Such investigations will lay the groundwork for investigating further modifiable risk factors (e.g. psychological stress) disrupting trajectories of regeneration. Funding provided by a Swiss National Science Foundation Early Postdoc Mobility Award.
Program Abstract #31
Polarized dissolution and condensation of Dishevelled in oocytes drives embryonic axis specification
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The body axes formed during embryogenesis are intimately linked to intrinsic asymmetries at the cellular scale in oocytes. Here, we report an essential symmetry-breaking event that occurs during meiosis in oocytes of the sea star Patiria miniata. The cytoplasmic Wnt signaling effector Dishevelled is required for specification of the anterior-posterior axis during development. Using 4D time-lapse imaging, we find that Dishevelled is uniformly localized in granules throughout the cortex in arrested oocytes, but becomes enriched at the vegetal pole after meiotic resumption. Dishevelled granules accumulate there by a dissolution-condensation mechanism, rather than by active transport, in a process tightly coupled to meiotic cell cycle progression. Using mechanical and microsurgical perturbations, we find that dissolution is influenced by diffusible gradients, while vegetal condensation requires pre-localized intrinsic cues. With high resolution imaging, we observe several distinct populations of Dishevelled that assemble on different cellular compartments, including Lamp1 positive endosomes and the cortex. With FRAP and computational analyses, we find that these discrete Dishevelled populations display distinct physical properties, with the endosome-associated granules being more mobile and liquid-like. Using precise function-separating Dishevelled mutants that differentially affect these localization behaviors, we find that these Dvl populations are essential for and anterior-posterior axis specification in embryos. Our results offer a paradigm that bridges previous models for Dishevelled that invoke lipid binding versus accumulation in biomolecular condensates, to achieve an important symmetry breaking event.
Funding sources: 1K99HD099315 to S.Z.S., R35GM126930 to I.C., pilot experiments conducted at Marine Biological Laboratory Embryology Course

Program Abstract #32
Identification of extrinsic cues promoting target-selective axon regeneration
Lauren Walker, Camilo Guevara, Michael Granato
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Regenerating axons in the peripheral nervous system must extend over long distances to reconnect with their original synaptic targets for functional recovery. However, re-establishing a complex trajectory that includes navigating multiple choice points and then selecting the appropriate target long after this circuitry was established during development, represents a unique challenge. To visualize regenerating axons as they navigate stepwise choice points, we established the larval zebrafish pectoral fin, equivalent to tetrapod forelimbs, as a vertebrate model system in which to study this process. Each pectoral fin is innervated by four motor nerves containing dozens of axons that branch to stereotypically-innervate specific regions of two muscle layers. Using a laser, we transect the fin motor nerves and monitor axon regeneration in real time. We have characterized the stepwise choices faced by regenerating axons including sorting at the plexus to select the correct muscle layer, selectively fasciculating with the appropriate axonal partners to grow into the fin, and then defasciculating to reinnervate their original individual muscle fibers. By labeling single axons, we observe robust, specific, and functional regeneration of motor axons back to their original domains within 48 hours post injury, indicating the existence of local cues within the fin to guide selective reinnervation. To identify extrinsic injury-dependent guidance cues in the pectoral fin, we have employed an RNAseq approach. We present results from this RNAseq analysis from denervated fins at timepoints that precede important axon guidance decisions including choosing a muscle layer, sorting at the plexus, and specific target selection. We predict that changes in gene expression may reflect regional cues important for axon growth and guidance and are testing mutants in genes that are upregulated after injury to determine their functional role in axon guidance. Funding: NINDS NRSA F32NS103219

Program Abstract #33
Different paths to the same cell type
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In the classical view of fate specification, a given cell type arises by a unique transcriptional program from a single developmental lineage. In contrast, during "convergent differentiation", an identical cell type is produced by multiple, unrelated lineages. This phenomenon, now appreciated to be prevalent across species, has long been appreciated in C. elegans but not understood at the molecular level. Therefore, we focused on the development of identical, radially symmetric cells called ILso glia that are produced by distinct lineages. Through an unbiased genetic screen, we found that the Forkhead transcription factor UNC-130 is required for the specification of the dorsal pair of ILso glia but not for the ventral or lateral pairs, which are produced from different lineages. UNC-130 is also required by other cell types that arise from the dorsal ILso sublineage. We identified point mutations in the DNA-binding domain that result in graded defects in UNC-130:DNA binding in vitro, and correspondingly, to partial specification defects in vivo. Fate specifying factors often act as activators, however, we show that UNC-130 acts as a transcriptional repressor to promote fate specification. In its absence, regulators of alternative fates, UNC-86 and RNT-1, may be spuriously activated, as loss of these factors in unc-130 mutants partially rescues fate defects. In addition, UNC-130 binds a canonical Forkhead motif and can be functionally replaced by its human homolog, FOXD3. Interestingly, FoxD3 also acts in a lineage-specific manner in vertebrates, promoting the specification of glia and sensory neurons as in C. elegans. Thus, in contrast to "terminal selectors," which are required to specify discrete cell types, UNC-130 acts as a "lineage selector," a lineage-specific factor required for cell fate specification. This provides evidence that molecularly distinct differentiation programs can produce the same cell type. Funding: Milton and Hearst Funds (Harvard)

Program Abstract #34
Single-Cell Spatial Transcriptomics at Embryo-Scale
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Spatial patterns of gene expression, which are established within and across tissues, underlie key embryonic developmental processes. While available spatial transcriptomic methods have greatly improved of late, many in situ methods for profiling gene expression are limited in the number of transcripts or the region of interest they can measure, while other methods give local aggregate measurements. We developed sci-Space to allow for spatial transcriptomics with single cell resolution while simultaneously capturing heterogeneity across large sections of embryos. When we applied sci-Space to fresh-frozen E14 mouse embryo sections we were able to use spatially applied barcodes to approximate cells' spatial coordinates in conjunction with the measurement of their transcriptomic profiles. By mapping transcriptomes to the original embryo-space we could produce "digital in situ" for individual genes and dissect spatial patterns of gene expression by cell type. As an example, we identified spatial regulation of the Hox-family transcription factors known to be expressed in an anatomically patterned manner. We resolved the spatial pattern of expression by excitatory neurons and demonstrated that the pattern was not the result of discernable cell subtypes. We showed that variation in other genes' expression could be explained by spatial context but not cell state differences. We developed a new statistical approach for quantifying the contribution of spatial context to total variation in global gene expression patterns. Finally, for endothelial cells, we identified gene modules and subpopulations that show tissue-specificity and others that were more universal. This work demonstrates that the sci-Space method for spatially resolving single cell transcriptomic data across large tissue sections can provide essential information regarding spatial state regulation during development. NIH DP2HL137188; MR-Washington Research Foundation Postdoctoral Fellowship

Program Abstract #35
Chytrid fungi and our evolving view of cell motility
Lillian Fritz-Laylin
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Various cells scattered throughout the eukaryotic tree crawl across surfaces or through three-dimensional environments. Evidence now indicates that cell crawling is not a single behavior, but rather a collection of processes, driven by different molecular mechanisms. Understanding these mechanisms and their evolutionary
relationships first requires narrowly defining mechanical modes of locomotion, and then identifying phenotypic and molecular markers of each. The best studied mode of cell migration is the adhesion-based migration of highly adherent animal cells, including fibroblasts and epithelial cells, a mode limited to cells of the animal lineage. In contrast, a mode we refer to as “alpha-motility” is widely dispersed among eukaryotic phyla and is characterized by dynamic, actin-filled pseudopods and weak adhesion to external substrates. We are currently defining gene complements required for each mode, and using the resulting data to predict crawling motility in new species. We use this approach to predict alpha-motility in the amphibian-killing Chytrid fungus \textit{Batrachochytrium dendrobatidis}, a prediction we have verified using microscopy and small molecule inhibitors of actin cytoskeletal components. By developing mechanistic definition of distinct modes of crawling motility, and expanding our phylogenetic analysis to many eukaryotes, we are identifying genetic markers to understand the evolution of this key eukaryotic behavior. The Fritz-Laylin lab is funded through the generous support of the NSF, NIH NIAID, Pew Charitable Trust, Smith Family Foundation, and UMass Amherst.

Program Abstract #36
Cell-matrix interactions during cell migration in vivo
Qian Xue, James Carrington, Minna Roh-Johnson
University of Utah, USA

How do cells make cell migration decisions in complex environments? Interactions with the environment inform cells to migrate singly or collectively, and towards or away from signaling sources. This communication is critical for normal development, as cells are often not born in the location of function. This process is also critical in cancer, in which the ability to migrate is an essential feature of metastasis. On 2-dimensional substrates, many types of cells migrate through a net forward movement of cell protrusion at the front and cell retraction at the back. Central to this form of single cell migration is cell attachment to the underlying substrate through focal adhesion complexes. Despite years of research on focal adhesion formation, the function and organization of these structures in their native environment are still unclear. What is the composition and dynamic regulation of focal adhesion structures in vivo? How do surrounding cells and extracellular matrix composition affect focal adhesion assembly and function? To answer these questions, we have developed a unique system in which we can directly visualize the formation of focal adhesion-like structures in highly migratory melanoma cells on a relatively planar surface of the larval zebrafish skin. While the surface is planar, the matrix composition and skin are intact, providing ideal detection of the focal adhesion-like structures in cells within their physiologically relevant tissue. We have used this system to dissect the composition and dynamics of focal adhesion-like structures \textit{in vivo} and have identified unique properties of focal adhesion formation and dynamics in cells in their native environments. In addition to revealing the cell biology and biophysical regulation of cell migration, this work will also extend our understanding of the basic mechanisms contributing to malignant melanoma progression, and reveal potential treatment strategies for this disease. This work is funded by R00CA190836.

Program Abstract #37
Regulation of cell adhesion dynamics coordinates migration and fate decisions in the neural crest
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How do migratory cells make robust fate decisions at the right time and place? Neural crest cells (NCCs) migrate as multipotent progenitors to form a huge variety of cell types. Many studies have shown gene functions in early NCC emigration, but few in specific NCC lineages at these early stages. We identified two genes in zebrafish, Ovol1a and Lmo7a, which when knocked down cause subpopulations of NCCs to aggregate at the dorsal midline. Strikingly, both alter adhesion dynamics at NCC membranes and Wnt signaling, with aggregates adopting distinct fates. While Ovol1a disrupts signaling downstream of N-cadherin, Lmo7a is required for focal adhesions. By expressing a photoconvertible EOS under control of a NCC-specific promoter, we FAC sorted NCCs and performed scRNA-seq on subsets from different migratory locations and stages as well as bulk RNA-seq on aggregate cells in \textit{ovol1a- and lmo7a -deficient} embryos. scRNA-seq data indicate unexpected heterogeneity in early migrating NCCs and detail the emergence of pigment and cartilage progenitors. Surprisingly, despite similar phenotypes, NCC transcriptomic profiles differ between Ovol1a and Lmo7a knockdowns. Computational models suggest that cells
acquire transitional lineage identities and maintain lineage/migratory decisions through cell-cell contacts and/or short range signals, including Wnt. We investigate these signaling dynamics by integrating RNA-seq data from a transgenic Wnt reporter and temporal scRNA-seq data. Consistent with this idea, NCC aggregates in Ovol1a knockdowns form pigment and cartilage progenitors while in Lmo7a mutants they become progenitors of both pigment cells and glia. These differences correlate with transcriptomic signatures and differential responses to Wnt. Such dynamic decision-making likely applies to many migratory cell populations both in embryos and adults. NIH R01 DE13828, HD073182

Program Abstract #38
Prickle isoforms determine handedness of helical morphogenesis
Bomsoo Cho, Song Song, Jeffrey Axelrod
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Subcellular asymmetry directed by the planar cell polarity (PCP) signaling pathway orients numerous morphogenetic events in both invertebrates and vertebrates. Here, we describe a morphogenetic movement in which the intertwined socket and shaft cells of the Drosophila anterior wing margin mechanosensory bristles undergo PCP-directed apical rotation, inducing twisting that results in a helical structure of defined chirality. We show that the Frizzled/Vang PCP signaling module coordinates polarity among and between bristles and surrounding cells to direct this rotation. Furthermore, we show that dynamic interplay between two isoforms of the Prickle protein determines right- or left-handed bristle morphogenesis. We provide evidence that, Frizzled/Vang signaling couples to the Fat/Dachsous PCP directional signal in opposite directions depending on whether Pkpk or Pksple predominates. Dynamic interplay between Pk isoforms is likely to be an important determinant of PCP outcomes in diverse contexts. Similar mechanisms may orient other lateralizing morphogenetic processes. (funding; R01 GM097081, R37 GM059823)

Program Abstract #39
Cell-size differential drives aberrant clone dispersal in epithelial tissue
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How animal cells of diverse shapes and sizes pack to form functional tissue is a fundamental question in biology. Nevertheless, the precise role of differential cell shape in tissue organization and homeostasis is limited. My presentation will focus on the physical and biological basis of how cells of divergent morphologies self-organize and form epithelial tissue. As epithelial tissues develop, groups of cells related by descent tend to associate in clonal populations rather than dispersing within the cell layer. While this is frequently assumed to be a result of differential adhesion, precise mechanisms controlling clonal cohesiveness remain unknown. We employ computational simulations to modulate epithelial cell size in silico and show that junctions between small cells frequently collapse, resulting in clone cell dispersal among larger neighbors. Consistent with similar dynamics in vivo, we further demonstrate that mosaic disruption of Drosophila Tor generates small cells and results in aberrant clone dispersal in developing wing disc epithelia. We propose a geometric basis for this phenomenon, supported in part by the observation that soap-foam cells exhibit similar size-dependent junctional rearrangements. Combined, these results establish a link between cell-size pleomorphism and the control of epithelial cell packing, with potential implications for understanding tumor cell dispersal in human disease. Funding: This work was supported by the Stowers Institute for Medical Research and grants from the National Institutes of Health (R01GM111733-05 to M.C.G).

Program Abstract #40
Cell crawling and junction contraction: “Frenemies” in convergent extension
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Convergent extension (CE) is a ubiquitous and conserved collective cell movement that elongates developing tissues via iterative cell intercalation, and is also implicated in human diseases, including neural tube defects and
congenital heart diseases. To complement ongoing efforts to identify genetic and molecular regulators of CE, we have applied a multi-scale mechanical analysis of CE to unravel the biomechanical principles that underlie cell behaviors and determine tissue properties. In the *Xenopus* dorsal mesoderm during CE, we discovered a tight integration between junction contraction and cell crawling, two cellular modes of CE with distinct mechanical patterns. We showed that the two act in concert to facilitate cell intercalation. To link this mechanical feature to the genetic systems governing CE, we have focused on ARVCF, a poorly studied catenin that is required for CE. Unlike other well-characterized genes controlling CE, ARVCF knockdown (KD) does not disrupt cell polarity or cell intercalation. Rather, by characterizing the mechanical patterns, we found that loss of ARVCF results in a shift of the contraction-crawling spectrum. Moreover, we linked this relatively minor change in intercalation behaviors to large scale defects in tissue mechanics, including reduced force-coupling and force propagation leading to impaired tissue-scale extension force and tissue stiffness. This work not only reconciles the junction contraction and cell crawling modes but offers an additional measure to characterize CE phenotypes and provides a more comprehensive understanding of how biomechanics across different scales contribute to CE and CE relevant birth defects. Lastly, we sincerely acknowledge our funding source NICHD R01 GM014853 for supporting our work.

Program Abstract #41

**Development of an ex vivo human brain organoid model to study severe malaria-associated brain injury**

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Human cerebral malaria (HCM) is a severe neurological manifestation of infection with *Plasmodium* species, with high mortality occurring especially in children. We have previously demonstrated that increased intravascular erythrocyte hemolysis during parasite multiplication releases free heme, leading to blood-brain barrier leakage and neuronal injury in cerebral malaria and placental disfunction during pregnancy. Current experimental models limit translationally relevant studies of malaria pathogenesis at the level of the brain *in utero* or postnatally. Recently, human iPSC have shown great promise as models for studying neuronal development in humans. Using iPSC approach, we have generated 3D *ex vivo* human cortical organoids to recreate a developmental heme-induced brain injury model. Our *hypothesis* is that heme induces human iPSCs and brain cortical organoid injury that can be attenuated by neuregulin (NRG-1). To test this hypothesis, we employed flow cytometry, western blot, multiplexed immunoassay procedures and immunohistochemistry to assess pluripotency, confirm neuronal and astrocyte markers including NRG-1, its receptor ErbB4, and apoptosis markers at various time points during cortical organoid development. Our *results* show that iPSCs and cortical organoids express NRG-1 and ErbB4, consistent with observations in the human brain. Heme induced iPSCs apoptosis and differentiation of iPSC at ~30 M concentration and beyond. In addition, heme reduced organoids growth and altered their architecture and structural integrity. NRG-1 attenuated the heme-induced iPSC and cortical organoid injury via ErbB4 mediated pathway. In *conclusion*, we demonstrate that cortical organoids can be used as a model for studying heme-induced brain injury in CM and can be used to test NRG-1 as a neuroprotective agent. Funding: NIH/NINDS R01NS091616, NIH/ NINDS R21TW006804 The Georgia Clinical and Translational Science Alliance (GCTSA; NIH/NCATS)

Program Abstract #42

**Hepatoblast organoids have bipotential fate in engineered liver tissue**

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Recent advances in tissue engineering have led to development of artificial human liver tissue containing human hepatocytes, blood vessels, and stromal cells. These tissues can perform key liver functions after implantation in mice, but they lack an organized biliary network – an integral component of liver structure and function. Incorporating hepatocytes and cholangiocytes (the cells that line bile ducts) into engineered liver tissues remains a critical challenge. Towards this goal, we generated hepatoblast organoids from fetal liver tissue and encapsulated these organoids in fibrin hydrogels. We hypothesized that upon implantation in mice with liver injury, endogenous signaling would drive hepatoblasts to differentiate into both hepatocytes and cholangiocytes.
After implantation, we found by histology and immunohistochemistry that numerous cell clusters resembled densely packed hepatocytes and stained positively for hepatocyte markers cytokeratin 18, arginase-1, alpha-1-antitrypsin, and albumin. Human albumin was identified in mouse blood serum, confirming successful engraftment and integration with host vasculature, as well as suggesting that organoid tissues are functionally active by synthesizing human protein. In addition to functional hepatocyte clusters, we also identified numerous cholangiocyte-like cells that had self-assembled into bile-like duct structures and stained positively for cholangiocyte marker cytokeratin 19. These results suggest that endogenous signaling upon engraftment can direct immature hepatoblasts within a tissue engineered construct towards both hepatocyte and cholangiocyte phenotypes. This work is an important step toward building clinically relevant human liver tissues and provides a new model to study human liver development. Future work will focus on directing maturation of differentiated cells to generate more complex and fully functional engineered liver tissues.

Program Abstract #43
Towards a complex in vitro model of human endometrium
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While the extensive endometrial remodeling taking place at the onset of pregnancy is not generally thought of as a classical developmental system, these events share multiple hallmarks with basic development. This includes differentiation of endometrial stromal fibroblasts (ESFs) to decidual stromal cells, signaling via secretions from uterine glands and influx (migration) of immune cells, all needed for subsequent maternal-fetal interface establishment, fetal implantation and immunotolerance. However, studying basic biology of mammalian reproduction in model organisms is often challenging, especially specifics of ape and human pregnancies. 2D cell culture systems can't adequately represent 3D uterine environment, while recently developed human endometrial gland organoids rely upon samples collected from biopsies and first trimester elective abortions that are difficult to obtain and are riddled with ethical concerns. We established endometrial gland organoids from term placental membranes and showed that they express known markers of glandular tissue, can be passaged and respond to hormonal treatment. These organoids are comprised of glandular epithelial cells only, so we sought to expand the model by adding another cell type, ESFs, isolated from the same donor. Cultured alone, ESFs died in the organoid Expansion Medium (ExM), but survived when co-cultured with the organoids or in ExM previously conditioned by organoids for 3 days. Mass spectrometry analysis of standard vs. organoid-conditioned ExM identified 11 significantly enriched proteins as potential ESF survival factors. Next, we will test how each of these secreted factors individually affects ESFs in culture. These experiments are the first step in developing a complex in vitro model of the maternal-fetal interface. Adding myometrial and immune cells will further expand our model, helping us shed light on the dynamic changes taking place in uterine tissue. March of Dimes Prematurity Research Center Grant

Program Abstract #44
Making vertebrate limbs from non-limb fibroblasts
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The limb bud progenitors (LPs), which originate from the somatopleural layer of the lateral plate mesoderm, give rise to the majority of tissues within the mature patterned limb including bones, cartilage and tendons. To identify factors involved in establishing limb progenitor properties in lateral plate mesoderm, we took a reprogramming approach, reasoning that the full set of the factors specifying "limbness" might be sufficient to convert non-limb fibroblasts into LP-like cells. First, we attempted to establish a robust culture for endogenous mouse LPs (mLPs) that would underlie reprogramming assays. By taking advantage of 3D culture systems (hyaluronan-based hydrogels or Matrigel), we have succeeded in maintaining LP marker expression in cultured mLPs for 10 days. To screen a set of potential candidates, we generated a list of factors expressed specifically in the early limb buds by using RNA-Seq. As a result, 18 factors were deemed as candidates, and overexpression of them was indeed sufficient to induce the expression of LP marker genes such as Sall4 and Lhx2 in 3D-cultured mouse embryonic
fibroblasts. We further narrowed down a minimum set of factors by examining the effect of withdrawal of individual factors, and identified that the combination of Prdm16, ZBTB16 and Lin28a greatly upregulated expression levels of LP genes. Furthermore, single-cell RNA-Seq analyses revealed that a fraction of the reprogrammed cells had similar transcriptome profiles to legitimate mLPs. Strikingly, when placed in high-density “micromass” culture or in the chick limb buds, the cells differentiated into cartilage, suggesting that they acquired differentiation potentials to some extent. Together, these results suggest that the three factors could convert into iLPs (induced limb progenitor-like cells), and they may play pivotal roles in cell specification of LPs during limb development. We will also discuss if fibroblasts from other vertebrates could be reprogrammed.

Program Abstract #45
Temporal regulation of green and red cone photoreceptor specification in human retinas and retinal organoids
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The mechanisms that diversify neuronal subtypes during human development are poorly understood. Trichromacy is unique to primates among mammals, enabled by specification of blue (S), green (M), and red (L) cones. During retinal development, cones choose between M- and L-cone subtype fates. Two non-exclusive models have been proposed: a stochastic model, in which a regulatory DNA element randomly loops to either the M- or L-opsin promoter to drive expression, and a temporal model, in which cone subtypes are generated in a developmental progression. Here we present data that support a temporal mechanism for M and L cone specification. We find that M-opsin is expressed prior to L-opsin in human fetal retinas and retinal organoids. We examined ratios of M and L cone subtypes in 750 adults and found associations with polymorphisms in genes involved in retinoic acid (RA) signaling. We developed an in situ method to visually distinguish between the highly similar M- and L-opsin mRNA transcripts and examined expression in organoids. Organoids differentiated in extended RA conditions displayed a dramatic increase in L-opsin-expressing cones and decrease in M-opsin-expressing cones, suggesting that RA is sufficient to induce L cones and suppress M cones. Together, our data support a model in which temporal specification of M and L cones is controlled by RA signaling. These studies advance human retinal organoids as a model to study developmental processes unique to humans and primates. Funding sources: Pew Scholars Program, NIH NEI R01EY025598-01, Johns Hopkins University Catalyst Award, NIH NEI F31EY0299157-01.

Program Abstract #46
Cellular and molecular mechanisms of Kabuki Syndrome neurodevelopmental defects in zebrafish and human iPSC-derived brain organoids
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Children with Kabuki Syndrome (KS) have a spectrum of neurodevelopmental defects (NDD) and various mutations in the histone methyl-transferase KMT2D involved in epigenetic regulation. Our previous work generated zebrafish null mutants of kmt2d and found that Notch pathway is hyperactivated in the cardiovascular system (1). Using our zebrafish model, we found that Kmt2d promotes neuronal progenitor differentiation of a specific neuronal population through regulation of the Notch pathway. Kmt2d-dependent alterations in Notch signaling and a misbalance between neuronal progenitors and neuronal differentiation leads to NDD. Using confocal imaging and FACS, we found that NDD in kmt2d null mutants were not due to alterations in apoptosis, proliferation, or neurovascular development. Strikingly, pharmacological inhibition of Notch signaling was able to rescue the transition of neuronal progenitors to neurons in kmt2d mutants. To test these mechanisms in human neural development, we produced two KMT2D KO iPSC lines that we used to generate cortical brain organoids. The differences between mutant and isogenic human brain organoids will allow us to investigate cellular and molecular mechanisms of NDD in human KS. Our research uses zebrafish to model patient-specific mutations in distinct Kmt2d domains to assess the multiple functions of the protein in neural development, and human iPSC-
derived brain organoids to understand the novel roles of KMT2D during neurogenesis. Through these multiple approaches, we are uncovering novel molecular and cellular mechanisms of NDD. The long-term goal of our program is to develop possible patient-specific NDD prognostics and therapeutic interventions to ameliorate neurodevelopmental disease in KS children. Research was supported in part by UM1 HL098160 to HJY and AHA Postdoctoral Fellowship to AS. (1) Serrano MA, et al. PLoS Biol. 2019; 1–39.

Program Abstract #47
Unraveling the similarities between hereditary and viral microcephaly
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Microcephaly, a major developmental disorder in which brain and head size is severely reduced, is often accompanied by cognitive deficits and other developmental delays. Microcephaly can develop in utero and has both genetic and environmental causes. Interestingly, there are many clinical similarities between microcephaly caused by Zika virus and that caused by mutations in ANKLE2. We previously identified a physical interaction between Zika virus non-structural protein 4A (NS4A) and human ANKLE2 using a global proteomics approach. NS4A expression in Drosophila inhibited brain development in an ANKLE2-dependent manner. Thus, similarities in viral and hereditary ANKLE2-associated microcephaly can be leveraged to dissect a fundamental developmental process and how it is dysregulated during disease. We hypothesize that pathogenic variation in ANKLE2 and Zika virus NS4A expression impact brain development in vertebrates through similar disruption of ANKLE2 protein interactions. Here, we present evidence that NS4A expression and ankle2 mutations inhibit brain development in a vertebrate zebrafish model. We also outline how global proteomics approaches can be used to identify the molecular level changes in neurodevelopmental disease states. By coupling global proteomics with high-throughput model organisms, we can discover new links between diseases with distinct etiologies and unravel their molecular similarities. This work was supported by the Gardner Fellowship from the Center for Immunology and Infectious Diseases at University of California, Davis and NIH T32 GM007377.

Program Abstract #48
The esophageal gland-mediated host immune evasion by blood fluke Schistosoma mansoni
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Schistosomes are parasitic flatworms that cause schistosomiasis, a major neglected tropical disease affecting over 200 million individuals. Their complex life cycle involves multiple body plans as they alternate between asexual (in a molluscan host) and sexual (in a mammalian host) reproduction. As such, their robust propagation requires stem cells to undergo frequent transitions in their cycling and differentiation during parasite homeostasis and reproduction. As infectious larvae (cercariae) arise from snails, a handful of stem cells packed inside the larval body serve as a likely origin for intra-mammalian parasitic development. However, how these early stem cells contribute to organogenesis remains unknown. Surprisingly, we discovered that the esophageal gland, an anterior accessory organ of the digestive tract, develops before the rest of the digestive system, and prior to blood feeding, suggesting that it may play a role in processes beyond nutrient uptake. To explore the function of the esophageal gland, we characterized Sm-foxA (foxA), a gene encoding a forkhead-box transcription factor, that is highly enriched in the esophageal gland. Knockdown of foxA completely blocked development and maintenance of the gland, without affecting other somatic tissues, as well as parasite viability, reproduction, and behavior in vitro. Intriguingly, schistosomes lacking the gland died after transplantation into naïve mice, while they were able to survive in immunodeficient mice lacking B-cells. Furthermore, feeding of GFP-expressing immune cells revealed that the gland-lacking parasites fail to lyse ingested immune cells within the esophagus before passing them into the gut. Together, our results unveil a novel immune-evasion mechanism mediated by the esophageal gland, which is essential for parasite survival and pathogenesis. Funding: Howard Hughes Medical Institute (P.A.N.).
Program Abstract #49
The Effects of Sexually-Transmitted ZIKV infection on Preimplantation Development
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Adults contracting Zika virus (ZIKV) exhibit little to no symptoms, whereas fetuses exhibit defects ranging from mild growth retardation to spontaneous abortion. Aside from transmission via mosquito, ZIKV can be sexually transmitted, which introduces the possibility that ZIKV infection could occur shortly after conception. However, the effects of ZIKV infection during preimplantation development are not understood. I hypothesize that the more severe congenital defects are a result of sexually transmitted ZIKV infection. Consistent with this hypothesis, I have discovered that candidate ZIKV receptors are present in early embryos. Among these, MERTK is present in both mouse embryo-derived stem cell lines and preimplantation embryos. A high concentration of ZIKVPR and ZIKVUG caused two-cell embryos to undergo developmental arrest. Moreover, infected blastocyst exhibited reduced SOX2 expression, an epiblast cell marker, and preferential infection of trophectoderm cells. The removal of the zona pellucida further exacerbated these effects by additionally reducing the expression of CDX2, a trophectoderm cell marker. Therefore, my results suggest that preimplantation ZIKV infection causes embryonic demise or embryonic cell fate defects depending on the time of infection. My studies are significant to human health because they provide knowledge of the impact of ZIKV infection on early pregnancy outcomes as well as set a precedent for molecular and pharmacological therapeutics for the pathogen. This work is supported by NIH T32 HD087166 awarded to J.W. and the James K. Billman, Jr., M.D. Endowment awarded to A.R.

Program Abstract #50
A broad translational program regulates the progenitor population during spermatogenesis
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The continuous production of sperm via spermatogenesis over an animal’s lifetime relies on spermatogonial stem cells and the transit-amplifying progenitors they produce. In men, the most common known genetic cause of spermatogenic failure is associated with deletions of the DAZ gene. The broader DAZ family of germline-specific, RNA-binding proteins is required for fertility across metazoa. However, the mechanistic basis for the DAZ family’s role in spermatogenesis remains poorly defined. Here we examine whether family member DAZL directly regulates mitotic progenitor spermatogonia using a conditional genetic mouse model and in vivo biochemical approaches combined with chemical synchronization of spermatogenesis. We find that the absence of Dazl impairs both expansion and differentiation of the spermatogonial progenitor population. Based on iCLIP in undifferentiated spermatogonia, DAZL binds the 3’ UTRs of ~2,500 protein-coding genes. DAZL’s targets include critical regulators of spermatogonial proliferation and differentiation as well as broadly expressed, dosage-sensitive factors central to transcription and RNA metabolism. DAZL binds 3’ UTR sites conserved across vertebrates at a UGUU(U/A) motif. By assessing ribosome occupancy in undifferentiated spermatogonia, we find that DAZL increases translation of its targets. In total, DAZL orchestrates a broad translational program that amplifies protein levels of key spermatogonial and gene regulatory factors to promote the expansion and differentiation of the progenitor population during spermatogenesis. Our mechanistic characterization of DAZL function in mouse spermatogonia provides novel insights into how DAZ may contribute to the most common known genetic cause of male sterility. This work was funded by HHMI.

Program Abstract #51
Molecular mechanisms modulating neural development in cblX syndrome.
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cblX is an X-linked recessive disorder that is associated multiple clinical manifestations that include abnormal cobalamin metabolism, intractable epilepsy, craniofacial abnormalities, intellectual disability, and failure to thrive. cblX is the direct result of mutations in the HCFC1 gene, which encodes for a transcriptional cofactor known to
regulate cell proliferation and survival. Various model systems have been developed to understand the neurodevelopmental function of HCFC1. However, functional analysis in mice has been restricted to tissue specific knockdowns owing to embryonic lethality in males and homozygous female carriers of Hcfc1 mutant alleles. Zebrafish have two HCFC1 paralogs, hfc1a and hfc1b. The strength of this model lies in the ability to coordinately knockdown expression of each paralog individually in order to study the overall function of HCFC1. We have developed the first two-germline mutations in the zebrafish hfc1a gene, termed the Co60 and Co64 alleles. These alleles differ in that the Co60 allele is a nonsense haploinsufficiency model and the Co64 allele is a missense mutation that is homozygous viable. Using immunohistochemistry and flow cytometry of several markers of brain development, we detected increased proliferation of neural precursor cells (NPCs) and increased expression of the markers indicative of the differentiation of neuronal and glial cells. Furthermore, our coordinated analysis of the Co60 and Co64 alleles, revealed a novel molecular mechanism by which Hcfc1a regulates NPC expansion via the expression of asxl1, a gene that is essential for cell proliferation. These cellular deficits are accompanied by a hypomotile phenotype in 5-day old larvae, suggesting a role for HCFC1 in motor function. Collectively, our data supports a unique mechanism by which brain development and motor function are regulated by HCFC1. Financial support for this project was provided by NINDS award 1K01NS099153-01A1.

Program Abstract #52

Caenorhabditis elegans processes sensory information to choose between freeloading and self-defense strategies

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Hydrogen peroxide is the preeminent chemical weapon that organisms use for combat. Individual cells rely on conserved defenses to prevent and repair peroxide-induced damage, but whether similar defenses might be coordinated across cells in animals remains poorly understood. Here, we identify a neuronal circuit in the nematode Caenorhabditis elegans that processes information perceived by two sensory neurons to control the induction of hydrogen-peroxide defenses in the organism. We found that catalases produced by Escherichia coli, the nematode’s food source, can deplete hydrogen peroxide from the local environment and thereby protect the nematodes. In the presence of E.coli, the nematode’s neurons signal via TGFβ-insulin/IGF1 relay to target tissues to repress expression of catalases and other hydrogen-peroxide defenses. This adaptive strategy is the first example of a multicellular organism modulating its defenses when it expects to freeload from the protection provided by molecularly orthologous defenses from another species. The research was supported by National Science Foundation CAREER grant 1750065 to J.A. and National Institutes of Health grant R01AG051659 to A.G.

Program Abstract #53

The roles of Macrophage Migratory Inhibitory Factors (MIFs) in sea urchin development

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Macrophage Migratory Inhibitory Factors (MIFs) are a conserved family of atypical chemokines involved in a myriad of cell processes in animals and plants, but how they operate throughout development is not known. Since the MIFs’ discovery in the 1960’s and full cloning in mammals in 1993, these molecules have been shown to be important in vertebrate specific CD74 and chemokine receptor binding, as an upstream activator of ERK1/2, AMPK, and AKT signaling pathways, and an in vitro enzymatic role acting as a phenylpyruvate tautomerase. In invertebrate systems, MIF-like genes were cloned and perturbed in the larval starfish Patiria pectinifera to examine the larval immune system but their tissue expression patterns and mechanistic action in embryo development was not addressed. Our work examines the MIF homologues present in the sea urchin Lytechinus variegatus in early to late larval development. Using a combination of sequencing, qPCR, and in situ hybridization data, we show expression patterns of MIFs in the cleavage stage embryo and mesenchymal cells of gastrulae. By perturbing the MIFs and candidate interacting partners through drug treatments and morpholinos, we’ve made progress in defining their position in a developmental gene regulatory network, and in identification of a possible
functional role given that perturbation of MIFs causes an apparent cellular reprogramming event. This work is supported by the NSF GRFP Grant No. DGE-1644868 to RLA, and NIH HD14483 to DRM.

Program Abstract #54
The role of intestinal TOR signaling in metabolic responses to bacterial infection.
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Upon oral infection with pathogenic bacteria, Drosophila adults mount organism-wide immune and physiological responses in order to provide infection resistance and promote tolerance. The intestine plays a central role in mediating these effects. Upon infection, damaged intestinal epithelial enterocytes (ECs) promote local stem cell-mediated regeneration to promote tissue repair. The intestine also signals to other tissues to control host metabolism and physiology. Interestingly, we found a rapid increase in TOR kinase signalling in the intestine specific to infection stress, which was prominent in ECs, but also apparent in enteroendocrine (EE) and stem cells. TOR signaling is a well-established regulator of cellular, tissue and whole body metabolism. We therefore explored whether the intestinal TOR induction might contribute to metabolic responses to infection. We found that oral infection led to local intestinal upregulation of many lipid metabolic genes and reduced levels of whole body lipids. Furthermore, pharmacological inhibition of TOR blocked many of the infection-mediated changes in gut lipid gene expression. In contrast, over-expressing TOR in the ECs was sufficient to induce many of the metabolic gene expression changes and reduction in whole body lipids caused by infection. These data suggest that intestinal TOR induction may mediate both local and whole body metabolic responses to infection. EE cells secrete many different endocrine hormone peptides that can signal both locally and remotely to control metabolism, which were up-regulated upon EC specific TOR induction. We are currently exploring whether signaling through these peptides may couple the infection induced intestinal TOR activation to host lipid metabolic changes, and how these changes may contribute to infection tolerance. Funding sources- CIHR, Clark H. Smith Graduate Studentship.

Program Abstract #55
Cell type evolution and biosynthetic innovation in animals
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A long-standing challenge in biology is to explain how the functionality of complex multicellular organs emerges from the underlying evolution of cell types. Here, we deconstruct the molecular assembly of new cellular functions comprising a biosynthetic novelty in animals. A chemical defense gland in rove beetles (Staphylinidae) is the putative catalyst behind the global radiation of a clade of >17,000 species. Using single cell sequencing to guide genetic interrogation, we show how defense gland function was pieced together from ancestral molecular source material to create two, clade-specific secretory cell types, each capable of synthesizing distinct compound classes. Production of a short-chain alkane and two esters by one cell type evolved from assembly of a multi-enzyme fatty acid pathway; the alkane branch bears identical enzymatic logic to the ancient biosynthesis pathway for insect pheromones, but has been convergently pieced together from enzyme paralogs. Production of noxious benzoquinones by the second cell type derives from tyrosine metabolism, and evolved from a duplication within the laccase family of diphenoloxidases with ancestral roles in exoskeleton formation. The alkane and esters provide a solvent for the benzoquinones, creating a potent, bioactive secretion, and this chemical cooperativity confers the adaptive, ecological value of the gland. Evolutionary assembly of each novel cell type was thus shaped by coevolution between the two cell types. These findings connect the molecular evolutionary steps underlying new cellular functions to selection for new, organ-level properties. This work was supported by a Simons Fellow of the Life Sciences Research Foundation to A. Bru?ckner, and a Rita Allen Foundation Scholars Award, a Shurl and Kay Curci Foundation grant, an Alfred P. Sloan Foundation Scholarship, anda Klingenstein-Simons Fellowship Award and an Army Research Office MURI award W911NF1910269 to J. Parker.
Program Abstract #56

Pattern Formation and Regeneration in a Single Cell

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Cells are not amorphous bags of enzymes, but rather highly complex and orderly structures. Despite an extensive knowledge of the cellular "parts list", we still do not know the mechanisms by which cells attain their complex internal structures and external shapes. The giant ciliate Stentor coerulesus is a classical model organism that permits microdissection studies of a type normally employed in embryology. A single Stentor cell undergoes axiation, pattern formation, and even induction, thus illustrating that a single cell can display the key processes that characterize developmental biology at the organismal level. Research in Stentor over the past 100 years has unveiled a rich set of morphogenetic and regulative processes, but did not result in a molecular understanding of how these processes work. We have sequenced the Stentor genome and developed methods for perturbation of gene function. Using a combination of proteomics and transcriptomics, we are linking patterns of gene expression to key morphogenetic events that take place during Stentor division and regeneration. This project is funded by NIH grant R35 GM130327.

Program Abstract #57

Evolutionary shifts in the genetic regulatory network controlling fruit development across eudicots

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Fruits are the result of extreme ontogenetic transformations of the carpel walls after fertilization of the ovules. Their tissue re-patterning is diverse and it is linked to seed dispersal strategies. Fleshy fruits with predominant soft tissue rely on animal seed dispersal. Conversely, in dry dehiscent fruits, seed release depends on mechanical forces. The fruit genetic regulatory network (GRN) is established in the model Arabidopsis thaliana, with dry dehiscent fruits having a persistent medial layer (i.e. replum). In A. thaliana, FRUITFULL (FUL) controls proper valve cell proliferation and SHATTERPROOF (SHP) controls the formation of the dehiscence zone. These two MADS-box transcription factors (TF) negatively regulate REPLUMLESS (RPL), a HOX gene that controls replum development. SHP positively regulates the bHLH genes ALCATRAZ (ALC) and INDEHISCENT (IND) to form the unlignified and lignified layers of the dehiscence zone, respectively. The GRN is negatively regulated by APETALA2. All these TF families have undergone several duplication events, thus, the genetic repertoire is very different outside Brassicaceae, where A. thaliana belongs. We have aimed to compare the fruit GRN across eudicots, particularly in the Solanaceae (core eudicot) and the Papaveraceae (basal eudicot). In Solanaceae, both dry and fleshy fruits occur. Members of the Solanaceae, lack IND orthologs and use FUL, SHP and ALC homologs for fruit wall patterning. In Papaveraceae, indehiscent or dehiscent dry fruits occur. The latter, can open longitudinally, poricidally, or transversely. Papaveraceae species lack IND and SHP orthologs and recruit local FUL, ALC, RPL and AP2 paralogs to specify the fruit wall versus the dehiscence zone. Our data show that the fruit GRN has diversified dramatically across angiosperms. Funding: This work was funded by the ExpoSeed (H2020 MSCA-RISE 2015-691109) EU grant, and the Convocatoria Programáticas 2017-16302 at UdeA.

Program Abstract #58

Of Ants and Embryos: Pathways to Major Evolutionary Transitions

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Major evolutionary transitions in individuality occur when independently replicating individuals integrate to form a higher-level individual. Key transitions include integration of unicellular organisms into a multicellular organism, solitary individuals into a eusocial colony, and distantly related species into an obligate endosymbiosis. Although such transitions are thought to play a key role in the evolution of biological complexity, the evolutionary and developmental steps towards integration remain poorly understood. In this talk, I present evidence that the obligate endosymbiosis between the bacteria Blochmannia and hyperdiverse ant tribe Camponotini originated and elaborated through radical alterations in embryonic development. The general implications for pathways to major transitions to individuality will be discussed.
Program Abstract #59
How (and why) the jerboa got its long legs
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The vertebrate limb is a paradigm of developmental biology. It is a complex musculoskeletal structure that is formed by many of the same genes that construct other parts of the bodies of all animals. Decades of research has revealed mechanisms of limb induction, outgrowth, and pattern formation, but evolution produced astounding phenotypic diversity over hundreds of millions of years that is not represented in ‘traditional’ laboratory animals. This is likely due to differences between the experimental elimination of gene function versus the process of natural selection that refined expression of many genes in time and space to reshape species. Our research aims to discover mechanisms that diversified limb form and function using the laboratory mouse and a closely related bipedal rodent, the three-toed jerboa. While the jerboa forelimb has “mouse-like” proportions and five fingers, the elongated hindlimb has three toes, disproportionately long metatarsals in the feet that fuse into a single bone, and a complete absence of intrinsic foot muscle. Our work has identified an unusual ‘atrophy-like’ mechanism of natural foot muscle loss without evidence of cell death. Foot muscle loss coincides with the rapid and disproportionate elongation of the metatarsals to which these muscles attach, and we showed the increased rate of metatarsal growth is associated with a larger pool of growth plate chondrocytes and larger size of hypertrophic chondrocytes in the jerboa. We have taken a ‘cross-species’ intersectional approach to RNA-Seq analyses that identified genes and gene regulatory networks associated with muscle loss or with disproportionate bone growth. In addition to revealing new mechanisms fundamental to development of these tissues, these data form a foundation to identify modular cis-regulatory elements that may allow evolutionary divergence specific to the foot without similarly altering the rest of the musculoskeletal system. Supported by NIAMS and NSF IOS.

Program Abstract #60
A novel framework for understanding and projecting insect responses to climate change
Leslie Ries
Georgetown University, USA
Growing degree day models have been used for decades to predict emergence timing for plants and insects (especially crops and pests in agricultural systems). By accumulating degrees as a proxy for energy available for growth and determining a specific budget for how many of these proxy degrees are required to get to each developmental milestone, specific timing or number of broods can be predicted locally and also over large geographic regions. It has long been recognized that the minimum threshold for developmental activity appears to be close to 10°C for many ectothermic species, with some clear latitudinal gradients to promote earlier growth in colder climates. However, the minimum threshold for growth corresponds only to the temperature where some growth or metabolic activity is possible, but is usually much lower than the temperature required to actually survive and complete development. Few other developmental thresholds have been measured consistently enough to determine if those have consistent patterns among species. This makes it difficult to develop a framework for understanding the different thermal niches occupied by a wide range of taxa and also to allow us to project how communities may shift under changing climates. I will present recent advances in using growing degree day models to explore insect responses to climate change and a novel framework for projecting those models into future climate profiles.

Program Abstract #61
Coral symbiosis cell biology in the age of climate crisis: Turning discovery into solutions for saving reefs
Virginia Weis
Oregon State University, United States
Corals engage in a mutualistic symbiosis with intracellular photosynthetic dinoflagellates. This intimate partnership forms the trophic and structural foundation of coral reef ecosystems. This presentation will examine some of the cellular mechanisms underlying healthy symbioses and processes driving dysbiosis and coral bleaching. In addition I will point to areas where a strong understanding of host developmental biology will
advance the study of these mutualisms. Focus in the presentation will be on three areas. (1) Host innate immunity and symbiont strategies for modulating this immune response are central to the stability of the symbiosis and are the primary theme of the Weis Lab research program. (2) The spatio-temporal patterning of symbionts in hosts and their impact on host development and growth is a recent area of interest in the Weis group. Finally, (3) I will discuss global efforts to develop solutions to the coral reef climate crisis and where coral cell and developmental biology can play a role: in the value of sea anemone model systems and in the need to develop cryopreservation techniques and germplasm repositories. This work has been supported for many years by the National Science Foundation, most recently through an NSF-EDGE award: Enabling Discovery through Genomic Tools.

Program Abstract #62
Temperature stress introduces variability in embryogenesis via cell type-specific effects on developmental rate
Michael Dorrity, Lauren Saunders, David Raible, Christine Queitsch, Stanley Fields, Cole Trapnell
University of Washington, USA

Although vertebrate development proceeds properly in the face of a variable environment, stress can cause this developmental robustness to break down. The stereotyped, consistent developmental defects associated with stress (e.g. from temperature, hypoxia, environmental toxicants) suggest that some cell lineages and developmental programs are more sensitive to stress than others, but this phenomenon has not been thoroughly examined. For example, the effects of increasing temperature, which accelerates whole-animal development, have not been comprehensively analyzed at the level of individual cell-types. Here, we use single-cell RNA-sequencing to profile >100,000 cells of *Danio rerio* embryos raised at five different temperatures to ask: Which cell lineages are most sensitive to temperature stress during early development? Do cell lineages show coordinated acceleration of developmental rate in response to temperature? The scRNA-seq approach allows us to capture not only transcriptional responses, but the relative abundances of cell types in the embryo. In this way, we can link changes in transcription to changes in anatomy and composition of the embryo developing under stress. We find 1) that temperature-dependent increase in developmental rate is not uniform across cell types; 2) that some cell types show temperature-dependent decreases in abundance and 3) that cell types show unique transcriptional responses to temperature stress that do not include canonical heat-shock proteins. These cell-type specific differences in developmental rate and the abundance may underly the consistent phenotypic defects observed under stress. Overall, this work highlights the power of scRNA-seq to sensitively measure the effects of perturbations on whole developing animals to identify cell lineages that limit developmental robustness. This work is supported by the NIH.

Program Abstract #63
Development of sensory, secretory, and contractile functions of an organ for settlement and metamorphosis of tunicate larvae
Alberto Stolfi¹, Florian Razy-Krajka², Christopher Johnson¹, Ute Rothbacher²
¹Georgia Institute of Technology, USA; ²University Innsbruck, Austria

Tunicates are marine filter-feeding chordates, the majority of which alternate between motile larval and sessile adult stages. Their larvae do not feed but rather disperse in search of a suitable substrate on which to settle and metamorphose. Many tunicate species are highly invasive and a growing global threat to biodiversity driven by both human activity and ocean acidification. Thus, understanding their life histories and the molecular mechanisms underlying their substrate attachment preferences and metamorphosis pathways are key to controlling them in a world threatened by rapid climate change. Here we report our recent findings on the specification and morphogenesis of the anterior adhesive organs, or papillae, of tunicate larvae. First, using single-cell RNAseq we identified specific markers of the four distinct cell types that comprise the papillae and carry out its many functions in larval settlement and metamorphosis. Using tissue-specific gain-of-function and CRISPR/Cas9-based loss-of-function manipulations, we identified the key cell fate specification events that define each cell type and drive the self-organization and morphogenesis of the entire organ. We show that we can use these manipulations to alter papilla function and subsequently block larval metamorphosis. Furthermore, we describe a previously unrecognized myoepithelial cell type that expresses orthologs of vertebrate smooth muscles
and underlies the retraction of the adhesive organs during larval attachment. Examining the transcription factors that are expressed in this lineage, we found many orthologs of those expressed in vertebrate salivary glands and, unexpectedly, in cardiac mesoderm. We discuss these findings in the context of potential deep evolutionary connections between epidermal glands, smooth muscles, and cardiomyocytes. Work in the lab of AS is funded by NIH award R00 HD084814 and CJJ is funded by an NSF graduate research fellowship.

Program Abstract #64
From the depths: deep comparative phylogenomics in fishes to identify genetic mechanisms of evolution, development, and disease
Jacob Daane1,2,3, Juliette Auvinet3, Michael Peters1,3, H William Detrich3, Matthew Harris1,2
1Boston Children’s Hospital, USA; 2Harvard Medical School, USA; 3Northeastern University, USA
Traits that are pathological in humans are often adaptive in the context of other species and environments. Can we make use of this natural variation to identify genetic regulation of ‘disease’ phenotypes and the means by which species overcome the deleterious aspects of these traits? To approach these questions, we performed targeted sequencing of over 250,000 protein coding exons and conserved non-coding regions (CNE) across hundreds of fish species, allowing us to probe both genetic and evolutionary mechanisms of trait development through analysis of selection across a phylogeny. Here, we will focus on the loss of red blood cells by “white-blooded” Antarctic icefishes (Notothenioidei: Channichthyidae), a remarkable trait facilitated by the oxygen-rich waters of the Southern Ocean. Through integration of paleoclimate records with our phylogenomic datasets, we reveal relaxation of purifying selection in icefish across erythrocyte-associated genetic regions following a rapid decline in global temperatures and the formation of stable ice sheets. This trend continues in modern Antarctic notothenioids, including red-blooded species. For example, we detect mutations in the beta-spectrin of dragonfishes at the same amino acid sites as in human patients with spherocytic anemia, and we find that dragonfishes also have spherical erythrocytes. Further, we observed a bias in drift in icefish CNEs near genes that are expressed late in erythropoiesis, consistent with the presence of early-stage erythroid progenitor cells in icefish hematopoietic tissues. The accelerated CNEs are thought to be gene-regulatory sequences that control erythroid maturation. Study of these and other natural experiments will enable discovery of conserved mechanisms of development and disease. Supported by NSF PLR-1444167 (H.W.D.), AHA 17POST33660801 and Harvard Medical School Fund for Genetics of Climate Change (J.M.D)

Program Abstract #65
Evolution and development of seasonal wing patterns in butterflies
Robert Reed
Cornell University, United States
Many organisms display seasonal plasticity, where their genomes encode alternative phenotypes that are triggered by environmental cues such as photoperiod and/or temperature. Little is known, however, about the developmental genetic basis of how seasonal plasticity evolves. Here I will describe work our lab has been doing to characterize the molecular mechanisms underlying he evolution and development of seasonal color patterns in butterflies. We have used a combination of genetic assimilation, mapping, endocrine and chromatin assays, and genome editing to characterize several genes that underlie natural variation in seasonal color pattern reaction norms. We found that color pattern seasonality is largely controlled by cis-regulatory variation at downstream wing patterning genes, and not upstream cue detection or signal transduction genes. We propose that this mode of evolution is observed because it allows tissue- and trait-specific tuning of reaction norms, while avoiding pleiotropic effects that would be caused by changes to core sensory and endocrine mechanisms. This work was funded by the U.S. National Science Foundation.

Program Abstract #66
Cochlear extension and patterning require Myosin II and E-cadherin
Elizabeth Driver, Matthew Kelley
National Institute on Deafness and Other Communication Disorders, National Institutes of Health, USA
The elongated sensory epithelium of the cochlea, the mammalian auditory organ, is comprised of an exquisite
mosaic of mechanosensory hair cells and non-sensory supporting cells. Correct patterning of this mosaic is necessary for auditory function and requires the coordination of multiple processes of cell growth, migration, and rearrangement. We have previously shown, using a combination of live imaging, in vitro explants, and a myosin II inhibitor, that myosin II activity is required for multiple aspects of cochlear extension. To begin to determine the cell-type specific roles of myosin II, we have expressed a dominant-negative allele of myosin II (Myh10DN) in specific subsets of cochlear epithelia cells, revealing both cell- and non-cell-autonomous roles for myosin II activity. In pillar cells, which form a boundary between the medial and lateral domains of the cochlear epithelium, myosin II is required non-cell-autonomously for maintaining cellular organization in the lateral outer hair cell region. Expression of Myh10DN in outer hair cells causes a failure of both the normal increase in hair cell size, which contributes to later stages of cochlear elongation, and the accompanying decrease in supporting cell luminal surface area. In contrast, loss of the cell-adhesion protein E-cadherin in the same lateral region of the cochlea leads to a marked decrease in the surface area of supporting cells, such that hair cells are often found directly adjacent to each other, appearing to form cell-cell contacts. The interaction of myosin II and E-cadherin is known to be important in many different contexts of epithelial remodeling. These results suggest that in the cochlea, myosin II and E-cadherin may act in a complementary manner to control the size of hair and supporting cells and the junctions between them, which must be regulated to ensure proper patterning of the cochlear sensory epithelium. Supported by funds from the NIDCD Division of Intramural Research (DC000059).

Program Abstract #67
Dynamic cell behaviors drive axial mesoderm morphogenesis
Marissa Gredler1,2, Kathryn Anderson1, Jennifer Zallen1,2
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The notochord is an embryonic structure that has crucial roles in patterning adjacent tissues, breaking left-right symmetry, and elongating the head-to-tail axis. Before the final notochord forms as a solid rod within the body, notochord precursors, known as the axial mesoderm, go through a series of complex morphogenetic movements: they develop as a flat sheet of cells on the surface of the embryo that lengthens along the anteroposterior axis and shortens mediolaterally. Lineage tracing has demonstrated that the axial mesoderm arises from multiple origins, but the specific cell behaviors that bring these lineages together to produce the contiguous, elongated notochord structure are not well understood. Combined with its step-wise morphogenesis and known lineage, the accessible position of the axial mesoderm on the surface of the embryo make it an attractive candidate for studying mammalian epithelial morphogenesis using live imaging. To identify the mechanisms driving tissue shape changes in the developing axial mesoderm, we track individual cells over time in cultured whole mouse embryos using confocal microscopy. We find that extension of the axial mesoderm occurs in non-discrete phases which are defined by different cell behaviors along the anteroposterior axis, including mesenchymal-to-epithelial transition, three-dimensional tissue shape changes, and cell intercalation. To test the hypothesis that cell polarity modulation is required for axial mesoderm morphogenesis, we are examining the effects of deleting Pard3, a known regulator of planar and apical-basal polarity. We find that Pard3 is required for convergence of the axial mesoderm along the mediolateral axis. By using live-imaging and computational approaches in combination with transgenic mouse models, we are characterizing the molecular and cellular mechanisms that drive axial mesoderm development. This work is supported by an NIH fellowship to MLG (F32 GM123718) and the Howard Hughes Medical Institute.

Program Abstract #68
Super-resolution imaging of 3D DNA folding and RNA transcription during Drosophila development
Leslie Mateo, Alistair Boettiger
Stanford University, USA

Many of the differences in cell-type arise from specific interactions between distal enhancers and their target promoters. Both long-range activity and target specificity are believed to arise from the cell-type specific three-dimensional (3D) genome organization. Our limited understanding of 3D genome structure has improved due to sequencing-based technology; however, these approaches lack information pertaining the spatial organization of cells in tissues, and largely provide population level information, not single cell data. I will describe a novel
microscopy approach termed optical reconstruction of chromatin architecture (ORCA), to follow the nanoscale DNA path in steps as small as 2 kb, giving an unprecedented view of chromatin organization at the single-cell level. Additionally, we can simultaneously image both the nascent RNA transcript and mature cytoplasmic RNAs of 30 genes within the same cells. These measurements allow for direct correlation between chromatin structure and gene transcription. With ORCA, I find cell-type specific 3D DNA folding of the bithorax complex (BX-C) within the *Drosophila* embryo. Using embryos with genetic perturbations allow me to determine the genetic elements that drive proper cell-type specific enhancer-promoter contacts and gene expression. I will also present data and analyses of embryos that lack zygotic chromatin architectural proteins, which are known to be important for genome organization, to determine their impact of DNA folding and gene expression during development. Using our microscopy approach, we are beginning to uncover the mechanisms of genome organization. This work was supported by a Burroughs Wellcome CASI grant and Searle Scholar’s grant to Dr. Alistair Boettiger and HHMI Gilliam Fellowship to Leslie Mateo.

**Program Abstract #69**

**Hyaluronan-hydraulics and contractile-cytocinches drive inner ear morphogenesis**

Akankshi Munjal

*HARVARD MEDICAL SCHOOL, USA*

How embryonic tissues form complex organs remains an open question. An excellent example is the semicircular canals (SCC), which form from a single-layered embryonic tissue called the otic vesicle (OV). The form of SCC is well-conserved and required for sensing balance and acceleration. SCC formation initiates when different regions of the OV bud to form longitudinal projections. The projections fuse to form pillars and the space around corresponds to the SCC. Mechanisms underlying these topological transitions remain unknown. We use the accessible inner ears of zebrafish to study SCC development with a combination of quantitative imaging, genetics, physical perturbations and single-cell transcriptomics. I find that stereotypic morphogenetic processes such as cell proliferation, intercalation and tissue buckling do not contribute to SCC formation. Instead, SCC formation is a local process where pre-patterned regions of the OV synthesize a unique extracellular matrix (ECM) rich in hyaluronic acid (HA) and other glycosaminoglycans. HA exists as large polymers that swell by water absorption to make an amenable viscoelastic hydrogel. Spatially restricted HA-ECM swells to drive morphogenesis in the form of projection growth and cell stretching. Strikingly, HA-synthesizing cells produce specialized lamellipodia-like structures, named 'cytocinches'. Cytocinches extend beneath adjacent cells, are actomyosin-rich and tensed, and asymmetrically distributed with a bias orthogonal to the longitudinal axis of the projections. I find that cytocinches render the isotropic pressure from HA to cause anisotropic growth of projections. I am currently exploring the genetic factors that pattern the OV to affect these unique morphogenetic processes for SCC development. Overall, our study presents a novel framework, where ECM-driven remodeling and cytocinch-driven shaping of a simple epithelial tissue results in the morphogenesis of a complex organ. Funding: LTF-HFSP, K99-NICHHD

**Program Abstract #70**

**Determining how noisy transcription controls stochastic fate specification in the developing fly eye**

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How gene expression noise is harnessed to generate cellular diversity during development is poorly understood. To determine how cells randomly choose between fates, we studied the random mosaic of two color-detecting photoreceptor subtypes in the fly eye. The on/off expression of the transcription factor Spineless (Ss) controls R7 photoreceptor subtype fate, resulting in 65% Ss<sup>on</sup> R7s and 35% Ss<sup>off</sup> R7s. Patterning is controlled by a two-step mechanism. In step 1, s<sub>s</sub> is transcribed in a pulse in R7 precursors. In step 2, the transcriptional pulse ceases, cells differentiate, and S5 turns back on in a random subset of terminal R7s. Manipulating regulatory DNA elements and transcription factors tunes the strength of ss expression in the early pulse, leading to changes in the on/off ratio in terminal R7s. These findings suggested that the level of ss expression in an individual R7 precursor determines the on or off state as the cell differentiates into a terminal R7. To assess transcriptional variability in the early pulse, we developed a three-color RNA FISH approach to visualize the 5′, middle, and 3′ region of the
nascent ss transcript. This quantitative approach showed that the early pulse of transcription is noisy and that this variability is independent of developmental timing. To monitor transcription in real time, we utilized the MS2/MCP system. We inserted MS2 stem loops into different positions and found that upstream insertions into the 5' UTR and the first intron are expressed in more cells than downstream insertions in a middle intron and the 3' UTR, indicating that elongation regulates transcriptional variability in the early pulse. Our current work aims to use live imaging to link noisy transcription to terminal cell fate. This work utilized imaging approaches to identify a source of transcriptional variability that acts to randomly pattern cell fates during development. This work is funded by the NIH R01EY025598.

Program Abstract #71
Molecular Control of Organ Regeneration: Insights from Platypus, Armadillos, Bats and Whales
Guo Huang
University of California San Francisco, USA
Across phylogeny and ontogeny, pronounced differences are evident in the ability of organisms to regenerate damaged tissues and organs. However, the underlying mechanisms driving this phenomenon have remained enigmatic. Mammalian hearts lose regenerative potential due to cardiomyocyte cell-cycle withdrawal and polyploidization coinciding with the development of postnatal endothermy. Through phylogenetic analysis of vertebrate cardiomyocyte ploidy as a proxy for cardiac regenerative potential, we uncover that certain monotreme, edentate, cetacean, chiropteran species have unusually high percentages of diploid cardiomyocytes in the adult heart. Moreover, cardiomyocyte abundance across 41 species conforms to Kleiber’s law, the ¾-power law scaling of metabolism with bodyweight, and decreases when the standard metabolic rate, body temperature, and thyroid hormone level increase during the ectotherm-to-endotherm transition. In parallel, we identify thyroid hormones as potent developmental triggers of mammalian cardiomyocyte polyploidy and cell-cycle arrest in vivo. Inactivation of thyroid hormone signaling reduces mouse cardiomyocyte polyploidization, delays cell-cycle exit, and retains cardiac regenerative potential in adults. Conversely, exogenous thyroid hormones inhibit zebrafish heart regeneration. Thus, our findings support a model where loss of cardiomyocyte regenerative potential is driven by increases of thyroid hormone levels, and implicate that the limited regenerative capacity in various adult mammalian tissues including the heart may be a tradeoff for the acquisition of endothermy during animal development and evolution.

Program Abstract #72
Telomere length is exclusively maintained by the ALT mechanism in a regeneration-competent vertebrate, the newt Pleurodeles waltl
Qinghao Yu1, Phillip Gates2, Ivan Mikicic1, Martina Lachnit1, Florian Salomon1, Antonio Caldarelli1, Ahmed Elewa3, Andras Simon3, Maximina Yun1,4
1TUD CRTD-Center for Regenerative Therapies TU Dresden, Germany; 2University College London, UK; 3Institute Karolinska, Sweden; 4Max Planck Institute of Molecular Cell Biology and Genetics, Germany
Telomere shortening places a key limitation on the lifespan of a cell, constituting a key hurdle in highly regenerative contexts. In all known vertebrates, this limitation is overcome by a telomerase-dependent telomere extension mechanism, whose failure results in premature ageing and functional impairments in regeneration as telomeres erode. An alternative mechanism for lengthening telomeres (ALT), dependent on homologous recombination, has recently been shown to compensate for telomerase loss in a limited proportion of cancer cell lines, though never at the whole-organism level. Here, we show that the newt Pleurodeles waltl, an organism which can undergo repetitive regeneration cycles throughout lifespan, controls telomere length through ALT during regeneration, development and homeostasis. Telomerase activity is absent from developing, larval and replicative adult tissues, long-tracks of telomere consensus sequence are absent from its DNA, and its chromosomes show altered binding of shelterin components at their ends. By mining the newt genome, we found that the telomeric sequences of P. waltl are comprised of a series of interspersed, highly recombined telomere consensus variants, characteristic of ALT activity. Furthermore, we show that newt tissues contain extra chromosomal telomeric DNA circles, sub-products of the ALT mechanism, and newt cells are specifically sensitive to an ALT inhibitor. Importantly, we show that this mechanism is enhanced during salamander limb regeneration.
Finally, our data suggests that ALT operates throughout the Salamandridae family. Altogether, we demonstrate that telomere length can be exclusively maintained by ALT in a vertebrate, a finding which has important implications for regenerative, developmental and cancer biology.

Program Abstract #73
Pleiotropic effects of mitochondrial dysfunction: A characterization of Multiple Mitochondrial Dysfunctions Syndrome 1
Peter Kropp
NIDDK/NIH, USA
Mitochondrial diseases present an interesting challenge to clinicians and researchers alike as symptoms and phenotypes are characteristically diverse, inconsistent, and of variable penetrance. However, careful analysis of mitochondrial diseases reveals delicately balanced feedback loops and intersection of homeostatic pathways that function in both cell-autonomous and cell-nonautonomous manners. We have used the nematode *Caenorhabditis elegans* to study rare mitochondrial diseases including Multiple Mitochondrial Dysfunctions Syndrome (MMDS1), an autosomal recessive disease caused by mutations to the iron-sulfur cluster (ISC) biogenesis factor NFU1 (LPD-8 in *C. elegans*). Lost NFU1/LPD-8 function prevents generation of lipoic acid, an essential cofactor for oxoacid dehydrogenase complexes including pyruvate dehydrogenase and a-ketoglutarate dehydrogenase. Using *C. elegans* we have recreated five exact patient mutations of NFU1/LPD-8 to better understand the consequences of each individual mutation. *C. elegans* are an excellent model in which to do this work because of ease of genetic and chemical modification, invariate cell lineage, the ability to parse cell-type specific phenotypes and tractability to humans. We have discovered an allelic series of phenotypic severity amongst the patient alleles indicating progressive impairments in oxidative metabolism, accumulation of oxidative stress, and subsequent activation of multiple mitochondrial stress response pathways. Our work suggests that impaired oxidative metabolism alone does not contribute to the observed phenotypes, but also iron toxicity caused by accumulation of labile iron in cells. Ongoing work indicates cell-nonautonomous effects with neuronal dysfunction causing dysregulation of other tissues. We are also working to identify genetic suppressors of these phenotypes. Funding: National Institute of Diabetes and Digestive and Kidney Diseases, Intramural Research Program

Program Abstract #74
Regulation of Damage-Responsive Maturity-Silenced enhancers in Drosophila
John Quinn
Arizona State University, USA
Regeneration is a complex process that occurs in a variety of different organisms. Although the mechanism of regeneration can vary, a consistent observation is that regenerative ability declines with maturity. Understanding how regenerative ability is lost could lead to insights into how to regrow damaged tissue in humans. In early larval stages the imaginal discs of Drosophila, the precursors to the adult appendages, have a significant capacity to regenerate, which is lost as the fly matures toward pupariation. Using a genetic ablation method developed in our lab we found that several genes involved in disc regeneration are regulated by damage-responsive and maturity-silenced (DRMS) enhancers. These regulatory elements are activated upon damage to induce regenerative gene expression, but epigenetically silenced as the organism approaches pupariation. The goal of my research is to investigate what activates DRMS enhancers and progressively silences them as the disc matures. The genes *wg* and *Wnt6* are required for regeneration and controlled by a single DRMS enhancer (DRMSWnt). We found that JNK signaling is necessary for the activation of DRMSWnt but not sufficient, since developmental JNK signaling during embryogenesis does not activate DRMSWnt. Using a GFP reporter for DRMSWnt we found that reducing JAK/STAT pathway activity decreases damage-induce activation of the reporter. We confirmed this using a novel in-vitro culture method and a chemical inhibitor of JAK/STAT signaling. To further understand enhancer dynamics, we also built a fluorescent-based system to visualize DRMS activity in real-time with single-cell resolution. This versatile tool will enable us to study DRMS silencing in different tissues with live imaging. Together, this research has the potential to reveal important insight into the cis-regulatory mechanisms that control regeneration. This work is supported by startup funding from the Harris lab at Arizona State University.
Program Abstract #75

Molecular mechanisms that enable synapse stabilization and restoration

Emily Heckman, Chris Doe
University of Oregon, USA

The continuous function of the human nervous system is dependent on the maintenance of trillions of synaptic connections between neurons. Despite synapse loss being a hallmark of many neurodegenerative diseases, little is known about how synapses are stably maintained during homeostasis. Cell surface molecules (CSMs) are key regulators of synapse establishment. We therefore sought to understand the extent to which CSMs are utilized in the persistent maintenance of synapses after they are established. Using previously published RNAseq data, we identified 129 CSMs belonging to the IgSF, LRR, and cadherin protein families that are enriched in the larval and adult Drosophila central nervous systems. We used the powerful spatiotemporal specificity of Drosophila genetics to determine which of these CSMs promote circuit function in a single pair of larval interneurons called Pair1. Pair1 are descending inhibitory neurons whose activity pauses forward locomotion. Of the 129 CSMs that were screened, we identified 13 candidate regulators of synapse maintenance whose knock down either suppressed or enhanced Pair1-mediated pausing. Here we focus on one of these CSMs, the conserved receptor protein tyrosine phosphatase, LAR. Importantly, we find that knock down of LAR in Pair1 results in synapse depletion. We find that LAR is expressed throughout larval life in Pair1 specifically, as well as brain-wide. We will describe future experiments aimed at unraveling the role of LAR in synapse maintenance, as well as in the restoration of synapses in a model of Alzheimer’s disease. This project was supported by HHMI, UO Developmental Biology Training Program, and NIH.

Program Abstract #76

How to build a gliding mammal: patterning mechanisms in mammalian skin

Ricardo Mallarino
Princeton University, USA

Our lab aims to uncover the mechanisms by which form and structure are generated during vertebrate development and how these processes get modified across evolutionary time to produce phenotypic variation. Specifically, we explore questions relating to patterning and the evolution of novelty in mammalian skin. Skin displays remarkable phenotypic variation, both between species and within an organism, suggesting that the developmental program patterning this tissue harbors a latent reservoir of anatomical complexity. In this talk, I will describe the steps we are taking to uncover the molecular basis of a remarkable adaptation: the mammalian gliding membrane, or patagium. The patagium is an ideal model for studying fundamental processes of morphogenesis, such as the mechanism by which positional information is conveyed to drive localized outgrowth of a tissue. In addition, this tissue is fascinating from an evolutionary biology perspective because it has arisen independently in divergent mammalian groups, including colugos, rodents, and Australian marsupials. Of these, Australian marsupials are particularly tractable because the patagium develops externally, offering an opportunity to study this tissue as it forms and expands. Moreover, three closely related species in this group have evolved gliding independently, enabling comparative studies at the genomic level. First, I will show how we are combining functional genomics and experimental manipulations to identify gene regulatory networks underlying patagium formation in a novel model system, the marsupial sugar glider (Petaurus breviceps). Second, I will discuss how we are using comparative genomic approaches to contrast closely related gliding and non-gliding marsupials and pinpoint loci that have repeatedly evolved to pattern convergent morphologies. By uncovering how positional information is encoded in mammalian skin, this work will provide mechanistic insights into the origin of phenotypic novelty.

Program Abstract #77

HOX genes and the cephalopod body plan

Caroline Albertin1, Therese Mitros2, Hannah Schmidbaur3, Oleg Simakov3, Daniel Rokhsar2, Clifton Ragsdale4
1Marine Biological Laboratory, USA; 2University of California, Berkeley, USA; 3University of Vienna, Austria; 4University of Chicago, USA

Cephalopods have a highly derived body plan and a suite of morphological innovations with no obvious correlates
in other animals. They also demonstrate a loss of the spiralian early developmental program characteristic of other molluscs. How this highly derived body plan and its novel developmental program relate to those of other animals has been obscure. To address these questions, we studied the genomic arrangement and expression of the HOX genes in *Octopus bimaculoides*, the California two-spot octopus, and *Doryteuthis pealeii*, the longfin inshore squid. Chromosome-scale genome assemblies for these species recover a single, intact, but massively expanded, HOX cluster in each of these species. We find that cephalopod HOX expression exhibits the canonical nested domains of expression, unlike what has been described in many other molluscs and spiralians, including another cephalopod (Lee et al. 2003). Moreover, the early expression of the HOX genes reflects the derived arrangement of cephalopod development: expression domains radiate out from the center of the embryo, forming the nested, bilaterally symmetrical sectors expected for a polar coordinate system. These data support an ancestral role for HOX genes in patterning the cephalopod body plan despite the dramatic increase in the size of the genomic cluster and the radial geometry of the early embryo. We also observe tantalizing differences in both the HOX gene complements and expression patterns between squid and octopus that may point to a key role for HOX genes in the evolution of their body plans. This work was funded by NSF grant IOS-1354898, the Grass Foundation, and the Hibbitt Early Career Fellows Program at the MBL.

**Program Abstract #78**

**Regeneration enhancers and the uneven distribution of regenerative capacities in vertebrates**


1*Stowers Institute for Medical Research, USA; 2Howard Hughes Medical Institute, USA; 3Department of Genetics, Stanford University, USA; 4Glenn Laboratories for the Biology of Aging, USA*

Species such as bony fishes display extensive regenerative capacities, while others such as mammals regenerate poorly. The mechanisms underlying the broad disparity of regenerative capacities in animals remains elusive. Here we report on a comparative epigenomic and transcriptomic approach which identiﬁed an evolutionarily conserved regeneration response program (RRP) in vertebrates. By defining the *cis*-regulomes and transcriptomes of early stages of regeneration in the distantly related zebrafish *Danio rerio* and the African killifish *Nothobranchiussfurzeri*, we were able to discriminate between species-speciﬁc and evolutionarily conserved genomic responses to amputation. Importantly, functional testing by systematic transgenic reporter assays of the conserved *inhibin beta A* (*inhba*) regeneration responsive enhancer (RRE) from zebrafish, killifish, and humans identiﬁed species-speciﬁc variations. Furthermore, deletion of the killifish *inhba* RRE signiﬁcantly perturbed caudal ﬁn regeneration and completely abrogated cardiac regeneration. We also show that *inhba* RRE activity requires the presence of predicted binding motifs for the Activator Protein 1 (AP-1) complex. Interestingly, AP-1 binding motifs can be identiﬁed in the conserved and non-conserved teleost RREs reported in this study, indicating that AP-1 may be required for both injury and regeneration responses. We propose that changes in RREs driven by natural selection are likely a crucial source of loss of regenerative capacities in vertebrates, including humans. (This research was funded by Stowers Institute for Medical Research and Howard Hughes Medical Institute.)

**Program Abstract #79**

**From head to toe: uncovering mechanisms of craniofacial and limb variation in domestic pigeons**


1*University of Utah, USA; 2University of Georgia, USA; 3University of Colorado, USA*

Deciphering the mechanisms of morphological variation remains a critical challenge in evolutionary and developmental biology. Canalized development typically produces only modest variation within a species. However, as a result of millenia of artiﬁcial selection, the domestic pigeon (*Columba livia*) displays spectacular diversity within a single species. Morphological variation is particularly striking in the pigeon head and hindlimb; beaks can be either miniaturized or dramatically enlarged, and feet can be covered in scales or elaborate ornamental feathers. To identify the genes and developmental pathways that control these remarkable examples of morphological variation, we use a combination of traditional laboratory crosses and comparative genomic
analyses. Recently, we characterized an evolutionarily conserved limb identity network downstream of TBX5 and PITX1 that is associated with scale vs. feather formation in pigeon hindlimbs (Boer et al., Dev Bio 2019). We have also identified genetic regulators of pigeon craniofacial morphology. By comparing the genomes of small and medium beak pigeons representing diverse breeds, we found several loci associated with variation in craniofacial morphology. In one highly differentiated genomic region, we identified an amino acid substitution in the non-canonical Wnt receptor ROR2 as a candidate regulator of pigeon beak size. To identify the genetic architecture of specific dimensions of craniofacial size and shape, we are combining traditional and geometric morphometrics with quantitative trait loci mapping in two F2 laboratory crosses. Notably, in our F2 population, beak length is strongly associated with the ROR2 coding variant identified in our genomic analyses. Because the genes that underlie normal development are highly conserved, the extraordinary variation in pigeons can provide new clues about the molecular control of vertebrate morphological diversity. This work is supported by NIH F32DE028179 and R35GM131787.

Program Abstract #80
Architecture and explosive discharge of a cellular weapon
Ahmet Karabulut, Boris Y. Rubinstein, Sean A. McKinney, Matthew C. Gibson
Stowers Institute for Medical Research, USA
The venomous harpoons of cnidocytes, the stinging cells of cnidaria, are one of the most remarkable micromachineries found in nature. Before firing, the harpoon is kept inside an intricate organelle (cnidocyst) as a coiled thread wrapped inside a pressurized capsule. Upon mechano-chemical stimuli, the pressurized capsule discharges rapidly, and ejects a venomous harpoon that penetrates and delivers a cocktail of neurotoxins into a prey. The mechanics of the individual stages of ultra-fast capsule discharge are still poorly understood. Here, we show that the harpoons of Nematostella vectensis are formed by two sub-structures -- a shaft and a tubule that are initially connected and become separated during the discharge. At the initial stage of discharge the highly compacted shaft is ejected by the capsule pressure. Next, the shaft everts and pulls out the tubule from the capsule by a slingshot-like mechanism. By using reactive dye labeling, we found that the Nematostella harpoon tubule traverses through the shaft formed by three helical fibers. At the final stage, the twisted tubule unwinds delivering venom into the prey. These results suggest that the composition of the cnidocyte harpoon is quite complex and the discharge process utilizes multiple mechanisms. Understanding the structure and mechanical behavior of the cnidocyte harpoon will reveal insights into the intracellular assembly of functional structures that form complex micromachinery. This research is funded by Stowers Institute for Medical Research

Program Abstract #81
Mechanisms of Development and Regeneration in Hydra
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The adult Hydra continually renews all of its cells using lineage-restricted stem cells and can regenerate its entire body from a small piece of tissue. This combination of properties allows us to dissect both homeostatic and regenerative development in parallel and make direct comparisons and connections between the two. Our research goals are to: 1) elucidate the signaling pathways and gene regulatory networks that drive homeostatic development and 2) determine how injury signals trigger these developmental programs during regeneration. To gain a comprehensive view of the gene expression changes that occur during homeostatic development, we used single-cell RNA-seq (scRNA-seq) to capture the spectrum of cell states that occur as cells differentiate. We used this information to build developmental trajectories for each lineage and identify transcription factors expressed at key developmental decision points. This has given us a starting point to build gene regulatory networks to describe Hydra homeostatic development. To understand how these developmental pathways are activated during regeneration we have surveyed the transcriptomic and chromatin changes that occur over the first 12 hours of head and foot regeneration. These data allowed us to identify putative regulatory connections between the conserved response to injury and the activation of Wnt signaling, which is necessary and sufficient for head development. Surprisingly, we found that Wnt signaling is activated in all injury contexts, including foot regeneration, but is rapidly down regulated in contexts that don’t lead to head regeneration. In summary, we aim
to take advantage of the relative simplicity of Hydra to uncover fundamental principles of the regulatory logic controlling development and regeneration. This work is supported by UC Davis start-up funds and NIH R35 GM133689.

Program Abstract #82
TMED2 is Required for Trophoblast Migration, Differentiation, and Proliferation
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Transmembrane emp24 domain (TMED) proteins are cargo receptors with an N-terminal Golgi dynamics (GOLD) domain critical for heterodimerization and cargo recognition. Although the structure of TMED2 is known, the function of TMED2 in placental development remains poorly understood. Mouse embryos homozygous for a null mutation in the signal sequence of TMED2 died at midgestation from failure to form a placental labyrinth layer. Moreover, ex vivo cultures of pre-attachment chorion and allantois suggest that TMED2-mediated transport of fibronectin and VCAM1 is required for labyrinth layer formation. Thus, we hypothesize that trophoblast migration and syncytiotrophoblast fusion also require functional TMED2. To test the role of TMED2 in cell migration and survival, we conducted scratch and viability assays in HEK293 cells overexpressing TMED2. We also tested the effects of mutating residues in the GOLD domain of TMED2, E48 and K91. We found that cells overexpressing wild-type TMED2 and TMED2-E48Q displayed similar cell migration and elevated ER stress. However, cells overexpressing TMED2-K91R showed increased cell migration and viability. Next, we conducted fusion studies in two human choriocarcinoma cell lines: JEG-3 and BeWo. Overexpression of wild type TMED2 in JEG-3 cells was sufficient for syncytiotrophoblast fusion. However, knockdown of TMED2 in BeWo cells inhibited fusion, supporting a requirement for TMED2 in this process. Overall, our data suggest that levels of TMED2 must be tightly regulated for cell migration and viability. In addition, we found that the K91 residue of TMED2, which is predicted to help mediate heterodimerization with TMED10 is important for TMED2 function. We propose that abnormalities displayed by embryos and cells deficient in or overexpressing TMED2 reflect the inadequate transport and localization of fibronectin and likely other unidentified proteins critical for trophoblast proliferation and migration. This work is funded by NSERC.

Program Abstract #83
Characterization of the spatiotemporal expression pattern of Dispached-3 during chick cranial neural crest development
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The neural crest is an embryonic, multipotent, vertebrate cell population that forms diverse cell types such as neurons, bone, cartilage, and smooth muscle. Dispatched-3 (DISP3), a transmembrane protein of the Dispatched family that releases hedgehog ligands from cells, was identified by our lab as a putative regulator of cranial neural crest development. DISP3 influences neural stem cell fate decisions and could play a similar role in neural crest development. However, DISP3 has never been studied during embryogenesis. Thus, the aim of this project is to characterize the spatiotemporal expression pattern of DISP3 in chick embryos to determine whether DISP3 is expressed at the right time and place to regulate cranial neural crest development. To accomplish this, I used whole mount in situ hybridization to visualize DISP3 expression in Hamburger and Hamilton stage 6 to stage 12 (16 somites) embryos. I found that DISP3 is expressed by the cranial neural folds, where premigratory neural crest cells reside, and the neural plate during neurulation. Interestingly, DISP3 is differentially expressed in the cranial region, with DISP3 restricted to the cranial neural folds in the anterior midbrain but expressed throughout the neural plate and cranial neural folds in the posterior midbrain and hindbrain. DISP3 is also expressed in the trunk neural plate and somites. Later, DISP3 is expressed in migratory cranial neural crest cells, which I confirmed using double whole-mount in situ hybridization for DISP3 and SOX10, a transcription factor expressed by neural crest cells. DISP3 is also expressed by the pronephros and blood islands, and DISP3 persists in the trunk neural plate and somites. These results indicate that DISP3 and thus hedgehog signaling may play a role in early cranial neural crest development. Funding: NSF IOS-1354809; UMN Undergraduate Research Opportunities Program; UMN TRIO McNair Scholars Program.
Program Abstract #84
Characterizing Meteorin as a midline signaling molecule that determines radial glial cell fates during zebrafish CNS development.
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Midline morphogenic signaling is well known to play a critical role in patterning the cell fates of the developing nervous system. In vitro studies have suggested that Meteorin is a putative glial cell differentiation factor; however, its prominent expression in the developing vertebrate nervous system has yet to be investigated. We used the zebrafish model system to investigate the role of meteorin, and its paralog meteorin-like in radial glial development across the CNS. We have determined that both metrn and metrnl are expressed dorsally in a restricted but overlapping pattern, most concentrated at the midline. To analyze the functional requirement of these two genes in radial glial development, we have conducted both gene knock-down and knockout approaches for metrn and metrnl genes in zebrafish. Loss of function of Meteorin results in tail axis curvature and cyclopia. Interestingly, overexpression of metrn mRNA similarly causes cyclopic embryos. Importantly, combinatorial analyses suggest synergistic functions between metrn and metrnl. Currently, we are assessing cell type expression patterns of these Meteorin genes, as well as characterizing the cell fate patterns following gain and loss of function manipulations. We propose that Meteorin functions as a novel dorsal midline morphogenic signal that serves to regulate progenitor cell development in the early CNS. This work has been supported by the Tomlinson Fund and the Clark Science Center at Smith College, as well as the National Science Foundation.

Program Abstract #85
Embryonic exposure to ibuprofen causes neural crest defects in vertebrate embryos
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Non-steroidal anti-inflammatories (NSAIDs) such as aspirin, ibuprofen, and naproxen have undergone a rigorous process to prove that they are safe for adult consumption and for use without the supervision of a medical practitioner. However, recent studies indicate that multiple cell types and physiological processes are affected by exposure to these drugs. Further, very few analyses have been performed to determine the effects of exposure on developing embryos. Here, we have analyzed the effects of exposure to the cyclooxygenase (COX) inhibitor, ibuprofen, on developing chicken (Gallus gallus) and axolotl (Ambystoma mexicanum) embryos. Based on previous work, we hypothesized that exposure to ibuprofen would create craniofacial and heart defects, and therefore we focused our analyses on the developing neural crest cells. Neural crest cells create the craniofacial bone and cartilage, pigment cells and the peripheral nervous system, and abnormal development of these cells cause defects such as cleft palate, albinism, and deafness. Using morphological analysis and immunohistochemistry, we identified a dosage-specific response to ibuprofen. Low doses of ibuprofen increased survival compared to controls, whereas high levels of ibuprofen created cranial developmental anomalies in both species, expanded expression of the neural crest progenitor marker, PAX7, and reduced the expression of the definitive neural crest marker, SOX9. These data suggest that signaling through the COX pathway is necessary for the progression of neural crest cell development from progenitor to bonafide neural crest cells. Using comparative anatomical, molecular, and biochemical analyses our future work will determine the links between COX-signaling and our observed phenotypes in addition to identifying if the detrimental effects are specific to ibuprofen or are a global NSAID phenotype. Funding provided by NIH BUILD PODER

Program Abstract #86
Uncovering functional roles in development for differentially expressed ribosomal protein eRpL22-like using a conditional gene knockout strategy
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The Drosophila melanogaster eRpL22 ribosomal protein family contains two structurally divergent & developmentally essential paralogs: eRpL22 and eRpL22-like - the latter exhibits tissue-specific expression across
development; the former is ubiquitously expressed. Multi-tissue co-localization comparison of eRpL22-like and core ribosomal components indicates eRpL22-like may have functional roles both within the ribosome itself and apart from ribosomal processes. To elucidate roles for eRpL22-like, we characterized morphological defects in an eRpL22-like heat-shock conditional knockout homozygote (CKO). Preliminary ovary-specific phenotypes include: sudden mid-oogenesis arrest, ectopic rounded follicular epithelium cells, and double-anteriorized eggs. Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs (also expressed within the ovary, with homologs associated with human disease) involved in cell polarity, suggesting that paralogue-specific “specialized ribosomes” translate specific mRNAs. Immunohistochemical characterization of our eRpL22-like CKO mutant allows us to tease-apart individual ovarian phenotypes, assess downstream protein expression changes of differentially enriched mRNAs, elucidate putative extra-ribosomal functions of eRpL22 paralogues, and potentially reveal a novel model useful for studying specific human conditions arising from a spectrum of epithelial polarity defects. These preliminary data broaden the context for investigation of the role of eRpL22-like as an essential player across multiple developmental processes. This work was funded by a Lehigh University Department of Biological Sciences Undergraduate Research Grant and Langer-Simon Scholar Award.

Program Abstract #87

Tubes, Coils, or Branches? Developing Novel Ways to Characterize Uterine Glands
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The premise that structure is critical to function is fundamental to study of all biological systems. Evolution shapes structure to optimize for specific functional outcomes; hence, quantitative morphological analysis carries great promise in yielding insight into the mechanisms and function of tissues in general. Here, we present our use of novel 3D imaging modalities to quantitatively analyze the branching morphology of uterine glands in mice during early pregnancy. Using new image analysis tools, we have generated quantitative structural comparisons between different stages of early pregnancy to better understand structure-function relationships during early pregnancy. Using 3D image reconstructions of gland populations generated from whole-mount confocal imaging, we characterize the uterine gland as a coiled, branched, tubular structure rather than a simple tubular structure as reported previously. When compared to a non-pregnant uterus, we show an increase in branch number and gland length without a change in gland volume at the peri-implantation stages. These preliminary findings support the conclusion that uterine glands reorganize but likely do not proliferate and that structural changes are pre-requisite to gland function essential for embryo survival and implantation success. To confirm this, we are using a nuclear marker to develop an algorithm for quantification of cell number per gland to ascertain how proliferation and apoptosis contribute to change in gland shape and structure during early pregnancy. We are also developing an automated segmentation tool to produce large numbers of gland lumen surfaces to classify luminal morphologies of several glands at multiple time points during early pregnancy. Our findings thus far demonstrate the promise of a structurally analytical approach for future studies of the gland function and its role in regulating the peri-implantation uterine environment.

Program Abstract #88

Community-Building Strategies Toward Improving Science Literacy in Secondary Education and Beyond
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Stem cell research, genomics and bioengineering are having an immense impact on human medicine, and are increasingly represented in college curricula. However, in the United States and internationally, a majority of people do not attend university. As a result, integration of these topics into secondary school syllabi can potentially have a dramatic effect on science literacy. This is particularly important in the area of stem cell science.
with the rise of unproven therapies and under-regulated stem cell “clinics.” In New York State, challenges to incorporation of quality learning activities include substantial teaching loads, limited time for new curriculum development, lack of resources and infrastructure for wet lab activities related to these topics. Immigrant communities often face additional hurdles in learning to navigate popular media and medical literature in their non-native languages. Rensselaer’s Pre-College Teachers Training Program in Stem Cell Biology & Bioengineering provides an intensive summer research experience with emphasis on team-building. In contrast to the Research Experience for Undergraduates (REU) model that pairs each participant with a researcher for most of the program, teachers spend 90% of the course working as a group. A third of that time is devoted to curriculum development, while the additional 10% is given to forming on-going professional relationships with mentor labs. Teachers are empowered to create activities appropriate for their grade level and school district. Over 3 years, 32 teachers have participated, representing 15 school districts with a combined enrollment of more than 170,000 students. Further, the unique format has made it possible to offer the program remotely during Summer 2020. Innovative hands-on activities developed through this program span grades 7 through 12, and may also be appropriate for introductory undergraduate classes. Funding was provided by the New York Stem Cell Initiative (NYSTEM C30161GG).

Program Abstract #89
Doing the Molecular Splits: Hands-on Demonstration Tips to Help Student Engagement Using Split Inteins in Molecular Biology
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Engaging undergraduates in the mechanisms of post-translational modifications lies at the heart of molecular and cell biology research. An important challenge is how science educators develop inclusive and equitable approaches along with hands-on demonstrations to clarify post-translational modification mechanisms, such as protein splicing called split inteins. Here, we describe step-by-step assembly of recycled materials that instructors can use to help clarify the molecular action of split inteins during inclusive classroom teaching settings. The science education work is supported through NSF REU DBI #1852032 and science education grant from Society for Developmental Biology.

Program Abstract #90
The Genomics Education Partnership: A Nationwide CURE that Enhances Research Opportunities for Students and Faculty at Diverse Institutions
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The Genomics Education Partnership (GEP; http://gep.wustl.edu) is a growing community of practice that provides authentic course-based undergraduate research experiences (CUREs) in genomics. Our members include faculty from over one hundred institutions, including community colleges, primarily undergraduate institutions, minority-serving institutions, and research-intensive universities, and we are currently recruiting new members. We use shared resources and curriculum, and publicly-accessible databases to provide opportunities for students to contribute meaningfully to scientific investigations which we coordinate. Students participating in GEP projects gain hands-on experience investigating eukaryotic gene structure using basic bioinformatics tools like a genome browser and BLAST, and those who complete projects are eligible for authorship on a scientific research paper. The GEP leverages the relative low cost, ease of scalability, and portability of our projects to reach a variety of students, including online settings during the Covid19 pandemic. The large consortium of faculty implementing a GEP CURE in a variety of ways has provided opportunities to examine best practices for lab course design and implementation. Through the use of faculty logs, assessment of student learning gains, and responses to surveys and focus groups, we have examined actions that impact student learning. Recent findings show that our students experience “formative frustration”, where initial failure, followed by exploration, re-evaluation, adjustment, and re-analysis becomes a beneficial learning experience. The low-cost, low-stakes structure of genomics
investigations encourage faculty to let their students experience this formative process. Supported by NSF IUSE-1915544 and NIH IPERT-1R25GM130517-01 to LKR.

Program Abstract #91

**Novel role of transcription factor NF-Y in Regulation of Cilia genes during Zebrafish Development**

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Birth defects resulting from dysregulation of cilia (ciliopathies) affect 1:20,000-1:150,000 U.S. newborns. Cilia are outward projecting microtubule-based organelles, which can be classified broadly as motile or primary cilia. Cilia assembly requires the transcription factors (TFs) RFX and FOXJ1. However, these TFs are not sufficient to account for the complete cilia gene expression program, specifically that of primary cilia. We hypothesize that NF-Y, a TF that binds CCAAT boxes at promoters and enhancers, acts to broadly regulate cilia gene expression. To study the genes that NF-Y regulates, NF-Y was depleted, and phenotypic abnormalities were found in the eyes and head cartilage of the early embryo. Additionally, RNA-seq and ChIP-seq was performed. We found 2,210 differentially expressed genes and 11,169 DNA binding sites. Overlap between the RNA and ChIP-seq experiments identified 347 potential NF-Y regulated genes. GO-Term analysis of these 347 genes show involvement in cilia processes. Since NF-Y is believed to be a pioneer TF, we determined if NF-Y influences the chromatin environment at cilia genes using H3K4me3, H3K27ac and H3K4me1 across time. We found that there was no difference between the global chromatin marks and specific cilia genes, suggesting that NF-Y is acting at enhancers at both sites during early development. Additionally, NF-Y and RFX2 reportedly share DNA binding site in human cell lines, suggesting that RFX2 and NF-Y may regulate cilia genes cooperatively. To determine the degree of overlap, we used ENCODE ChIP-seq data and found that NF-Y and RFX have around 60% overlapping DNA binding site. We hypothesize that NF-Y is acting at enhancers, possibly with RFX, to regulate cilia gene expression. To test if NF-Y deficiency leads to cilia defects, we are currently measuring cilia characteristics and ciliogenesis in NF-Y depleted animals. Supported by NIH grant NS038183.

Program Abstract #92

**Mechanical regulation of ephrin/Eph signaling in the developing Xenopus brain**

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Eph receptors and their membrane-bound ligands, ephrins, provide key signals in many developmental processes including neuronal guidance. However, despite immense progress in our understanding of ephrin/Eph signaling, discrepancies between in vitro and in vivo work remain. As neuronal growth is regulated by both chemical and mechanical signals, and the mechanical regulation of ephrin/Eph signaling is currently poorly understood, we here investigated the role of mechanical cues in this signaling pathway. *Xenopus* retinal ganglion cell axons cultured on glass responded to ephrinB1 as previously described: growth cones from the EphB receptor-bearing ventral retina collapse significantly more than those from the ephrinB ligand-bearing dorsal retina in response to ephrinB1. However, when the same cells were cultured on a soft substrate mechanically resembling brain tissue, we observe the opposite effect of ephrinB1 application. Furthermore, preliminary in vivo atomic force microscopy data suggest that the developing *Xenopus* brain is mechanically heterogenous, with a change in mechanical properties at the diencephalon-tectum boundary, where retinal ganglion cells begin to unbundle to target specific locations. Since ephrinB/EphB signaling in *Xenopus* RGCs is affected by substrate stiffness in vitro, and a complex mechanical landscape exists in the optic tectum at the time of innervation, our data suggest that mechanical cues could be important in tuning retinotectal mapping through chemical signaling. This work is supported by the Wellcome Trust (215156/Z/18/Z to J.S.) and European Research Council (Consolidator Award 772426 to K.F.).

Program Abstract #93

**Effects of Physical Perturbations on Calcium Activity in Developing Embryos**

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Calcium serves as an important universal messenger in both mature and developing nervous systems. Though a plethora of research has been conducted on the role of calcium signaling in the developed nervous system, its role in the developing nervous system is not well characterized. This study aims at determining the effects of physical perturbations on calcium activity during the early stages of embryonic development using *Xenopus laevis* as a model organism. In order to do this, physical pressure is exerted onto developing *Xenopus laevis* embryos at controlled frequencies, intensities, and durations of pressure through a custom-designed device. Calcium imaging of the developing embryos is done as the physical perturbations occur, and RNAseq gene assays are done on the embryos after the physical perturbations have been completed. Physical perturbations on embryos in the blastula stage increase calcium activity in a localized, time-restricted manner suggesting that pressure-dependent changes in calcium activity begin early in embryonic development. By elucidating the effects of physical perturbations on calcium activity present in developing embryos, this study aims to further our understanding of what role calcium signaling has on embryonic development. Funding: Grants, NSF 1257895, and NIH 1R15NS067566-01, 1R15HD077624-01, and 1R15HD096415-01, to MSS.

Program Abstract #94
Analysis of transcriptome changes in zebrafish ZGA mutants using RNA-sense tool
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Oct4 (Pou5f1) and Sox2 transcription factors are crucial for pluripotency maintenance in mammalian embryonic stem cells. Their homologs, Pou5f3 and Sox19b activate transcription during the zygotic genome activation (ZGA) in zebrafish embryos. Previous studies indicated that Pou5f3 and Sox19b share a group of common target genes. However, the genetic ablation of Pou5f3 and Sox19b leads to distinct embryonic phenotypes: maternal-zygotic Pou5f3 mutants MZspg are arrested at gastrulation and lethal, while MZsox19b mutants are delayed in gastrulation and viable. To understand the common and distinct biological functions of Sox19b and Pou5f3 during ZGA and their connection to gene expression dynamics, we performed transcriptome analysis on the wild-type, MZsox19b, MZspg, and double MZspgsox19b mutants at eight time points during ZGA. We developed a pipeline for comparison of RNA-seq time series, which we calledRNA-sense. RNA-sense tool employs user-defined cut-offs and can be easily adapted for other biological systems with dynamic gene expression. RNA-sense tool works in three steps. In the first step, all the expressed genes are classified to time profile groups, based on maximal up- or down- switch in expression levels between the time points. In the second step, differentially expressed genes between the two genotypes and mutant are scored for each time point. In the third step, correlations between time profiles (1) and differential expression gene groups (2) are calculated and visualized. We present RNA-sense analysis of single and double mutants, which suggests partially independent roles for maternal Sox19b and Pou5f3 in ZGA. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) DFG-ON86/4-2, and DFG-EXC294

Program Abstract #95
Comparative transcriptomics of non-model colonial hydrozoans: understanding the signaling toolkit involved in the body plan diversification
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Cnidaria is a phylum that comprises a diverse group of aquatic animals which occupy a key phylogenetic position as the sister group to all bilaterians. Hydrozoa is a rather varied group of Cnidaria of mostly colonial forms. The relatively simple organization of Hydrozoans, combined with their extreme diversity in terms of colony architectures makes them an ideal assemblage to investigate the evolution of key aspects of body plan diversification. However, only limited genomic datasets are available for hydroids, and this issue restricts the ability to engage in comparative research. Thus, our work is focused on partially filling this knowledge gap using NGS technology to generate and analyze reference transcriptomes of the two non-model marine colonial
hydrozoans with different types of colony organization: Dynamena pumila (Linnaeus, 1758) with monopodial growth, and Gonothyraea loveni (Allman, 1859) with sympodial growth. For transcriptomes sequencing, we used Illumina RNA-seq data and standard protocols of assembly using rnaSPADES software. Obtained transcriptomes provide us information regarding toolkit of developmental signaling pathways such as WNT or TGF-beta and species-specific regulatory genes in hydrozoans with different body plans. Our high-quality transcriptomic data expands opportunities and opens up promising avenues for comparative evolutionary studies using in situ hybridization methods, differential gene expression analysis tools, and gene knockdown methods. This work is funded by RFBR, #20-04-00978A.

Program Abstract #96
Effects of Polyploidy in C. elegans
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Polyploidy is a major cause of miscarriages and infertility, and the driving force of cancer development. Additionally, polyploidization is a key step in normal development, wound healing, adaptation, and speciation. Despite its importance, there is a major gap in our understanding of how whole animal polyploidy is established and what are the effects of - and long-term consequences. This is, in part, because most animal models for laboratory studies are embryonic lethal or sterile as polyploids. We have developed a methodology to rapidly and easily generate viable and fertile tetraploid (4n) animals from diploids (2n) of nearly any genetic background in Caenorhabditis nematodes. Using multiple tetraploids, we have surveyed effects of polyploidization on; meiosis and mitosis, and biological scaling and scaling relationships. The effects of increased genome on biological scaling and cell divisions are context dependent. Comparison of tetraploid and diploid strains meiotic and mitotic divisions, show that the effects of polyploidy on centrosome size, final spindle length, and the speed of chromosome segregation, were different either between the two meiotic divisions or in the first mitosis of the embryo. Tetraploid animals are 30% larger than the diploids. This increase in animal size is due to increased cell size, not cell number. Interestingly, the scaling of cell and organ size relative to the animal size depends on the type or cell or the organ. This differential scaling results in body shape differences between diploids and tetraploids that are not due to a proportional size change. This and other data suggest that specific-gene expression rather than global increase in gene expression might be associated with C. elegans tetraploidization. We will report on our initial comparison between diploid and tetraploid strains transcription profiles.

Program Abstract #97
Sloth1/2 are two small peptides encoded from a single transcript that regulate mitochondrial function in Drosophila
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Naturally produced peptides (Drosophila encoded by conserved smORFs, Sloth1 and Sloth2. These peptides are translated from the same bicistronic transcript and share sequence similarities, suggesting that they encode paralogs. We provide evidence that these peptides are highly expressed in neurons, localize to mitochondria, and form a complex. Double mutant analysis in animals and cell culture revealed that sloth1 and sloth2 are not functionally redundant, and their loss causes animal lethality, reduced neuronal function, impaired mitochondrial function. These results suggest that phenotypic analysis of smORF genes in Drosophila can provide a wealth of information on the biological functions of this poorly characterized class of genes. J.A.B. was supported by the Damon Runyon Foundation. This work was supported by NIH grants R01GM084947, R01GM067761, R24OD019847, and NHGRI HG009352 (S.E.C). N.P. is an investigator of the Howard Hughes Medical Institute.
Program Abstract #98
An Enzymatic Method for Creating CRISPR sgRNA Libraries
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Forward genetic screening has led to the identification of many genes involved in a wide range of developmental processes. However, the time and resources necessary to conduct these screens has greatly hindered the pace of gene discovery. Recent advances in sgRNA library synthesis have made it possible to more rapidly conduct these screens using CRISPR technology, but synthesis on this scale is expensive and requires an in-depth knowledge of the genome. Thus, the availability of these libraries is largely limited to targeting the whole genome of a few well-characterized organisms, and they are not easily customized to particular tissues or processes. To reduce the barriers of entry for CRISPR screening and allow highly customized libraries to be generated quickly and inexpensively, we have developed a protocol for enzymatically creating CRISPR/Cas9 libraries from DNA or RNA collected from any tissue at any time point in any species—even if the genome sequence is unknown. This method can be completed in as little as 4 hours and at costs orders of magnitude less than sgRNA synthesis. We are applying our technology to two forward-genetic screens: a pancreatic beta-cell screen conducted in culture, and a heart-looping morphogenesis screen in zebrafish. Using our method will greatly increase the speed of gene discovery in both model and non-model organisms while making forward-genetics available to labs that do not have the resources necessary for traditional methods. Our method also creates a flexible platform for sgRNA library creation that can be applied to other CRISPR-based methods. Funded by NICHD AREA Grant R15HD098969

Program Abstract #99
CRISPR-Cas13d induces efficient mRNA knock-down in animal embryos
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Early embryonic development is driven exclusively by maternal gene products deposited into the oocyte. Although critical in establishing early developmental programs, maternal gene functions have remained elusive due to a paucity of techniques for their systematic disruption and assessment.CRISPR-Cas13 systems have recently been employed to degrade RNA in yeast, plants and mammalian cell lines. However, no systematic study of the potential of Cas13 has been carried out in an animal system. Here, we show that CRISPR-RfxCas13d is an effective and precise system to deplete specific mRNA transcripts in zebrafish embryos. We demonstrate that zygotically-expressed and maternally-provided transcripts are efficiently targeted, resulting in a 75% average decrease in transcript levels and the recapitulation of well-known embryonic phenotypes. Moreover, we show that this system can be used in medaka, killifish and mouse embryos. Altogether our results demonstrate that CRISPR-RfxCas13d is an efficient knock-down platform to interrogate gene function in animal embryos. This work was supported by Ramon y Cajal program (Ryc-2017-23041) and grants PGC2018-097260-B-I00 and MDM-2016-0687 from Spanish Ministerio de Ciencia, Innovación y Universidades and the Springboard program from CABD (M.A.M-M) and the Stowers Institute for Medical Research (A.A.B.). M.A.M-M was recipient of the Genome Engineer Innovation 2019 Grant from Synthego. J.R.M-M. is supported by BFU2017-86339-P and MDM-2016-0687 grants (Spanish Ministerio de Ciencia, Innovación y Universidades). E. M-T and J.A.-NP are supported by INNOVATE PERÚ grant 168-PNICP-PIAP-2015 and FONDECYT travel grant 043-2019. The CABD is an institution funded by Pablo de Olavide University, Consejo Superior de Investigaciones Científicas (CSIC) and Junta de Andalucía.
Program Abstract #100
Evolution of the Amphipod Crustacean Body Plan: from End to End
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Crustacean body plans, particularly when it comes to the morphology and function of limbs, are extremely variable, especially within Amphipoda. Studies in the amphipod crustacean, Parhyale hawaiensis, have provided insights into the mechanisms of limb patterning through studies of Hox gene function via CRISPR-Cas9 gene editing. Here, we describe two related amphipod species, Monocorophium insidiosum and Caprella cf mutica, which show remarkable departures from the typical body plan exemplified by Parhyale.

In M. insidiosum, the second antenna has a distinctly limb-like morphology and function, and our initial analysis reveals differences in the formation of joints and muscles in this appendage between M. insidiosum and Parhyale. Using knowledge acquired from Drosophila studies on genes that control the distinction between leg and antennae, we hope to be able to elucidate the molecular nature of the morphological alteration between the second antennae of these two amphipod species.

In C. cf mutica, the adults lack an abdomen, as well as limbs on the T4 and T5 segments. Our developmental analysis has revealed that C. cf mutica does form an abdomen during embryogenesis, but the abdomen undergoes apoptosis during later stages of embryogenesis. Interestingly, the region that undergoes apoptosis is the Abdominal-B expression domain, and we are currently testing if Abd-B does regulate abdomen loss in C. cf mutica. By comparing Parhyale to unique amphipod body plans, like those seen in Monocorophium and Caprella, we are able to further understand the roles that Hox genes, and other early patterning genes, have played in the evolution of the crustacean body plan.

Program Abstract #101
The Two Body Problem: An investigation of development in adult and larval body plans of the indirect developing hemichordate Schizocardium californicum.
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While indirect development, with distinct larval and adult body plans, is the most common developmental strategy in many animal phyla, much of what we know about development comes from direct-developing species. For indirect-developing species, a larval body plan transforms into an adult body plan, often via a dramatic metamorphosis. In species where metamorphosis occurs by remodeling of larval tissue into adult tissue, this transition provides an opportunity to investigate the cellular biology of this process and how proliferation and apoptosis are balanced through this important threshold in development. We propose that Schizocardium californicum, an indirect developing hemichordate worm, with dramatically different larval and adult body plans, provides an excellent model for examining the processes that remodel and reshape one body plan into another. We are using a combination of genomics, cellular studies, and developmental biology to answer the following: given that the two life phases of Schizocardium californicum are drastically different, how can one genome build the two distinct larval and adult body plans? Through a combination of cell labeling assays, irradiative cellular depletion, and RNA sequencing, we characterize cell death and somatic cell proliferation in both of these body plans. In efforts to understand how the formation of two such disparate body plans we are exploring single cell RNA sequencing data to compare these unique but linked life-history states. We aim to uncover: Do larvae and adults share overlapping cellular complements or does metamorphosis represent a profound change, not only to body plan architecture, but also to cellular composition? Funding Support: P.B. was supported by a National Science Foundation Graduate Research Fellowship (DGE – 1147470). Research was supported by CZ Biohub Intercampus Research Awards: Beyond model systems: Insights into genome evolution and cellular innovations.

Program Abstract #102
Morphogenetic basis for tetramerous symmetry in scyphozoan polyps
Igor A. Kosevich
Currently we agree that cnidarians possess bilateral symmetry, at least at the level of genetic regulatory toolkit. However, representatives of corals display morphological six-fold or eight-fold radial symmetry, hydrozoans – radial symmetry, scyphozoans and cubozoans are characterised by the dominance of tetramerous symmetry. For the first time the tetramerous symmetry of scyphozoans becomes apparent at the polyp stage due to the four septa in the gastric cavity. The mechanisms governing the septa development are still unknown. Some time ago, Berking (2007, 2010) proposed the model of septa development regulation in corals and scyphozoans. The main idea is that the lining of the gastric cavity of the developing polyp should be composed of two or three compartments (stripes) with different properties. The border between two different compartments will demarcate the place of septum development. Detailed investigation of the Aurelia aurita larva metamorphosis supports this idea. The endoderm of competent larva consists of three compartments: the vacuolated anterior one, the middle granulated dark one, and the vacuolated posterior one. During metamorphosis, these compartments of endoderm behave differently. The posterior endoderm participates in the development of the first pair of tentacles. The middle endoderm participates in the development of the second pair of tentacles and most of the gastrodermal lining of the polyp calyx. The anterior endoderm transforms into the polyp peduncle gastrodermis. The four septa of the primary polyp develop exactly at the place where the posterior and middle endoderm compartments contact in the circumference of the polyp oral disk between the bases of the primary four tentacles. At these points, the inner manubrium lining (originating from the ectoderm) has direct contacts with the epidermis at the base of the tentacles that can serve as additional signal for septa initiation. The work was supported by RFBR Grant #19-04-01131-a.

Program Abstract #103

**cWnt signaling is essential for the colony-level patterning in thecate hydrozoans**

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Colonial hydrozoans at the polyp stage form highly elaborated colonies with a variety of branching patterns that makes them ideal models for studying the mechanisms of body plan diversification. Although patterning in hydrozoan polyps has been well studied, little is known about the patterning of architecturally complex hydrozoan colonies. In order to investigate the role of Wnt signaling in the patterning of the complex hydrozoan colonies we used non-model thecate hydrozoan, Dynamena pumila. Investigation of spatiotemporal expression patterns of Wnt ligands and endogenous Wnt signaling inhibitors during Dynamena colony development revealed that Wnt signaling involved not only in single-polyp-level but also in colony-level patterning. Using a functional approach, we have shown that pharmacological hyperactivation of the canonical Wnt pathway abolishes the formation of colony shoots, but not stolons in a young Dynamena colony. This leads to the development of a stolonal colony characteristic for other hydrozoan species (for example, Clytia hemisphaerica). Inhibition of the Wnt pathway arrests the formation of shoots as well as stolons, but the subsequent restoration of Wnt signaling leads to the transdifferentiation of shoots into stolons. Our findings demonstrate that specification of all structures (i.e. hydrants, shoots, and stolons) depends on the Wnt signaling activity in a dose-dependent manner and suggest the idea that fine-tuning of the canonical Wnt signaling could be one of the leading factors in the evolution of the spatial organization of hydrozoan colony. This work is funded by RFBR, grant ? 20-04-00978A.

Program Abstract #104

**The Global Obesity Epidemic -Are You What Your Mother Ate?: Environment Shapes Adult Phenotype**

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Obesity is a current global health epidemic. Chronic, low-dose exposure to obesogens, like heavy metals, airborne pollutants, and pesticides, shape embryonic development and ultimately shew adult phenotype toward fat storage. Long-term consequences of sub-lethal environmental exposures are often unknown. The round worm *C. elegans* is an excellent model organism to investigate how embryo environment shapes the adult phenotype. To determine developmental windows when environmental exposures act to alter adult phenotype, we exposed *C.
elegans to chronic sub-lethal doses (0.1-100 µM) during specific larval stages or at adulthood and measured changes in growth, fertility, and energy balance (fat storage, pumping, and thrashing activity). Environmental exposures include: estrogen mimics Bisphenol A (BPA) and diethylstilbestrol (DES), metals and metalloids (cadmium Cd, arsenic As), biocides (triclosan TRI, tributyltin TBT, fenthion FEN) and airborne pollutants (cigarette smoke CSE, nicotine NIC, benzo-a-pyrene BAP). Most environmental exposures shifted energy balance towards lipid storage only in offspring, while DES also affected both adults and offspring. Environmental exposures may act via nuclear hormone receptor (NHR) signaling, specifically PPAR to skew adult phenotype toward energy storage. C. elegans and other nematodes have an exceptionally expanded NHR gene family (~300 vs. ~40 in mammals), which hinders homolog identification of ligand-binding transcriptional factors such as PPARγ. Consequently, we created a multiple domain homology bioinformatics strategy to detect candidate PPARs in nematode genomes and found strong candidate homologs of PPARγ in worms, and validated candidates using RNAi and RNA-seq.

Program Abstract #105
Exploring the role of Blood Cells (Hemocytes) during Drosophila metamorphosis
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The blood vascular system of animals can be identified as one of the most versatile systems within the body. Apart from keeping every tissue in our body oxygen-rich, it provides a medium of communication and nutrient transfer amongst all the organs. Moreover, blood cells are recognized as the main mediator of immunity as they protect from invading pathogens. Out of many functions blood cells perform, one of its major aspects is to repair damaged or dying tissues and mediate the clearance from within the body. Like other animals, Drosophila blood cells termed as “hemocytes” also orchestrate immune responses, additionally, hemocytes are also associated with some non-immune functions that are crucial during various stages of development. However, less is known about the function and behavior of hemocytes during the metamorphic stage of Drosophila where immune responses are inert and fly experiences drastic remodeling of its tissues. Our work aims to identify the activity and behavior of hemocytes during this phase of fly development. We elucidate the activity, form, and behavior of the most abundant hemocyte known as the “plasmatocyte”, throughout metamorphosis. Our work reveals different forms of plasmatocytes laden with varying degrees of histolyzing debris (muscles and fats), far exceeding its larval size, thus identifying a new paradigm in the phagocytic capabilities of Drosophila plasmacytocytes. We also decipher that such engulfment is an actin-dependent process and degradation of such debris is mediated by an Atg8 (LC3 Homologue) associated lysosomal vesicles. Therefore our study potentially provides us with a genetically tractable in-vivo model for phagocytes that can be employed to dissect out the relevant components of macroendocytosis and lipid-loaded phagocytosis, which in the current scenario is crucial for addressing the emerging role of phagocyte physiology and pathophysiology. Funding Sources: CSIR fellowship and Wellcome Trust/DBT Senior Fellowship

Program Abstract #106
Elucidation of Pax2 ocular phenotypes beyond coloboma
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Pax2 encodes a paired-box domain transcription factor with multiple roles in vertebrate organogenesis. Pax2 mutant embryos exhibit coloboma—failed optic fissure closure and die from kidney and brain malformations. In the E9.5 optic vesicle, Pax2 is widely expressed, but later is restricted to the optic nerve head (ONH), a cellular boundary between neural retina and optic stalk (OS). Pax2 activity is essential for the ONH, but underlying molecular mechanisms remain unclear. We used a germline Pax2GFP mutant allele to explore Pax2 roles in the ONH and OS1. We compared GFP expression with endogenous protein, then validated Pax2 protein total loss in mutants. We noted that eye morphology is abnormal in Pax2 mutants and hypothesized it could be due to abnormal proliferation. This idea was tested by comparing the percentages of E11.5 and E13.5 M-phase and E13.5 S-Phase cells between Pax2GFP/+ and Pax2GFP/GFP eyes. These experiments revealed that while there is no overall difference in proliferation between Pax2GFP/+ and Pax2GFP/GFP optic cups, proliferation is increased specifically in the ONH of Pax2GFP/GFP eyes. The abnormal ONH morphology of Pax2 mutants correlates with ONH cells dividing more
than the controls. To understand the consequences of ONH overproliferation, we tested for apoptosis in E16.5 embryos, and saw a significant increase onsetting after overproliferation. We suspect that Pax2 coloboma results from abnormal overproliferation of cells within the ONH. Ocular morphogenesis occurs differently in mouse and zebrafish, but we found Pax2 acts analogously, since mouse mutants also have abnormal optic fissure closure, cup dysmorphology, ONH overproliferation, and cell death phenotypes. 1. Soofi et al Dev Biol 2012. 2. Lee et al Dev Biol 2008. 3. This study supported by a Choose Development! Fellowship to J.S-N; NIH grant EY13612 to N.L.B.

Program Abstract #107
Analysis of mutations in distinct domains of kmt2d: genotype-phenotype correlations in Kabuki Syndrome zebrafish.

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The histone methyl-transferase Kmt2d is a critical epigenetic regulator in vertebrates, and mutations in this gene cause human Kabuki Syndrome (KS): a rare condition that includes cardiovascular and neurodevelopmental defects, learning disabilities, craniofacial and other developmental abnormalities. However, the underlying mechanisms leading to phenotypic variability are unknown. In this study, we present preliminary results suggesting a relationship between the mutation site in kmt2d gene and the manifestation of specific phenotypes in our KS models. We previously demonstrated that kmt2d homozygous null mutants in zebrafish have lethal cardiovascular and brain defects (1). To assess whether mutations affecting different domains of the Kmt2d protein have distinct ranges of phenotypes, we used CRISPR/Cas9 technology to generate 9 mutant lines with either in-frame and frame-shift mutations in specific Kmt2d domains, and crossed these mutants with tg(fli1:GFP) transgenics to assess cardiovascular development. Phenotype evaluation was performed in homozygous mutant and wildtype siblings at 2 days post fertilization (dpf) and 3 dpf by gross-morphology assessment, immunofluorescence and confocal analysis on brain and heart. Adult phenotype analysis included weight and length, ultrasound for cardiac morphology and function, and behavioral assays for learning and phenotypes. Overall, our results suggest that mutations affecting different domains of Kmt2d protein have distinct phenotypic outcomes, specifically in mutations in the methyl-transferase domain that gave mild embryonic cardiovascular developmental delay but stronger adult phenotypes. In the long-term, these findings will contribute to our understanding of clinical variance seen among distinct KMT2D mutant alleles in humans. (1) MA Serrano et al. PLoS Biol. 2019 Sep 3;17(9):e3000087. Supported in part by UM1 HL098160 to HJY and AHA Postdoctoral Fellowship to AS.

Program Abstract #108
Investigating the Roles of Asteroid and Star during Oocyte Selection and Oogenesis in Drosophila

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The intricate process by which gametes are formed from the germline stem cells is a fundamental question in biology. In Drosophila, oogenesis begins by asymmetric division of the germline stem cells, and ultimately produces a cyst of 16 cells surrounded by a layer of somatic cells. One of these 16 germline cells is selected as the oocyte, the future egg, while the remaining become supporting nurse cells. A genetic screen in Drosophila identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination, leading to loss of mature eggs and fertility. Two of the genes identified in this screen are asteroid (ast) and Star (S). Strikingly, when the germline cells are mutant for S or ast, the resulting cysts contain 16 nurse cells, but no oocyte. Further characterization of asteroid mutants revealed a persistence of double-stranded DNA breaks (DSBs) during meiosis and localization to the selected oocyte in the late germarium. The protein encoded by ast and its human ortholog (ASTE1) both contain XPG domains, suggesting they act as nucleases, possibly during DNA repair. Interestingly, ASTE1 is mutated in a subset of patients with colorectal cancers, although its molecular function is yet unknown. To address the necessity of ast's predicted nuclease-encoding domain during Drosophila oogenesis, we recently made a CRISPR-generated allele lacking the XPG domain. Surprisingly, loss of this domain does not result in severe defects during oogenesis in Drosophila. We are now investigating the relationship...
between S and ast by generating individual gene knockouts using CRISPR/Cas9 gene editing. Further insight into the roles of asteroid and S during oogenesis will shed much needed light on the molecular mechanisms controlling oocyte fate determination. Supported by the American Cancer Society PF-14-041-01-DDC and the UExplore Undergraduate Research Program at the University of Evansville.

Program Abstract #109
Investigating Role of the COPII Vesicle Trafficking during Oocyte Determination in Drosophila melanogaster
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The establishment of polarity in a developing organism is vital for coordinating cell fate and proper development. Throughout oogenesis in Drosophila melanogaster, many different RNAs and proteins must be trafficked and properly localized within the germline for the oocyte to be selected from a cyst of 16 interconnected germline cells. Once specified, the oocyte initiates meiosis, while the remaining 15 cells, called nurse cells, initiate endoreplication. An ethyl methanesulfonate (EMS) mutagenesis screen in Drosophila identified several mutant lines that fail to establish an oocyte among the 16 germline cells. Mutant germline clones of Sec24CD, a gene identified in this screen, exhibits oocyte loss and clustering of the ring canals. Given the role of Sec24 in COPII anterograde trafficking, this result suggests a role for COPII trafficking during oogenesis. In addition, we found that Sec24CD co-localizes with the known oocyte-specifying factors Orb, Bic-D, and Egl at the posterior of the oocyte, and that microtubule disruption destroys this polarized localization. Strikingly, germline knockdown of Sar1, a GTPase needed for COPII coat assembly, yielded severe defects in oogenesis, suggesting it is also involved in germ cell development. We are now investigating the role of Sec23 during oogenesis using a CRISPR-generated allele. We aim to confirm the role of COPII-coated vesicle trafficking during oocyte determination and predict that Sec23 mutant egg chambers will exhibit a phenotype similar to mutation of Sec24CD. Together, data from these studies will shed light on how trafficking regulates oogenesis, and, in particular, oocyte determination. Future studies aim to elucidate the interactors and cargo associated with COPII-mediated vesicle trafficking during oocyte fate establishment and oogenesis. Supported by funding from the University of Evansville Honors Program.

Program Abstract #110
Characterization of CG4511 as a Novel Regulator of Spermatogenesis
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In Plasmodium berghei, Phosducin-Like Protein 3 (PhLP-3) has been found to contain redox activity and hypothesized to function as a co-chaperone in the folding and regulation of cytoskeletal proteins. CG4511 is the Drosophila homolog of the Plasmodium berghei PhLP-3. In order to explore the role of PhLP-3 and its homologues in regulation of the cytoskeleton, we are examining the role of CG4511 in Drosophila spermatogenesis. Drosophilaspermatogenesis is an excellent model for studying regulation of the cytoskeleton given the involvement of actin and microtubules, and a level of organization in the testis that allows one to view all stages of spermatogenesis simultaneously. Following meiosis, the microtubule-based axonemes elongate, while an actin-rich individualization complex (IC) aids in spermatid individualization and cytoplasm removal. We find that homozygous CG4511 males are sterile, and have reduced CG4511 RNA levels compared to wild type. In order to determine what may be happening in spermatogenesis to cause sterility we next examined sperm morphology in the distal testis and seminal vesicle. At this stage, wild-type sperm individualize and nuclei exhibit a needle-like appearance. However, in CG4511 homozygous mutants individualizing sperm were not observed and non needle-like nuclei were observed. Seminal vesicles also appeared smaller in size, consistent with decreased sperm production. In order to determine when spermatogenesis begins to fail in CG4511 mutants, immunohistochemistry is being used to determine if 16 cell cysts are present, and if spermatids transition to meiosis. Differential contrast microscopy is being used to examine if elongating sperm bundles are present. Further experiments will be conducted to determine if CG4511 affects tubulin and actin-based structures in spermatogenesis, since CG4511 may be a co-chaperone in the folding of cytoskeletal proteins. LUC Provost Award.
Program Abstract #111
Tracing the Migration Pattern of Primordial Germ Cells (PGCs) in Developing Monodelphis domestica Embryos
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Primordial germ cells (PGCs) are precursor cells that develop into the gametes in sexually reproducing organisms. They are set aside early in embryonic development and thus need to migrate to the yet-to-be-formed gonads as the embryo develops. Any error in the migration process can cause misplacement of the PGCs in extragonadal locations, which is a cause of germ cell carcinomas. For this reason, the migration pattern of PGCs has been studied extensively in laboratory mouse (M. musculus). However, PGC migration pattern varies in embryos with different topologies. Mouse embryos, which form egg cylinders during implantation, have a structure that is not seen in other mammals. We hypothesized that the folded epiblast in mouse egg cylinder allows a different PGC migration pattern in mouse, making it a less useful model for understanding the human PGCs. To test our hypothesis, we examined the migration of PGCs in Monodelphis domestica (grey short-tail opossum), a metatherian mammal. Opossum embryos are good candidates as they have flat epiblasts that are typical of all non-rodent mammals, both metatheria and eutheria. They can also be retrieved easily as they do not implant into the endometrium. To construct a migration map of the opossum PGCs, we stained opossum embryos aged from day 7 to day 12 using immunohistochemical staining method. Two PGC marker proteins were stained: STELLA and SDF-1. The expressions of the two markers delineated a migration pattern of opossum PGCs, which migrate anteroposteriorly within the lateral mesoderm along the primitive streak. Hence, opossum PGCs were found to follow a migration pattern different from that of mouse embryos, but more similar to that in flat-epiblast embryos. Thus, topology is an important consideration to be factored into the study of a migrating cell population in a developing embryo. Our work was supported by the Department of Biology, Oberlin College and the Robert S. Danforth Foundation.

Program Abstract #112
Physical Basis for Epithelial Cell Reintegration
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Proliferative epithelia face a challenge: tissue integrity must be maintained despite the potentially disruptive process of cell division. One way for tissues to address this challenge is by allowing dividing nuclei to move apically, freeing them from physical constraints (crowding) imposed by their neighbors. Our work addresses epithelial cell reintegration, the mechanism by which newly-born, apically-positioned daughter cells reincorporate basally. Reintegration depends on both lateral adhesion between cells as well as the ability to transmit force through adhesion to the cytoskeleton. We hypothesize that reintegration requires the adhesive force that draws the cell basally to overcome a resistive force from the underlying epithelium. To test this possibility, I will investigate the role of layer stiffness and cell contractility in reintegration by coupling 3D computational modeling with light microscopy. Computational simulation allows me to decouple underlying biological variables from physical variables. Confocal microscopy will be paired with a custom-built cell stretching device to mechanically alter cell properties. Together, these approaches will help to determine how basic physical attributes of a proliferative epithelium affect maintenance and development. This work is supported by NIGMS R01GM125839.

Program Abstract #113
Mechanical and signaling mechanisms that guide pre-implantation embryo movement
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How a mammalian embryo determines and arrives at its site of attachment is a mystery that has puzzled researchers for decades. While in monotocous mammals, it is essential to select a ‘good’ site of attachment, in polytocous species, embryos face a unique challenge of achieving adequate spacing to avoid competition for
maternal resources. Using confocal imaging, 3D image reconstruction, and quantitative location analysis, we evaluate murine embryo location along the longitudinal oviductal-cervical axis of the uterus. Our analysis reveals three distinct pre-implantation stages: a) Embryo entry; b) Unidirectional movement of embryo clusters; and c) Bidirectional scattering and spacing of embryos. We show that the unidirectional movement of embryo clusters is facilitated by a mechanical stimulus of the embryo as a physical object and is regulated by adrenergic uterine smooth muscle contractions. Surprisingly, we find that embryo scattering is independent of muscle contractions but instead relies on LPAR3 signaling mediated embryo-uterine communication. Our data support a model where murine uterine implantation sites are neither random nor predetermined and are a function of the number of embryos entering the uterine lumen. We propose that the presence of embryo clusters in the uterine horn provides an opportunity for the uterus to sense and count the embryos, followed by scattering and even spacing of these embryos along the given length of the horn. These studies have implications for understanding how embryo-uterine communication is key to determining an optimal implantation site, which is necessary for the success of a pregnancy. This project was funded by startup funds provided by Michigan State University.

Program Abstract #114
Mechanical bistability during Drosophila mesoderm invagination
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Actomyosin-mediated apical constriction is a common mechanism that drives localized folding in epithelia. The way by which contractile stress generated near the apical surface of an epithelial sheet drives out-of-plane bending of the sheet is not fully understood. To test whether actomyosin contractility is required throughout the folding process, we developed an optogenetic tool to acutely inhibit non-muscle myosin II (“myosin”) by controlling the subcellular localization of a dominant negative form of Rho1 (Rho1DN). Blue light-mediated plasma membrane recruitment of Rho1DN results in rapid Rho1 inhibition, as evidenced by the immediate Drosophila mesoderm invagination, a well characterized model for epithelial folding. We found that inhibiting myosin before a critical time point, Ttrans, which is approximately halfway through the folding process, resulted in immediate relaxation of the constricted cells. In contrast, inhibiting myosin after Ttrans did not delay the progression of invagination. The bipartite response to myosin inhibition suggests that Drosophila mesoderm invagination is a mechanically bistable process. Computational modeling demonstrated that mechanical bistability during invagination can arise from a buckling-like mechanism that depends on compressive stress from the lateral ectodermal tissue. In support of this model, laser ablation in live embryos showed that the lateral ectoderm becomes compressive immediately before Ttrans. Furthermore, disrupting the lateral ectoderm using laser ablation led to a specific arrest of mesoderm invagination at Ttrans. Taken together, our data suggest that compressive stress from the lateral ectodermal tissues facilitates mesoderm invagination by triggering mechanical bistability during the folding process. This research is supported by NIGMS ESI-MIRA R35GM128745.

Program Abstract #115
Zebrafish basal epidermal cells require Yap and Taz to survive
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Integrin signalling participates in basal epidermal cell proliferation and survival, which are important for skin homeostasis. Previously, integrin signalling in keratinocyte cultures was shown to shuttle YAP into the nucleus (Elbediwy, A., et al., 2016), in keeping with the mechanoresponsive property of YAP and TAZ, effectors of the Hippo signalling pathway. And in the same study, YAP/TAZ was shown to promote basal epidermal cell proliferation in neonatal mice. In the developing zebrafish embryo, we report that Yap and Taz are localized in the nuclei of enveloping layer cells and basal epidermal cells (p63 +ve). However, zebrafish yap;taz double homozygous mutants do not exhibit impaired cell proliferation, but instead display aberrant basal epidermal cell death at the 4-6 somite stage (ss). These mutant basal epidermal cells are apoptotic as they are immunoreactive to cleaved Caspase-3 antibody, and live imaging captured a series of apoptotic events in these cells – DNA condensation, fragmentation and membrane blebbing. Blocking the expression of Tp53 did not inhibit apoptosis in these yap;taz mutants. Similarly, aberrant basal epidermal cell death occurs in another zebrafish yap mutant

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which harbours an in-frame deletion affecting the Tead-binding domain, with concurrent loss of taz. Therefore, Yap-Teads mediated transcription of target genes in epidermal cells are involved in basal epidermal cell survival. Notably, genes expressed in the epidermis that encode the extracellular matrix proteins (Col7a1, Col1a1b) and integrins (Itga3b, Itga6b) are downregulated in the tailbuds of these yap;taz mutants at the 16-18 ss (Kimelman, D., et al., 2017). Thus, we hypothesize that mechanical cues regulate Yap/Taz activity in basal epidermal cells, which in turn is required for cell survival and modulate genes that define the cells’ mechanical environment in a positive feedback manner. [This project is funded by the MoE, Singapore]

Program Abstract #116
Injury and recovery of plasma membrane during cell invasion in vivo
Kieop Park
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Basement membrane (BM) is a dense sheet-like extracellular matrix (ECM) that underlies most tissues. Cells must invade through BM during development to form tissues and facilitate cell dispersal, and this behavior is co-opted in tumors to initiate metastasis. Invasion through ECM is thought to be a mechanically stressful process, and plasma membrane (PM) repair proteins are strongly associated with cancer progression. However, PM injury has never been observed during invasion in vivo, and has been exclusively studied in vitro using artificially induced PM injury. It is thus unclear whether invasive cells experience PM tears and how they are repaired in vivo. To address this question, I am examining anchor cell (AC) invasion in C. elegans. The AC is a specialized uterine cell that invades through BM in a highly stereotyped manner. I observed that ~5% of ACs were vacuolated only during invasion, a phenotype observed in dying cells. These observations suggest that invasion damages the AC, but then it recovers. I also looked at invasion in a mutant strain that lacks matrix metalloproteinase (MMP) activity. In animals lacking MMPs, the AC does not degrade the BM, but instead pushes the BM aside through physical force. Consistent with more damage during invasion, ~15% of invading ACs were vacuolated, but all still recovered. Therefore, I hypothesized that AC-BM interactions might injure the AC PM and cause vacuolation. To test this, I stained animals with FM1-43, a charged fluorescent dye that intercalates into cells’ exterior phospholipid bilayers and enters the cells when PMs are torn. Strikingly, in all cases of vacuolation, FM1-43 entered the AC at the site of BM invasion. I am currently conducting screens to identify genes that mediate PM repair. Together, my work has established that PM damage occurs during BM invasion and I expect in the future to reveal mechanisms of PM repair within invasive cells in vivo. (funding source: UPGG fellowship and R35 GM118049 to DRS)

Program Abstract #117
Mechanical force regulates the balance of scx and sox9 expression at the cartilage-tendon attachment interface during development.
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The interface at the tendon-bone(cartilage), also known as the enthesis is a specialized unit consisting of tenocytes that express both scx and sox9 genes. The combinatorial expression of these genes enables the formation of a fibrocartilage matrix at the attachment interface. Studies have shown a gradient of sox9 expression at the enthesis with an increased level of sox9 expression in tenocytes closer to the cartilage. Previously, we have shown that mechanical force from muscle contraction controls tenocyte gene expression and morphogenesis in a TGFβ dependent pathway. While studies have shown that paralysis affects the development of enthesis, the role of mechanical force on controlling the expression of sox9 and scx is not known. Here we have used Hybridization Chain Reaction (HCR) protocol for quantitative in situ in enthesis of head tendons in control and paralyzed embryos (caacnb1^-/-) to show that mechanical force is essential for maintaining scx expression in tenocytes at the enthesis. This work has been supported with funds from the R01 grant to TS from NIAMS, NIH.

Program Abstract #118
To branch ER not to branch: Uterine gland branching phenotypes in Estrogen Receptor knockout mice
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Uterine glands are branched, tubular, exocrine glands whose secretions are absolutely necessary for success of a mammalian pregnancy. Branching morphogenesis provides the advantage of increased branched tubular surface area and closer proximity to the target cell for secretion delivery. Although it is known that uterine glands are key for embryo attachment, implantation, and decidualization, their branching structure and variations during pregnancy is poorly understood. Similarly, it is known that estrogen signaling mediated by estrogen receptor alpha (ERα) is important for embryo implantation but its role in uterine gland branching morphogenesis has not been evaluated. Here we use 3D imaging to establish the changing structure of uterine glands at pre-pubertal, post-pubertal and pregnancy stages. Further, using tissue-specific deletion of ERα, we demonstrate that stromal estrogen signaling plays a role in uterine gland bud elongation and epithelial estrogen signaling plays a role in branch initiation. During pregnancy, we observed defects in alignment of the embryo axis with respect to the uterine axis, embryo attachment, and embryo invasion, all due to glandular defects as well as loss of estrogen signaling in the luminal epithelial compartment. Thus, estrogen regulates uterine gland branching morphogenesis which is essential for early pregnancy success. Furthermore, estrogen is essential for the branching of both mammary glands and uterine glands, suggesting selection of conserved pathways for exocrine gland branching morphogenesis in reproductive organs. This project was funded by startup funds provided by Michigan State University.

Program Abstract #119
Developmental lymphangiogenesis requires regulation of the transcription factor NFATC1
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The lymphatic system is essential for maintaining fluid homeostasis and immune surveillance and is implicated in a number of pathological conditions such as lymphedema and cancer metastasis. NFATC1 (Nuclear Factor of Activated T-cells 1) is a transcription factor expressed in lymphatic endothelial cells during development whose loss results in embryonic lethality and lymphatic hyperplasia in mice. To further examine the functional role of nfatc1 during lymphatic development, we analyzed several nfatc1 loss-of-function zebrafish models, including CRISPR-Cas9 mutants, oligonucleotide-targeted morphants, and pharmacological inhibition using Cyclosporin A. Using high-resolution confocal imaging, we demonstrate that nfatc1-deficient animals display thoracic duct hyperplasia. Time-lapse live imaging of nfatc1-deficient embryos also reveals that they exhibit ectopic sprouting from the posterior cardinal vein. These results indicate that nfatc1 is required for proper vascular development. Currently, we are further exploring the role of nfatc1 in lymphatic endothelial cells in vivo using transgenic endothelial-specific expression of constitutive active or dominant negative forms of nfatc1. Our studies are shedding new light on the vital role of a key regulator of lymphangiogenesis. This work was supported by the intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (ZIA-HD008808 and ZIA-HD001011, to BMW).

Program Abstract #120
Renal stromal netrin-1 signaling drives kidney arterial development
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The developmental steps underlying renal arterial morphogenesis are poorly defined. Building kidney arteries requires orchestrated actions of endothelial cells (ECs) and vascular smooth muscle cells (vSMCs). In all developing organs including the kidney, vSMCs enlace and stabilize arteries. vSMCs have been reported to arise from Foxd1+ stromal progenitor cells (SPs), which are themselves emerging as crucial players in kidney organogenesis. Whether and how stromal signals govern lineage-specific stromal fate to regulate arterial and kidney formation remains largely unknown. Here, we show that the chemotrophic cue netrin-1/Ntn1 is specifically expressed in Foxd1+ SPs and drives renal arterial formation. We conditionally ablated Ntn1 in SPs in vivo by Foxd1Cre (Ntn1SpKO). At E13.5, control kidneys develop a patent arterial tree. By contrast, Ntn1SpKO kidneys strikingly fail to form vSMC-enwrapped arteries, with a majority of αSMA+ vSMCs accumulating on the kidney surface. Mutants also exhibit
heightened renal hypoxia. RNA-seq analysis revealed significant reduction of Klf4 in Ntn1SPKO kidneys. As Klf4 is a critical determinant of cell differentiation and is abundant in E13.5 SPs during normal kidney development, its attenuation upon Ntn1 loss may suggest a functional role in stromal fate and/or arterial formation. We therefore generated SP-specific Klf4 null mice (Klf4SPKO) and observed similar surface αSMA+ vSMC accumulation as in Ntn1SPKO. Furthermore, Klf4SPKO kidney arteries are poorly developed, with limited vSMC coverage. Collectively, our results identify critical genetic programs necessary to build a mature renal artery. Furthermore, they suggest that renal SP-derived Ntn1 and Klf4 may determine Foxd1+ SP to vSMC differentiation and/or instruct NSC-derived vSMCs to nascent arteries to initiate renal arterial assembly, thereby regulating kidney growth. This work is supported by R24DK10673 (OC), R01DK124393 (OC) and AHA Postdoc Fellowship (XG).

Program Abstract #121
DRP1-Mediated Mitochondrial Fission is Essential for Mouse Cardiogenesis
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Mitochondria are a vital organelle with multiple critical functions, and they form a highly dynamic tubular network through fission and fusion. MFN1/MFN2 and DRP1 are dynamin-related GTPases required for fusion and fission, respectively. Studies on deletion of Mfn1/Mfn2 have established the essential role of mitochondrial fusion for mouse heart development; however, the role of mitochondrial fission during cardiogenesis remains unclear. We specifically deleted Drp1 in secondary heart field-derived structures. All conditional knockout mice died before E16.5 with severe hypoplastic right ventricles. We have thus provided the first evidence supporting the essential role of Drp1 in embryonic hearts. Drp1-deficient cardiomyocytes displayed abnormal cell proliferation, survival and orientation, and mitochondria in these cells showed hyperfused morphology. Furthermore, transmission electron microscopic tests revealed abnormal mitochondrial ultrastructure in mutant cardiomyocytes. We measured oxygen consumption using Oroboros O2k and observed that coupling of electron transportation with ATP generation was significantly impaired by Drp1-deletion. Drp1 is thus essential for mitochondrial dynamics, ultrastructure and activity. To better reveal how Drp1-deletion affects cardiomyocyte development, we performed single cell RNA-Seq and unexpectedly found that expression of a group of ribosomal protein genes was significantly decreased in mutant samples, suggesting that Drp1-deletion leads to decreased general translation. To our best knowledge, this is the first example showing that mitochondrial dysfunction leads to reduced protein synthesis through downregulating expression of ribosomal protein genes. We are currently testing the pathway connecting DRP1-mediated mitochondrial fission to reduced translation of ribosomal protein genes. In summary, our study has established that mitochondrial fission is essential for embryonic heart development. Supported by an R01 grant to KJ.

Program Abstract #122
Characterizing the role of RHOA signaling in regulating vascular integrity and development
Laura Pillay, Joseph Yano, Andrew Davis, Keith Barnes, Matthew Butler, Vanessa Reyes, Daniel Castranova, Amber Stratman, Gennady Margolin, Aniket Gore, Matthew Swift, James Iben, Brant Weinstein
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Cerebral cavernous malformations (CCMs) are clusters of abnormally dilated blood vessels prone to rupture. Vessel hyperpermeability in CCM patients leads to hemorrhagic stroke with neurologic dysfunction, disability, or death. CCM disease models exhibit increased activity of the GTPase RHOA and its effectors the RHO-kinases ROCK1/2, suggesting that RHOA signaling may negatively regulate vascular integrity. However, RHOA regulation of vascular development and integrity in vivo is still poorly understood, owing to the early embryonic lethality of RHOA knockout mice. To study the role of RHOA in blood vessels in vivo, we generated an allelic series of zebrafish RHOA-ortholog (rhoaa) mutants and transgenic embryos expressing wild type or mutant forms of rhoaa specifically in endothelial cells (ECs). Mutants with a hyperactive rhoaa allele, and transgenic embryos expressing constitutively active rhoaa in ECs exhibit cerebral hemorrhage and reduced cranial vessel sprouting. These data suggest that ectopic RHOA activity is sufficient to disrupt vascular integrity in vivo. Embryos expressing dominant-negative rhoaa in ECs also exhibit impaired cranial angiogenesis and hemorrhage, indicating that some RHOA is
required to maintain vascular integrity. Inhibition of ROCK1/2 produces a similar phenotype, suggesting that RHOA may regulate cranial vessel patterning and integrity by activating ROCK1/2. Our combined data suggest that vascular integrity in developing zebrafish is highly sensitive to Rhoaa activity and that RHOA regulates cranial blood vessel angiogenesis. To further elucidate the molecular mechanisms by which RHOA regulates vascular integrity, we conducted proteomic analyses on human ECs in culture with increased or decreased RHOA/ROCK activity. We identified novel members of the vascular RHOA signaling network and are characterizing them in vivo in zebrafish embryos. Our analyses may identify novel targets for stroke prevention and treatment. Funded by NICHD/NIH.

Program Abstract #123
Proteomics analysis to define stage-specific molecular networks regulating vascular development at the placental maternal-fetal interface
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The placenta is a highly vascularized organ that is paramount in supporting maternal health and fetal development during pregnancy. Placental dysfunction is associated with adverse maternal and fetal clinical outcomes including preeclampsia, fetal growth restriction, preterm birth, and loss of life. The cause(s) of placental dysfunction remain incompletely understood at the molecular level. Our central hypothesis is that placental dysfunction can arise from developmental origins that alter the structure and function of the specialized vasculature maternal-fetal interface (MFI). This unique vascular interface mediates communication between maternal and fetal blood streams; maternal blood directly interacts with trophoblasts on the maternal side of the interface while fetal blood interacts directly with endothelial cells on the fetal side of the interface. Trophoblasts and endothelial cells of the MFI develop in concert. The aim of this study was to determine stage-specific molecular networks that regulate the developmental course of the MFI. We employed tandem mass spectrometry proteomics and profiled mouse placenta samples between embryonic day (E) 8.5 and E17.5. This analysis quantified 3814 mouse proteins placental labyrinth which represents MFI in the mouse. In PCA samples clustered based on the gestational age. Network analysis of the kinetics of the protein abundance data identified groups of co-occurring proteins over the course of development. Application of functional enrichment analysis (GO, IPA, GSEA) and AI-based methods further allowed us to identify stage-specific molecular networks. Our future studies will test functional relevance of these stage-specific networks and will evaluate how they may be modulated to prevent or alleviate placental dysfunction. In conclusion, these data advance fundamental basic science knowledge of placenta vascular biology and support new avenues of placental health research.

Program Abstract #124
Evolution of the small molecule transport system in animal development
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ATP-binding cassette transporter (ABCs) and solute carrier transporter (SLCs) gene families encode the major proteins involved in small molecule transport (SMT). These proteins handle diverse signaling molecules and morphogens important for intercellular communication in embryos. It is presumed that most of these transporters evolved from microbial proteins adapted for transport of lipids and toxic xenobiotics, and later became more specialized/selective in ligands with developmental functions. Members of the SMT system belong to seven gene families including the SLC21, SLC22, SLCO, SLC47, ABCB, ABCC and ABCG groups. They include well known proteins such as P-glycoprotein (P-gp/ABCB1), as well as proteins like ABCC4/5 with well-known signal molecule ligands such as cyclic nucleotides and prostaglandins. Here we take advantage of multiple genomes, including choanoflagellates, diatoms, echinoderms, and vertebrates, to ask how SMT gene families have diversified at the major evolutionary transitions to multicellularity and deuterostome development. As part of this, we mined the newly generated transcriptome and genome (745MB) of *Lytechinus pictus*, for SMTs. We identified candidates spanning all of the major subfamilies and are currently using these candidates to assess the phylogenetic relationships of these genes across marine invertebrates, with attention to changes coinciding with
acquisition of multicellularity and novel developmental modes. We expect that the results will shed light on the selective pressures driving evolution of the SMT system, and point to functional roles of these proteins in development. This work is funded through a Diversity Supplement Award to KN from the parent grant NIH ES030318 awarded to AH.

Program Abstract #125

Using Nature's experiments to uncover insights into genetic regulation of form
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Experimental genetics provides a window into the regulation of biological form and function. However, insights are limited to genetically tractable model organisms and large effect mutations that are often out of balance with their genetic context. An alternative approach is to perform “forward genomics” by interrogating sequence variation within naturally occurring morphologies. We define a natural experimental condition and apply the logic of genomic screens to identify novel sequence variants that modulate form. In Lake Kronotskoye in Kamchatka, Russia, a species flock of Dolly Varden charr (Salvelinus malma) rapidly diversified 10-15 thousand years ago, forming seven, true-breeding, sympatric morphs. Each morph has distinct craniofacial proportions as well as differences in body size, coloration, and ecology. The diversification of this species flock presents a unique opportunity to parse out genetic variants underlying morphological changes. We leveraged targeted capture to isolate and interrogate genomic variation within these populations. Key loci underlying particular “natural mutant” phenotypes were identified by making comparisons across the clade. The custom array comprises over 500,000 conserved, coding and non-coding baits based on current annotations of known Salmonid genomes. Nearly 90% of targeted regions were recovered and reconstructed at a depth of 2x or greater. Importantly, we can identify fixed and variable sequence variants because species samples are comprised of pooled DNA. As this species flock represents a recent, rapid diversification, signatures of selective sweeps are detected. Candidate regions with clear selective signatures include genes implicated in craniofacial micrognathia, Pierre Robin sequence, and frontonasal carcinoma. We are employing gain- and loss-of function manipulations in zebrafish to identify conserved mechanisms modulating outgrowth of the upper and lower jaws. This work is supported by 1 F32 DE029362-01 to KCW.

Program Abstract #126

Dissecting plantlet development in K. marnieriana
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The genus Kalanchoe Adanson consists of approximately 150 species distributed mainly throughout Madagascar, South and East Africa, and Southeast Asia. Kalanchoe is roughly divided into three taxonomic sections based on photosynthetic carbon assimilation pathways. Most Section I (Kitchingia) species perform C3 photosynthesis; Section II (Bryophyllum) species are obligate CAM performers, mostly photosynthesizing diurnally; Section III (Eukalanchoe) species perform CAM exclusively at night. Asexual reproduction has evolved within Kalanchoe Section II species, commonly resulting in plantlet development in margins of leaves. Separate genetic studies have suggested formation of plantlets within Kalanchoe results from cooption of organogenesis and embryogenesis developmental processes to leaf margins, these studies focused on few genes, and fall short of a complete explanation of how asexual reproduction is ectopically triggered, especially concerning Section II species capable of both sexual and asexual reproduction. In order to further dissect the mechanisms underlying asexual reproduction in the genus Kalanchoe, we first carried out detailed morphological analyses of Section II species K. marnieriana. Twenty leaves were plucked from the parent and plantlet development was observed stereoscopically. Significant developmental changes were noted on days 1,3,5,7,9,11 and 15. For SEM, additional leaves were plucked from the parent plant, and the 7 significant growth days were imaged, further suggesting a trigger of the embryogenesis pathway leading to the development of seed-like embryos. Light microscopy further confirmed this observation by providing detailed analysis of the internal embryo morphology, especially the connection between embryo and the mother-plant. This morphological study of plantlet development in K.
*marnieriana* now serves as the basis for detailed molecular analysis, with special attention to the ectopic activation of embryogenesis genes at leaf margins.

**Program Abstract #127**

The larval bivalve shell: an evo-devo approach reveals life-stage specific transcriptional profile and hints at a developmentally conserved biomineralisation “tool-kit”

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Molluscs produce a plethora of shells; from spirals to spines, bivalves to cones, with many different colour patterns and microstructures. To understand the molecular control and evolution of shell production, mantle transcriptomes and shell proteomes of adult life stages have been surveyed in many species, finding surprisingly little conservation in the mollusc shell “secretome”. Molluscs use a diverse range of lineage-specific proteins to build their shells, and this diversity is facilitated primarily by co-option of both ancient and recently evolved genes, in addition to rapidly evolving repetitive low complexity domains. Comparatively little attention however, has been given to the molecular control of shell development. Here, we used histology, scanning electron microscopy and transcriptomic profiling to describe the development of the larval mantle, shell and putative shell development gene repertoire in the Antarctic clam, *Laternula elliptica*, to address two questions: Is the larval shell built using the same gene regulatory network and downstream effectors as adult shell in bivalves? And, does the pattern of evolutionary divergence in biomineralisation proteins hold true for early life stages when comparing across species? We find that a large portion of adult shell genes are not expressed during shell development, and instead, a development-specific gene repertoire controls early shell deposition. Comparison with recently reported shell development in other species allows us to infer that a transcriptionally unique larval shell is a conserved and likely ancestral feature of the bivalve lineage, and possibly more broadly across the mollusca. In addition, preliminary comparisons suggest that the larval shell “tool-kit” may be more conserved than that of the adult.

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**Program Abstract #128**

Early-life hypoxia alters adult physiology and reduces stress resistance and lifespan in *Drosophila*

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In many animals, short-term fluctuations in environmental conditions in early life often exert long-term effects on adult physiology. In Drosophila, one relevant environmental variable is oxygen. In their natural ecology, larvae grow by burrowing into rotting, fermenting food that is rich in microorganisms. This environment is likely low in oxygen and, as a result, Drosophila have evolved mechanisms to tolerate hypoxia. While the acute effects of hypoxia in larvae have been well studied, whether early-life hypoxia affects adults is less clear. We have begun exploring this issue. Here we show that Drosophila exposed to hypoxia during their larval period subsequently show reduced starvation stress resistance and shorter lifespan as adults, with these effects being stronger in males. We find that these effects are associated with reduced expression of brain-derived Drosophila insulin-like peptides and a decrease in whole-body insulin signaling. In contrast, we see elevated TOR kinase activity, a manipulation known to reduce lifespan. We also identify a sexually dimorphic effect of larval hypoxia on adult nutrient storage and mobilization. We find that males, but not females, adults exposed to hypoxia as larvae have elevated levels of lipids and glycogen. Moreover, we see that both males and females exposed to hypoxia as larvae show defective lipid mobilization upon starvation stress as adults, which may explain why they are starvation sensitive. Together, these data show how early-life hypoxia can exert persistent long-term effects on Drosophila adult physiology and lifespan. Funding sources: NSERC, CIHR.
Program Abstract #129

_Drosophila as a Model for Understanding Bisphenol A Promotion of Obesity via Lipolytic Reduction_

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Obesogens are chemicals that promote obesity by disrupting endocrine function (EDCs). Obesogens may increase adipose content, reduce calories burned at rest, favor calorie storage, or alter appetite and satiety signaling. Based on this and published work, our lab conducted studies to evaluate the impact of 8mM bisphenol A (BPA) on larval fat deposition in _Drosophila melanogaster_. These studies demonstrated that embryonic and larval exposure to BPA results in statistically increased lipid deposition levels (unpublished). RT-PCR data concluded that 8mM BPA exposure resulted in significant decreases in Brummer lipase transcription (main lipase in _Drosophila_ metabolism) and significant upregulation in Kr-h1 transcription factor (lipase inhibitor). Folic acid supplementation rescue experiments were performed using 1:1 Folic Acid and BPA. We report amelioration of the high lipid phenotype relative to BPA exposure without supplementation, mirroring that of the control in sucrose buoyancy assays. Oil Red O and Sudan Black staining were performed to better visualize changes in lipid droplet size and concentration in the fat body of 8mM BPA treated, 8mM Folic acid:BPA treated, and 8mM ethanol treated 3rd instar-larvae. This work was supported by the National Endocrine Society Research Fellowship and the Wyman-Potter Foundation.

Program Abstract #130

_The Identification of Maternal-Effect Genes in Zebrafish by a Maternal Crispant Screen_

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In vertebrates, early development is dependent on a pool of maternal factors, both RNA and proteins, and depletion of these maternal factors can be lethal. The role these factors play in adult fertility and early development is not entirely understood. By exploiting the biallelic editing ability of CRISPR-Cas9 and the benefits of the zebrafish model, we aim to identify and characterize maternal-effect genes in a single generation, or maternal crispant. We validated our ability to generate biallelic mutations in the germline by creating maternal crispants that phenocopied previously characterized maternal-effect genes: _motley/birc5b_, _tmi/prc1l/zgc:86764_, and _aura/mid1ip1_. Additionally, we identified two new maternal-effect zebrafish genes, _kpna7_ and _fhcd3_. The genetic identity of these maternal crispants was confirmed by sequencing haploid progeny from F0-injected females. These sequencing results showed minimal genetic variation within a clutch; specifically, we observed an average of two edited alleles per clutch. These findings are consistent with biallelic editing events occurring early in development in CRISPR-Cas9-injected embryos. Our studies show that maternal crispants allow for the effective identification and primary characterization of maternal-effect genes in a single generation, facilitating the reverse genetics analysis of maternal factors in embryonic development. Funding was provided by the Laboratory of Genetics and College of Agricultural and Life Sciences at University of Wisconsin - Madison, and the University of Wisconsin School of Medicine and Public Health an NIH grant to F.P. (GM065303).

Program Abstract #131

_A novel zebrafish mutant, stl159, exhibits defects in sensory axon and glial patterning_

Lauren Limbach, Afomia Ayele, Kristen Pitts, Elisabeth Bradford, Sarah Petersen

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The peripheral nervous system is an exquisitely patterned network that conveys motor and sensory information throughout the body. In vertebrates, these nerve impulses are carried by axons surrounded by a myelin sheath formed by the iterative wrapping of neural crest-derived glia, called Schwann cells. During development, Schwann cell precursors and elongating axons coordinate migration and maturation by direct, interdependent signaling. However, these processes are also affected by the external environment, and those non-cell autonomous molecular mechanisms critical for nervous system development are not fully defined. Starting with a forward genetic screen in zebrafish, we identified a novel mutant, _stl159_, that has defects in sensory axon patterning and impaired Schwann cell differentiation. We hypothesize that aberrant nervous system patterning in _stl159_ is a result of an early nonsense mutation in the bHLH transcription factor _tcf15_. Previous studies show that _tcf15_ is
responsible for proper muscle development and the expression of classic guidance factors on the muscle membrane. Because the developing sensory nerve is directly adjacent to tcf15-expressing muscle, we therefore predict that stl159 axon and glial phenotypes are due to the loss of guidance cues on the muscle surface. Currently, we are testing the hypothesis that tcf15 non-cell-autonomously promotes nerve development using CRISPR-Cas9 phenocopy and tissue-specific rescue of the stl159 phenotype. We are also using live in vivo imaging to characterize and quantify the behavior of glial cells migrating along axons in wild-type and stl159 mutants. Future work will identify muscle-derived guidance cues that are regulated by tcf15 and necessary for Schwann cell migration along nerves and muscle. Thus, we aim to uncover those molecular mechanisms functioning at the nexus of neural, glial, and muscle development. This work is supported by NSF CAREER Award #1941664.

Program Abstract #132
Fipronil affects the Cardiovascular System in Zebrafish (Danio rerio)
Zoe Krut, Kasey Cooper, Benjamin Walker, Alexander Kramer, Jonathan Morgan, Christopher Lassiter
Roanoke College, USA
Fipronil is a commonly used insecticide that acts by hyper-excitation of the insect nervous system. The drug also causes developmental defects and neurotoxicity in vertebrates. We investigated the cardiovascular system of zebrafish embryos after exposure to fipronil. The cardiovascular system showed abnormalities such as increased heart rate, a larger atrium, and the presence of edema with concentrations as low as 0.7 \( \mu \text{M} \). We hope to expand these studies by investigating aspects of neural development and the molecular pathways involved. Funding sources: Roanoke College Office of Undergraduate Research and Biology Department.

Program Abstract #133
Iroquois genes may play an important role in pharyngeal development
Kelsey Donahue
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Our study is focused on identifying targets of the Fibroblast Growth Factor (FGF) signaling pathway during pharyngeal development. The pharynx of vertebrate embryos gives rise to various craniofacial features, the thymus and parathyroid glands, as well as parts of the inner ear and aorta. The FGF pathway is known to play multiple roles during pharyngeal development during the early patterning of the pharynx as well as the later differentiation of various organ derivatives. We use Xenopus laevis as our model organism for vertebrate development. We performed a microarray to identify novel targets of the FGF signaling pathway in Xenopus embryos. We hypothesized that targets of the FGF signaling pathway may play important roles in the development of the pharynx and its derivatives. Two members of the Iroquois family of transcription factors were identified as possible FGF targets. We analyzed the expression patterns of the Iroquois family in Xenopus embryos with in-situ hybridization found that several are expressed in the developing pharynx. We inhibited Iroquois translation by injecting Xenopus embryos with morpholinos and analyzed these Iroquois loss-of-function embryos with in-situ hybridization to visualize pharyngeal gene expression and found changes in genes such as cyp26a1. We also analyzed Iroquois loss-of-function tadpoles with skeletal preparations and found defects in their craniofacial features compared to sibling control embryos. Overall, we have identified and begun to characterize the Iroquois genes as possible FGF signaling pathway targets in the pharynx. The more we learn about the genetic signaling cascades that help guide embryonic development, the more we will be able to understand why certain birth defects occur. Funding sources: Northern Kentucky University Faculty Senate and CINSAM; Kentucky Biomedical Research Infrastructure Network NIGMS grant # 8P20GM103436-14.

Program Abstract #134
Effects of tail amputation on the regeneration of electric fish species with myogenic and electrogenic electric organs under varied environmental conditions
Samantha Bowden, Anita Singh, Graciela Unguez
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Gymnotiforms are South American electric teleost fish that generate an electric organ discharge (EOD) via an electric organ (EO). In most groups of electric fish studied to date, cells that make up the EO, or electrocytes,
derive from skeletal muscle cells that suppress many muscle properties, do not contract, and differentiate into specialized electrogentic cells. We have studied this muscle-to-electrocyte conversion during tail regeneration in adult fish in the yellow-striped knifefish *S. macrurus*. Whether muscle transdifferentiation of only select muscle fiber types is conserved in other electric fish species after tail amputation in adults is not known. To test this hypothesis, we performed tail amputations in two additional electric fish species: *E. virescens* (*n*=10), which has a myogenically derived adult EO, and *A. albifrons* (*n*=10) with a myogenic-derived EO only in embryonic but not adult stages. In adult fishes, our immunolabeling studies with antibodies against MyHC 2A and MyHC 2B showed: a) distinct MyHC 2A- and MyHC 2B-positive fibers in *S. macrurus*, b) weakly-labeled MyHC 2A fibers and MyHC 2B fibers with some MyHC 2A immunolabeling in *E. virescens*, and c) a predominance of MyHC 2A fibers with little detection of MyHC 2B fibers. In the 2-week regeneration blastema of *A. albifrons*, muscle cells were mostly MyHC 2A-positive whereas those in *E. virescens* were both MyHC 2A- and MyHC 2B-positive. MyHC 2B-positive fibers were not readily detected and developing electrocytes were MyHC 2A-positive. Whether muscle-to-electrocyte conversion occurs after tail amputation in *A. albifrons* may require longer regeneration periods. Previously, we showed that *S. macrurus* fish maintained in darkness after tail amputation resulted in stunted regeneration blastema (Unguez and Mitchell, 2018). Interestingly, darkness did not affect blastema regeneration length in either *A. albifrons* or *E. virescens*.

**Program Abstract #135**

**Loss of jag2b leads to adult-onset hypertrophic cardiomyopathy in zebrafish**

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Cardiomyopathy is a huge clinical concern as it is the number one cause of cardiac failure in young adults and affects approximately 1 in 500 adults. Hypertrophic Cardiomyopathy (HCM) is a heart disease in which the walls of the ventricles thicken due to the enlargement of cardiomyocytes. HCM is generally considered to be an inherited disease, but the cause of this muscle thickening is yet unknown. The Yost lab has developed the first zebrafish model of adult-onset hypertrophic cardiomyopathy using transgenic tools that lineage-label and selectively ablate neural crest-derived cardiomyocytes (NC-Cms)(1). Genetic mutants of *jag2b* have adult-onset hypertrophic cardiomyopathy in adults, with phenotypes similar to ablation of embryonic NC-Cms. These similar phenotypes suggests that Jag2b plays a role in NC-Cms. *Jag2b* expression is enriched in embryonic NC-Cms, and NC-Cms interact with other cardiomyocytes to activate Notch response and to produce normal trabeculation patterning during embryogenesis. Using single cell RNAseq (scRNAseq,10X Genomics) data from our transgenic lineage-labeled zebrafish, we have observed that ventricular cardiomyocytes expressing Jag2b are enriched in one sub-cluster that displays a distinct transcriptome from other cardiomyocytes. We are investigating the specific roles of Jag2b and NC-CMs in regulating embryonic trabeculation and preventing adult-onset HCM. (1) S. Abdul-Wajid, B. Demarest, HJ Yost. Loss of embryonic neural crest cardiomyocytes causes adult hypertrophic cardiomyopathy. Nat Commun. 2018 Nov 2;9(1):4603. Supported in part by UM1 HL098160 to HJY and CIHR Postdoctoral Fellowship to CH.

**Program Abstract #136**

**A Tale of Two CENP-C's: Dynamic and Stable Populations of CENP-C in Oocyte Meiosis are Both Required for Accurate Chromosome Segregation**

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The centromere protein CENP-C is a critical component of mitosis and meiosis. Mutations in CENP-C are associated with infertility as well as cancer progression. CENP-C binds directly to CENP-A (the centromere histone) and is critical for accurate segregation by recruiting kinetochore proteins in prometaphase. Both CENP-A and CENP-C rely on each other for loading after mitosis in order to establish centromere identity for the next cycle. However, using *Drosophila melanogaster* oocytes as a model, we have shown that CENP-C is able to load onto centromeres during early and late prophase while CID (*Drosophila* CENP-A) is not. We have also shown that CENP-
C unloads during prophase indicating a period of replacement not observed in mitosis. This study also revealed that not all of the centromere-localized CENP-C is involved in this exchange, indicating there is both a stable and a dynamic population of CENP-C in meiosis. We have made use of existing CENP-C mutants, RNAi, and epitope-tagged CENP-C transgenes to probe the function of both the stable (G1) and dynamic (meiotic prophase) populations of CENP-C. We found that CENP-C regulate cohesion, possibly through the loading of cohesins, a unique function in early prophase I. CENP-C is also necessary for kinetochore building and bi-orientation of homologs late in prophase. Using mutants and RNAi resistant transgenes, we have shown that late prophase loading of CENP-C can rescue kinetochore and bi-orientation defects. Loading of cohesins onto chromosomes is restricted to the earliest stages of prophase, while kinetochore building is at its peak much later in prometaphase. Our results indicate that CENP-C is the only known centromere component in *Drosophila* to play a significant role in both processes via two separately regulated populations of CENP-C. This work is funded by NIH grants K12GM093854 to JEF and GM101955 to KSM.

Program Abstract #137
Characterizing HECD-1/Hect-family ubiquitin ligase during oocyte formation in *C. elegans*.
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Microtubule-severing plays important roles in organism development, including for cell structure and cell division. I focus on the meiotic to mitotic transition during oocyte development of *C. elegans* to study the regulation of katanin, a conserved microtubule severing complex. Katanin, encoded by *mei-1* (human KATNA1) and *mei-2* (*KATNB1*), severs meiotic spindle microtubules, but is inactivated in mitosis by two ubiquitin-based degradation pathways. Otherwise, expression of katanin in mitosis is lethal. My research focuses on elucidating the role of another type of ubiquitin ligase, HECD-1 (HectD1), in katanin regulation. In contrast to cullin-based ubiquitin ligases, which result in protein degradation, HECD-1 does not affect katanin levels. Interestingly, it also switches between activating katanin in meiosis and inhibiting it during mitosis. Although mammalian HECTD1 affects protein localization, mutant worm HECD-1 did not affect known katanin regulators (PPFR-1, MEI-2, MEL-26) localization. To find more katanin regulators in *C. elegans*, I took advantage of published work showing mammalian HECTD1 interacts in the striatin-interacting phosphatase kinase (STRIPAK) complex. My project studies HECD-1 regulation of katanin activity, and the involvement of the STRIPAK complex in this regulation. My knockdown and mutant data shows STRIPAK components genetically interact with katanin regulators. Core components of this complex act as inhibitors of katanin function whereas variable components act as activators. Using our functional CRISPR FLAG-tagged HECD-1, I found that HECD-1 localizes throughout the oocyte, during meiosis and mitosis. Future studies will look at HECD-1 localization in STRIPAK mutant worms to understand how STRIPAK affects HECD-1. My results will lead to a further understanding of the interplay of katanin and STRIPAK. Funding by NSERC.

Program Abstract #138
A Novel Role for the Chromosomal Passenger Complex in Germ Plasm Aggregation During Early Development
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In zebrafish, the specification of primordial germ cells is dependent on the inheritance of a compartmentalized membrane-less subcellular structure that contains a pool of germ plasm ribonucleoparticles (GP RNPs) and maternally expressed proteins. Interactions between cytoskeletal components are crucial for the movement and collection of GP RNPs into the furrow during early development. Synergy between the dynamic microtubule and actin networks is vital for germ plasm aggregation. Previous work has identified a maternal homolog of Survivin (*motley/ birc5b*), a component of the Chromosomal Passenger Complex (CPC), as a necessary maternal factor required for interactions between the cytoskeleton and GP RNPs. Traditionally, the CPC coordinates chromosomal segregation and cytokinesis during mitosis. We have identified another maternally-expressed component of the CPC, *cdca9*, a homolog of Borealin/Cdc8. Similar to *motley* mutants, *cdca9* mutants do not establish a furrow microtubule array, leading to defects in cytokinesis, indicating a conserved role in cellular division for these factors. These two mutations also result in decreased aggregation of GP RNPs, suggesting a previously unknown
function for the CPC in germ plasm aggregation. Localization data suggest that *birc5b* and *cdca9* interact within a CPC-like complex, acting as a linker between GP RNPs and microtubules. Our studies reveal a non-conventional role for a maternally-inherited CPC-like complex during early development as a critical interactor between the cytoskeleton and aggregating germ plasm components. Funding was provided by the Laboratory of Genetics and College of Agricultural and Life Sciences at University Wisconsin - Madison, and the University Wisconsin School of Medicine and Public Health and an NIH grant to F.P. (GM065303).

**Program Abstract #139**  
**Quiescence in primordial germ cells**  
Nathalie Oulhen, Stephany Foster, Gary Wessel  
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Primordial germ cells (PGCs) are the essential stem cell for reproduction in animals and the small micromeres are the putative primordial germ cells of the sea urchin. In animals for which the PGCs form early in development, their specification precedes gastrulation and the PGCs become quiescent in terms of cell division and transcription, compared to neighboring somatic cells. In the sea urchin, the PGCs differ significantly relative to their sibling somatic cells. The PGCs of the sea urchin reduce their activity of transcription, translation, cell division, and mitochondrial activity to less than 10% of their somatic counterparts. They also likely decrease their cytoplasmic pH, reflective of a shift in metabolism from oxidative phosphorylation to glycolysis. We discovered that this dramatic quiescence is transient; the PGCs restart their activities after gastrulation, demonstrating a rapid, predictable, and transient quiescent activity. How do the PGCs enter, maintain, and exit quiescence during the embryonic development? Nanos is a translational regulator required for the survival and maintenance selectively of the PGCs. In the sea urchin, Nanos2 is essential to maintain the quiescence of the PGCs. Nanos2 is embryonically activated in the PGCs shortly after their formation and requires the maternal Wnt pathway. Surprisingly, Nanos2 mRNA accumulation expands into adjacent somatic mesodermal cells during gastrulation. This secondary Nanos2 expression requires Delta/Notch signaling. Funding information: National Institutes of Health 1R01GM132222,9RO1GM125071,1P20GM119943

**Program Abstract #140**  
**Germline development is dependent on segment number and not the chronological age in the marine annelid Platynereis dumerilii**  
Emily Kuehn, Ryan Null, B. Duygu Ozpolat  
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Many animals rely on sexual reproduction to propagate. During development, primordial germ cells (PGCs) get segregated from the rest of the cells (soma) in the body, and eventually give rise to gametes. While germ-soma distinction is clear in model organisms (e.g. fruit fly, mouse), there are many organisms in which this distinction is less clear-cut. For example, some organisms express the classic germline markers (e.g. *vasa, piwi, nanos*) in non-germline cells such as regenerating somatic tissues or pluripotent stem cells. We use *Platynereis dumerilii*, a highly-regenerative marine segmented worm, to study germline development and regeneration. *Platynereis* has 4 PGCs that arise early in development and these cells are thought to give rise to the *vasa* germ cell clusters found across the body in the juvenile worms. The stages of germline development and how the 4 PGCs become the numerous *vasa* germ clusters are not well-documented. We investigated germline development across several life stages using *vasa* as a marker. We found that segment number (as a metric of growth state), and not the chronological age, predicted the state of germline development and the abundance of *vasa* clusters. We also asked if the *vasa* clusters in *Platynereis* play a role in regeneration (as similar cells are observed in *Hydra* and planarian regeneration) and found that *vasa* clusters were not required for regeneration in *Platynereis*, suggesting a strictly germline nature. Finally, we show that the germ clusters regenerate upon amputation. Studies are underway to investigate the behavior of *vasa* cells live in transgenic animals. Funding: Hibbitt Fellowship Startup Funds
Program Abstract #141
Heteromeric Kinesin II is required for flagellar assembly and elongation of nuclear morphology during spermiogenesis of Schmidtea mediterranea
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Development of spermatozoa requires microtubule-based mechanisms that drive formation of a compact head and flagellar tail structures. The Kinesin Superfamily Protein 3A (KIF3A) is a motor subunit of kinesin II, the main molecular machine responsible for transport along microtubules during ciliary assembly. KIF3A has been shown to contribute to tail formation and manchette function during vertebrate spermiogenesis. Here, we show that orthologs of KIF3A and the adaptor kinesin II component Kinesin Associated Protein 3 (KAP3) are required for multiple steps during spermiogenesis in the planarian Schmidtea mediterranea. Expression of Smed-KAP3 is enriched in the planarian testis lobes and accumulates during progression of spermatogenesis. Disruption of expression of Smed-kif3A or Smed-KAP3 by RNA-interference (RNAi) resulted in accumulation of partially elongated spermatids and loss of spermatozoa. Assessment of flagella formation using acetylated-alpha-tubulin antibodies revealed that spermatids with disrupted kinesin II function failed to assemble flagella, whereas visualization with DNA stains revealed reduced nuclear elongation. Disruption of Smed-kif3A or Smed-KAP3 resulted in the somatic phenotypes that include edema formation, reduced locomotion, and loss of epidermal cilia, which corroborate with previous studies of Smed-kif3B (the other motor subunit of heterotrimeric kinesin II). These findings suggest an ancestral role for kinesin II in driving structural changes during sperm head assembly and demonstrate the utility of planarian flatworms for the study of cytoskeletal rearrangements during spermiogenesis. This work was supported by the National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development [R15HD082754] to LR.

Program Abstract #142
Maternal exposure to tobacco products leads to skeletal deformities in offspring mice persisting into adulthood
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Maternal smoking during pregnancy (MSDP) has resulted in more than half a million infants that are prenatally exposed per year. Studies have shown that MSDP leads to low birth weight, increased asthma and wheezing, and behavioral abnormalities in the offspring. Although the effects of smoking have been studied, the ramifications of MSDP on development of the skeleton are largely unknown. To characterize the effects of maternal exposure to tobacco products on the developing skeleton for the first time, pregnant mice were injected with smokeless tobacco extract at E6.5 and E8.5 and skeletal morphology was studied in the offspring. Near-term, this exposure resulted in hypomineralized bones at multiple skeletal sites. In adult offspring, rib cage length was increased by 8.43% in females and 5.95% in males. Rib cage angles were increased by 1.31% and 3.94% in females and males, respectively. Barrel chest was observed at an average ratio of 0.62 (females) and 0.58 (males). Bone densitometry of the adult skeleton further showed that in-utero-exposed mice exhibited a decrease in mineralization. These novel, convincing results suggest that tobacco exposure during development leads to a hypomineralized phenotype in the bones later in life, at least in mice. To determine whether these detrimental effects of tobacco exposure extend to human skeletal development, we next used an in vitro osteogenic differentiation protocol from human embryonic stem cells developed in our lab. Indeed, exposure during differentiation inhibited calcification. Together, these findings illustrate that the inhibition of normal osteogenesis elicited by in utero tobacco exposure that result in rib cage deformities and hypomineralization in mice could translate to humans as well. Funded by Tobacco Related Disease Research Program grants 19KT-0017H, 20DT-0038 and 24DT-0002.

Program Abstract #143
The dynamic life of the thyroid gland: Environmental cues regulating development and function of the thyroid follicular cells.
Pierre Gillotay, Macarena Pozo Morales, Meghna Shankar, Sema Elif Eski, Ines Garteizgogoa Suner, Sabine Costagliola, Sumeet Singh
IRIBHM, ULB, BE
Cells do not exist in a vacuum. They are constantly communicating and interacting with their environment. Development and homeostasis requires local cell-cell interactions at the molecular and mechanical levels. To develop an integrated model of organ formation and function, we utilize the zebrafish thyroid gland as it provides a simple and tractable model. To understand the various interactions occurring in the zebrafish thyroid gland, we developed an in silico connectome, and focused on the role of immune and stromal cells on the physiology of the organ. For this, we first developed the single-cell mRNA atlas of the thyroid gland, which yielded the profile of 6249 cells. The profiling provided the molecular diversity within the thyroid follicular cells, the functional unit of the endocrine gland, along with transcriptional signature of endothelial, immune and stromal cells. Notably, the atlas highlighted unique molecular differences between blood and lymphatic vessels. Moreover, we observed six unique patterns of gene signature covering the mesenchymal fibroblast of the stromal compartment. GO analysis revealed exclusive functionality to each gene signature (ECM deposition, immune modulation, amino acid metabolism), thereby suggesting that the fibroblast population employs division of labor to fulfil the various tasks that it is required to accomplish. Additionally, we identified fibroblast secreted decorin as a potential regulator of thyroid gland’s growth. Finally, we observed the existence of macrophages in the thyroid gland, potentially regulated by CXCL12- CXCR4 signaling axis. Live imaging demonstrated macrophage presence in the gland from 2 dpf, raising an exciting possibility on the role of immune cells in thyroid morphogenesis. Our results identify the complex choreography undertaken by different cell-types to build and organize an organ, and shed lights on mechanisms that can lead to organ dysfunction and failure. Study funded by MISU, FNRS (34772792).

Program Abstract #144
Investigating the role of nicotinamide adenine dinucleotide synthesis in early Xenopus laevis organogenesis
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VACTERL association is diagnosed in infants with multiple congenital malformations in Vertebral, Anal, Cardiac, Tracheal, Esophageal, Renal and Limb (VACTERL) tissues. Recently, mutations in KYNU, HAAO or NADSYN1 genes of the nicotinamide adenine dinucleotide (NAD⁺) synthesis pathway have been identified in patients with VACTERL association. Similar defects were observed in offspring of mice undersupplied with NAD⁺ biosynthesis precursors, tryptophan or vitamin B3. VACTERL phenotypes have also been recapitulated in Kynu and Haao knockout mice and rescued by vitamin B3 supplementation. These findings strongly support the concept that NAD⁺ synthesis, previously considered a universal requirement in all cells, is especially necessary for the development of several, primarily mesodermal, organ systems. We hypothesize that nadsyn1 is differentially regulated in early development with higher expression or activity in mesodermal derivatives. To test this, we first examined the expression of nadsyn1 in early Xenopus laevis frog embryos. Nadsyn1 transcripts were detectable by in situ hybridization in the hatching gland and developing pronephric kidney in Xenopus. Given kidney defects are a key feature of VACTERL association, we are using CRISPR/Cas9 gene editing to test whether mutations in nadsyn1 result in developmental defects in the kidney. Preliminary evidence suggests that nadsyn1 mutations in Xenopus embryos cause diminished kidney pronephros complexity. These experiments will provide groundwork for understanding how compromised NAD⁺ synthesis results in the specific developmental defects that lead to VACTERL association. We acknowledge the Canadian Institute of Health Research (CIHR), the Children’s Health Research Institute (CHRI) and the National Sciences and Engineering Research Council of Canada (NSERC) for funding this research.

Program Abstract #145
Differential requirement of hand1 in the development of specific LPM lineages in Xenopus Laevis
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The transcription factor hand1 is expressed in the heart, lateral plate mesoderm (LPM) and neural crest cells during development. Hand1-null mice die early in embryogenesis due to defects in the heart and placenta. Due to
this lethality, our understanding of the range of functions for HAND1 in early development remains incomplete. In *Xenopus*, we observed that changes in the *hand1* expression patterns correlate very closely with development of specific LPM derivatives, leading us to hypothesize that these changes in *hand1* expression are required for normal development of specific LPM lineages. We compared the development of different LPM lineages in *hand1* knockdown, using Crispr/Cas9, and overexpression models in *Xenopus*. As in the mouse, we found that Hand1 is required for proper heart morphogenesis. Hand1 is also required for the formation of a complex vascular plexus within embryos by maintaining early endothelial cell populations, however, *hand1* overexpression is not sufficient to induce endothelial cell differentiation. Furthermore, downregulation of *hand1* is critical for expansion of the hematopoietic lineages. These findings confirm a conserved role of Hand1 in heart morphogenesis and suggest regulation of Hand1 levels is critical for normal lineage development in the LPM. This work was funded by the Canadian Institutes of Health Research and the National Science and Engineering Research Council.

**Program Abstract #146**

**From Wigglesworth to present: The role of branchless and their receptor during the tracheal system remodeling in *Rhodnius prolixus***

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Insect tracheal system is a tubular network that delivers oxygen to target tissues. The first studies on insects were carried out by Vincent Wigglesworth. In his works, a great characterization of *Rhodnius prolixus* tracheal system was provided, and the hypoxia-mediated plasticity of this organ was discovered. Nowadays, we have an extensive knowledge of *D. melanogaster*, turning it into an important model for branching morphogenesis. Often, these genes are functionally conserved and play an equivalent role during the vertebrate angiogenesis, among which the FGF pathway have a key role in the new branches extension. The aims of our work is to identify the members of FGFs and FGF-R gene family in *R. prolixus* and determine their role on the hemimetabolous tracheal system. We have identified two transcripts for FGF family in the *R. prolixus* genome: one ortholog to the FGF8 subfamily and another one with high similarity to *branchless (bnl)* - gene involved in tracheal development in *D. melanogaster*. On the other hand, we were only able to find a single transcript for FGF receptor. To evaluate the loss of function phenotype of *Rp-bnl* and *Rp-FGF-R* genes, we performed RNAi, injecting fifth-stage (N5) larvae with RNAds to observe how the tracheal system is modified post-molting. Interfered insects that molted to adults were analyzed for changes in the branching pattern of the dorsal epidermal trachea. For both genes, the interfered insects showed a significant reduction in the branching pattern of the tracheal system comparing to the control. Moreover, we have searched for changes in the tracheal supply to the CNS, finding that in the interfered insect the tracheal number and density decrees in this organ. Thus, our results provide valuable physiological and genetic information about *Rp-bnl* and their receptor function in the tracheogenesis and the tracheal system remodeling during the molting process in hemimetabolous insects. Funding: ANPCyT-PICT2015-2374,UNNOBA SIB19-0614

**Program Abstract #147**

**The PAR polarity complex promotes apical remodeling during intestinal development.**

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Epithelial tissues are comprised of adherent polarized cells that line organs and create selective barriers to the outside world. During development, epithelia face many assaults on their integrity such as cell division, shape change, and physical forces. The developing *C. elegans* intestine provides an excellent *in vivo* model to study how epithelia overcome these assaults while maintaining barrier function and apical continuity. The intestinal primordium consists of 16 polarized cells with apical surfaces facing a central midline, the future lumen. Four cells divide again, and the resulting 20 cells elongate to build a continuous intestinal tube. Using live imaging, tissue-specific protein depletion, and genetic screening approaches, we are investigating how this polarized epithelium maintains apical continuity through the challenges of (1) mitosis and (2) elongation *in vivo*. To understand what happens when polarized cells divide, we live imaged cytoskeletal, polarity, and junctional proteins during intestinal cell divisions and observed that the adherens junction protein HMR-1 and apical PAR proteins remain
localized during mitosis. In contrast, apical microtubules and associated proteins are transiently lost as the mitotic spindle is built, and returned after mitosis completes. We hypothesized that PAR proteins act as a memory mark, directing the return of apical microtubules after mitosis. Using intestine-specific depletion of PAR-6/Par6 and PKC-3/aPKC, we found that the apical return of microtubules is indeed disrupted following mitosis, supporting our hypothesis. In addition, we found that intestine-specific depletion of PKC-3 or PAR-6 causes apical surfaces between neighboring cells to become discontinuous during elongation, and ultimately causes larval lethality. These experiments reveal a role for PAR proteins in maintaining apical continuity through mitosis and elongation, which is critical for epithelial function across organisms. Funding: NIH K99, DP2

Program Abstract #148

hox5 genes pattern Weberian vertebrae in zebrafish

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An important function of HOX genes is to pattern and specify vertebrae. In mice, loss of Hox5 has been described to induce homeotic transformations in the spine—transformation of vertebral segments into a more anterior or posterior identity. For example, in Hox5 triple mutants, an anterior transformation of C3-T1 to a C2-like morphology has been described. However, the similar morphology of mammalian cervicothoracic vertebrae makes it difficult to unequivocally discern whether homeotic transformations or other morphogenetic processes are responsible for the mutant phenotype. We hypothesized that zebrafish hox5 genes regulate patterning of the Weberian apparatus, a unique specialization of anterior vertebrae in fish that connects the swim bladder to the auditory system. The Weberian vertebrae possess distinct ossicles on each vertebra, allowing for unequivocal demarcation of vertebral identities. We generated zebrafish hoxa5a, hoxb5a, and hoxb5b mutant alleles with premature stop codons in the final exon, upstream of the homeodomain. Vertebrae were analyzed using high-resolution microCT. In support of our hypothesis, we found that the Weberian vertebrae are under the control of hox5 paralogs. We show that zebrafish with severe loss of hox5 exhibit dysmorphic, fused, and loss of Weberian ossicles, but do not exhibit homeotic transformations. No malformations were observed in other vertebrae. We also show that moderate loss of hox5 genes, which does not induce gross changes in bone shape, reduces the size of Weberian ossicles. We conclude that loss of hox5 genes alters the size and shape of the Weberian ossicles, absent of homeotic transformations. Our studies elaborate on previous studies implicating hox5 genes in patterning anterior vertebrae, and highlight their role in regulating morphogenetic processes that are independent of specifying vertebral identity. This study was funded by the University of Washington Institute for Stem Cell and Regenerative Medicine.

Program Abstract #149

Topologically Associating Domain Boundaries are Commonly Required for Normal Genome Function

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At sub-megabase scale, the mammalian genome is organized into Topologically Associating Domains (TADs) that are typically flanked by CTCF- and cohesin-enriched boundaries. TAD architecture is well-conserved between cell types and across mammalian species. TADs facilitate enhancer-promoter interactions within their boundaries, while preventing contacts between regulatory elements and genes across boundaries. Rearrangements of the human genome involving TAD boundaries result in gene expression changes and developmental phenotypes, but it is unclear whether most TAD boundaries alone are sufficient for ensuring functional integrity of the gene regulatory landscape. To explore this relationship, we deleted eight independent TAD boundaries from the mouse genome and assessed consequences of their respective deletion in vivo. These loci were selected from >3,300 genome-wide TAD boundaries, prioritized for high CTCF and cohesin occupancy, high sequence conservation, and flanking TADs containing genes important for embryonic development but that were otherwise gene-sparse.

in vivo deletions ranged from 11.5 to 82 kb. Loss of a boundary near Smad3, resulted in embryonic lethality, while four other boundary deletions (near Sim1, Smad7, Tbx3 and Tbx5, respectively) resulted in partially penetrant prenatal lethality. TAD boundary deletions near Tbx5 and Twist1, resulted in surviving homozygous mutants having severely underdeveloped lungs and decreased body length, respectively. Overall, chromosome conformation capture (HiC) experiments showed alteration of local TAD architecture for six boundary deletion loci. Our data demonstrate a functional requirement of TAD boundary integrity in vivo, and underscore the context-dependent nature of local three-dimensional genome configuration and its importance during development. (Funding: NIH grant 5UM1HG009421 [to L.A.P. and A.V.] and US DOE contract DE-AC02-05CH11231)

Program Abstract #150
Genetic basis of behavioral evolution in the cavefish Astyanax mexicanus
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Colonization of a new environment often results in the evolution of morphological and behavioral traits. One striking example of this is the suite of traits that have convergently evolved in many species of cave animals throughout the world, including eye loss, albinism, enhancements in non-visual sensory systems and changes in behavior. While these traits have repeatedly evolved in cave species across taxa, the genetic and developmental relationships between these traits are largely unknown. To investigate this, we focused on albinism, one of the hallmarks of cave species, in the blind Mexican cavefish, Astyanax mexicanus. We found a strong relationship between albinism and sleep loss in cave-surface hybrid fish, raising the possibility that shared genetic architecture underlies the evolution of these traits. In cavefish, albinism is caused by mutations in oculocutaneous albinism 2 (oca2), a gene in the melanin synthesis pathway. Mutation of oca2 in surface fish induced albinism and reduced sleep, suggesting a pleiotropic role of oca2 in albinism and sleep loss. Further, crosses of oca2-mutant surface fish to albino cavefish support a role for this gene in the evolution of sleep loss in independently evolved cave populations. Together, these findings suggest that morphological and behavioral traits in cavefish have evolved through shared genetic bases, and reveal a role for pleiotropy in the evolution of a complex behavioral trait. Funding: This work was supported by an NSF award DEB1754231 to JEK and AK.

Program Abstract #151
The fish family poeciliidae as a model to study the evolution and diversification of regeneration capacity in vertebrates
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The capacity of regenerating a new structure after losing an old one is a major challenge in the animal kingdom. Fish have emerged as an interesting model to study regeneration due to their high and diverse regenerative capacity. To date, most studies have focused on the mechanisms that underlie fin regeneration, and little is known about why or how this remarkable trait evolves. Here, we advocate the livebearing fish family Poeciliidae as a promising new model system to study the evolution of fin regeneration. We address potential evolutionary pressures that modulate fin regeneration capacity: the Poeciliidae offers a large variety of species with contrasting lifestyles in a well-defined phylogenetic framework, allowing comparative studies of fin regeneration within and among species. We will propose a number of empirically testable theories for how natural and sexual selection can lead to the evolution of fin regeneration among closely related species, among populations and within populations. This work was supported by a scholarship from Agencia Nacional de Investigacion y Desarrollo (ANID) / Becas Chile, postdoctoral fellowship.

Program Abstract #152
The Role of SHH Signaling in Regulating Species-Specific Jaw Size
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Changes in jaw size during evolution have been crucial for the adaptive radiation of vertebrates. However, abnormal variation in jaw size is also associated with human birth defects and disease. Our prior work indicates that the evolution of species-specific differences in jaw size is due in part, to early developmental mechanisms that are regulated by neural crest mesenchyme (NCM), which is the progenitor population that migrates into the mandibular arch and gives rise to the jaws. One such mechanism involves precisely timed interactions between NCM and adjacent epithelium that facilitate the patterned outgrowth of the lower jaw. These interactions are mediated by multiple signaling pathways including Sonic Hedgehog (SHH). SHH signaling plays a role in a variety of developmental processes including cell proliferation. We examine duck and quail embryos and find that duck start with more NCM in their mandibular arch and have distinct proliferation dynamics including a longer cell cycle. We hypothesize that the response of NCM to SHH signaling in quail versus duck is species-specific, which affects proliferation and generates differences in jaw size. We test our hypothesis in vivo by quantifying expression of SHH pathway members in the mandibular arch of duck, chick, quail, and quail-duck chimeras. We find that NCM-mediates species-specific expression levels for key pathway members. We also test if the response to SHH signaling in NCM is intrinsically species-specific using in vitro mandible culture. We treat mandibles with different levels of rSHH protein or a pathway inhibitor (i.e., cyclopamine). We also overexpress a SHH binding protein, which alters jaw size. These experiments reveal that species-specific differences in SHH signaling are mediated by NCM and are cell-autonomous. Our work suggests that changes to the SHH pathway in NCM cells may modulate jaw size during development, disease, and evolution. Funding: R01DE025668, R01DE016402, and S10 OD021664 to RAS.

Program Abstract #153
Characterization of a yeast interfering RNA larvicide with a target site conserved in the Rbfox1 gene of multiple disease vector mosquitoes
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Combating established and emerging mosquito-borne infectious diseases will require new mosquito control strategies. In recent years, our laboratory has conducted siRNA screens that identified hundreds of larval lethal genes in Aedes aegypti, mosquito vector of multiple disease-causing arboviruses. Here we describe the characterization of a yeast larvicide developed through genetic engineering of Saccharomyces cerevisiae to express a short hairpin RNA (shRNA) corresponding to a target site in the A. aegypti ortholog of RNA-binding fox protein 1 (Rbfox1), a larval lethal gene that encodes a regulator of tissue-specific alternative splicing in Drosophila melanogaster. A. aegypti larval consumption of this yeast, which was heat-inactivated and dried prior to feedings, resulted in >90% larval mortality in both laboratory and semi-field trials. Based on these findings, it was hypothesized that the yeast, which has an shRNA target site conserved in multiple mosquito species, but which has not yet been identified in the genomes of non-target organisms, may function as a broad-range mosquito insecticide. In support of this, Aedes albopictus, Anopheles gambiae, and Culex quinquefasciatus mosquito larvae exhibited high levels of mortality following consumption of the yeast. These results suggest that this yeast larvicide may represent a new innovation that can be used as a biorational method of countering multiple species of vector mosquitoes. Future studies will further evaluate the potential use of this technology for mosquito control, continue to assess the functions of Rbfox1 genes in mosquitoes, and explore the use of RNAi-based yeast technology for comparative analysis of gene function in additional non-model arthropods. Funded by NIAID 1R21 AI128116-01, USAID AID-OAA-F-16-00097 and DoD PR161211 awards to MDS.

Program Abstract #154
Astrocytes close a critical period of motor circuit plasticity
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Critical periods – brief intervals where neural circuits can be modified by sensory input – are necessary for proper neural circuit assembly. Extended critical periods are associated with neurodevelopmental disorders, including schizophrenia and autism; however, the mechanisms that ensure timely critical period closure remain unknown.
Here, we define a critical period in the developing *Drosophila* motor circuit and identify astrocytes as essential for proper critical period termination. During the critical period, decreased activity produces larger motor dendrites with fewer inhibitory inputs; increased motor neuron activity produces smaller motor dendrites with fewer excitatory inputs. Importantly, activity has little effect on dendrite morphology after critical period closure. Astrocytes invade the neuropil concurrent with critical period closure, and astrocyte ablation prolongs the critical period. Finally, we use a genetic screen to identify astrocyte-motor neuron signaling pathways that close the critical period, including Neuroligin-Neurexin signaling. Reduced signaling destabilizes dendritic microtubules, increases dendrite dynamicity, and impairs locomotor behavior. Previous work defines astroglia as regulators of plasticity at individual synapses; here we show that astrocytes close a critical period of motor circuit plasticity, thereby allowing proper locomotor behavior. Funding was provided by HHMI (CQD), R01 HD27056 (CQD), R01 NS059991 (MRF) and NIH F32NS098690 (SDA). SDA is a Milton Safenowitz Post-doctoral fellow of the ALSA.

**Program Abstract #155**

What guides the leaders: Identifying factors involved in *C. elegans* pioneer axon extension

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During development, axons navigate complex environments to reach synaptic targets. Growth cones of early extending axons interact with environmental guidance cues, while later extending axons can interact directly with earlier “pioneering” axons, “following” their path. This is how the largest anterioposterior nerve bundle in *C. elegans*, the ventral nerve cord (VNC), is formed. The first axon to extend along the VNC is that of the AVG neuron. Approximately 50 axons then extend along AVG, including the interneuron axons of the motor circuit. In *lin-11* mutants, AVG fails to differentiate and many VNC axons are misguided. The mechanisms for AVG axon guidance are unknown. *NID-1/Nidogen* is a basement membrane component enriched along the VNC. In the absence of *nid-1*, AVG is misguided at 20% penetrance. We identified several genes that enhance AVG guidance defects in the absence of *nid-1*. One gene identified is *aex-3*, a *rab-3* guanine exchange factor. *aex-3* and *rab-3* genetically interact with *unc-5*, a receptor for the guidance cue UNC-6/netrin. Here we describe a new gene in this pathway: *F52H2.7* is a homologue of human C2CD5, a CDK5 binding partner. *F52H2.7* genetically interacts with *cdk-5*, *unc-18/Munc18*, *aex-3/MADD*, *rab-3/Rab3*, and *unc-5/UNC-5*. We also identified three IgCAMs that regulate AVG axon navigation: *wrk-1*, *rig-5*, and *rig-6*. *rig-5* genetically interacts with *rig-6*, but not with *wrk-1*. *rig-5* also suppresses *F52H2.7* AVG guidance defects, indicating that RIG-5 is regulated by *F52H2.7*, though the mechanisms for this remain unclear. Based on these results, we hypothesize that *F52H2.7* and CDK-5 regulate UNC-18 initiated exocytosis of AEX-3/RAB-3 associated vesicles, transporting factors needed for UNC-5 and RIG-5 mediated AVG axon guidance. While many aspects of this model remain unclear, the current work provides a wide reaching outline for future investigation into anterioposterior pioneer guidance systems in *C. elegans* and beyond.

Funding sources: CIHR, NSERC

**Program Abstract #156**

Unique early pioneer neurons in two dinophilid species (Lophotrochozoa: Annelida) neurogenesis

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Dinophilidae is a unique group among Polychaeta and its representatives combine morphological traits of different Lophotrochozoan taxa. Moreover, adult dinophilids demonstrate morphological similarity with trochophore larva. The earliest events in Dinophilid neurogenesis will help to shed light on our understanding of the evolution of this group: whether they demonstrate archaic origin or neoteny. We applied standard neuronal and pan-neuronal immunochemical markers (antibodies against serotonin, FMRFamide, and acetylated tubulin) to reveal the first nerve elements during larval development of two relative species *Dinophilus gyrociatus* and *Dinophilus taeniatus*. Contrary to all other Lophotrochozoa investigated, the earliest positive cells demonstrate reaction with acetylated tubulin only. The first nerve cell differentiates in episphere at the anterior pole of the embryo, and its caudally running fibers construct two ventral cords. Later three tubulin-positive cells adjacents to each ventral cord appear in hyposphere. They send fibers anterior and posterior along the processes of the first
cell and together construct the anlagen of the nervous system: brain neuropil, paired ventral cords with commissures, and esophageal ring. No specific neuronal markers were detected in all early cells. Serotonin- and FMRFamide-immunoreactive neurons differentiate much later within the brain and along ventral cords in a course of development, and their axons follow the path already established by the processes of the early cells. Such a scenario of early neurogenesis is unique for all Annelids representatives studied so far and allow to suggest the archaic origin of Dinophilids. The reported study was funded by RFBR, project number 19-34-60040.

Program Abstract #157

The evolution of centralization: neural architecture in the sea anemone *Nematostella vectensis*

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The evolution of centralized nervous systems from the diffuse nerve nets found in simple animals represents one of the most intriguing transitions in the history of life. The starlet sea anemone *Nematostella vectensis* is a member of Phylum Cnidaria (jellyfish, corals and anemones), which is representative of the earliest branching animals and generally thought to possess a simple nerve net composed of loose networks of interconnected neurons. Despite its simple morphology, very little is known about how this nerve net is organized to support coordinated movements and drive behaviors using a large repertoire of chemical neurotransmission-related genes. Recently we developed a novel transgenic line that broadly labels neurons, making it possible to illuminate the overall architecture of the *Nematostella* nervous system at high spatiotemporal resolution. One of the most striking and unexpected features that we observe are clusters of neurons accumulating at the base of tentacle junctions. The morphology of these cells resembles ganglionic structures seen in Bilateria, while their locations suggest a possible role in coordinating tentacle movement, integrating sensory inputs, or facilitating crosstalk between the oral and aboral regions of the body. This observation hints at a far greater degree of neuronal complexity than previously appreciated, making *Nematostella* an attractive model to better understand the origins of neural centralization. To characterize the subpopulation of neurons in the oral region and further identify potential markers to inform functional studies on the ganglia-like structures, here we will report on single cell transcriptional profiling followed by *in situ* hybridization to validate candidate genes in different neuronal populations. We also derived a neural-specific jGCaMP7s transgenic line and demonstrate its utility to visualize neural activity in behaving animals. This study was funded by the Stowers Institute for Medical Research.

Program Abstract #158

Exploring the variables defining synaptic partner choice of vomeronasal sensory neurons

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The functional identity of neurons is established and maintained by the tightly regulated expression of transcription factors (TFs) that control chromatin architecture and selective activation of genetic programs. Manipulating the function of these important TFs creates ambiguously defined neurons with altered target selection. The role of guidance cues and cell surface molecules that organize axonal convergence has been extensively explored, however, how the selection of a postsynaptic cell partner over another is defined remains unclear. To study the mechanisms behind neuronal identity and postsynaptic target selection we used the mouse vomeronasal organ (VNO) and accessory olfactory bulb (AOB). The VNO is an olfactory subsystem which detects pheromones that inform social and sexual behaviors in many vertebrates. The two main populations of vomeronasal sensory neurons (VSNs), apical and basal, are named for their spatial location in the neuroepithelium. Individual VSNs express 1 or 2 receptors out of hundreds encoded by two vomeronasal receptor (Vr) gene families, creating many VSN subtypes that project to different areas of the AOB. Unlike the olfactory system, where olfactory receptor expression strictly dictates which glomerulus will be targeted, VSNs expressing the same Vr gene can innervate up to 30 different glomeruli. We used single-cell sequencing (scSeq) to understand the genetic differences between VSNs expressing the same Vr that connect with different postsynaptic partners. From the scSeq results, we identified several candidate cell surface molecule genes and immunolabeled a Vr-specific reporter mouse line to measure variability in protein expression. Our preliminary data suggest that unique combinations and ratios of expressed cell surface molecules may be responsible for VSNs
expressing the same Vr to converge on distinct glomeruli. This work was supported by the NIDCD section of the National Institutes of Health under Grant No 1R01DC017149-01A1.

Program Abstract #159
Understanding retinal ganglion cell axon outgrowth using human retinal organoids
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During human visual system development, the extension and bundling of retinal ganglion cell (RGC) axons form a bridge, called the optic nerve, to convey visual information from the retina to the brain. RGC death causes degeneration of the optic nerve and glaucoma, a leading cause of vision loss in aging people. To preserve or restore vision through clinical therapies such as transplantation, it is critical to prevent RGC death and preserve RGC axons. Human retinal organoids, differentiated from pluripotent stem cells, are similar to developing human retinal tissue. Organoids provide an in vitro system to study human biology that overcomes the obstacles of genetic manipulation. Here, we outline a project aimed at understanding the biology of RGC axon outgrowth using human retinal organoids. RGC axon outgrowth is observed from cut organoids on matrigel-coated surfaces. These axons extend rapidly, followed by drastic changes in arrangement and morphology. We are currently examining the interactions between two cut organoids to understand interactions among RGCs and other types of neurons during visual system formation. We found that two cut organoids form a ‘fusion area’ where RGCs and other neurons intercalate with each other. After one organoid is detached by an applied force, the axons remain connected. The detached organoid is pushed closer to the affixed organoid, new axon outgrowth is observed, and the organoid is stabilized again. Follow-up studies focus on investigating how RGCs interact between organoids and identifying factors that stabilize axons in the fusion area. Our work advanced human retinal organoids as a model for revealing mechanisms of RGC survival and axon stabilization, with implications for glaucoma disease treatment involving organoid-based transplantation. This work is supported by BrightFocus Award G2019300.

Program Abstract #160
Mechanisms regulating cytokinesis in the early C. elegans embryo
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Cells divide throughout embryogenesis, and mitosis must be tightly regulated for cell fate. Cytokinesis is the process where a contractile ring physically pinches in the daughter cells at the end of mitosis. The prevailing dogma is that spindle-directed pathways dominantly regulate cytokinesis, based primarily on studies using cultured cells or pluripotent one-cell embryos. However, recent studies have uncovered spindle-independent mechanisms that regulate cytokinesis, and their requirement may vary with cell fate. Our lab discovered a Ran pathway that senses chromatin position to regulate cytokinesis in cultured mammalian cells, and functions redundantly with spindle pathways. Our model is that cortical proteins with nuclear localization signals are recruited equatorially by importin-α/β to position the ring at distances away from chromatin where Ran-GTP levels are high. We are using the two-cell C. elegans embryo to determine how the requirement for the Ran pathway varies with cell fate. The two-cell embryo has an anterior AB cell fated to be multiple tissues and a posterior P1 cell fated to be germline. Imaging with high temporal resolution revealed that each cell has unique cytokinesis kinetics, supported by differences in the accumulation of contractile proteins. We found that disrupting polarity (par-1,-3 or -6 RNAi) or ran-3 (RanGEF; RCC1) RNAi equalized kinetics between the two cells. Kinetics were slowed upon co-depletion of contractility regulators ect-2 (RhoA-GEF) or let-502 (Rho Kinase). Co-depletion of ani-1 (anillin, scaffold for the ring) and ran-3 suppressed cytokinesis phenotypes in AB, but not P1 cells, supporting different pathway requirements. Similarly, ima-3 (importin-α) and imb-1 (-β) had different requirements in AB vs. P1 cells. Our findings reveal cell-fate dependent differences in mechanisms regulating cytokinesis and emphasize the need to study cytokinesis in a developmental context. This research is funded by NSERC.
Program Abstract #161

ATXN10 as a novel ciliary associated protein involved in maintaining cell polarity in the developing heart and adult kidney.
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Primary cilia are microtubule-based structures present on the surface of nearly all mammalian cell types. Cilia are crucial regulators of multiple cell signaling pathways and are necessary during embryonic development. ATXN10 was identified in an in silico screen for genes encoding proteins suspected of having a role in cilia function or formation during mammalian development. We have identified ATXN10 as a crucial player involved in cardiovascular development and in maintaining kidney tubule organization in adult mice. In vitro studies indicate that ATXN10 is not necessary for ciliary assembly; however, fluorescently-tagged ATXN10 is enriched at the base of the cillum as determined by colocalization with basal body marker, γ-tubulin. In vivo studies have shown that during development, ATXN10 is almost exclusively expressed in the developing heart between E8.5 and E12.5. Following E12.5 expression expands from the heart to include the majority of the embryo. In ATXN10 congenital mutant mice lethality occurs by E11.5 due to severe pericardial effusion. Using histology and contrast enhanced μCT we have observed reduced ventricular wall thickness and volumetric expansion of the developing common ventricular chamber without an increase in cell number or proliferation. By using conditional Atxn10 allele with tissue specific Cre we show that loss of Atxn10 in the myocardium drives the ventricular phenotype seen in the heart. Loss of Atxn10 in the endothelium results in lethality with embryos exhibiting vascular abnormalities including hemorrhaging and blood pooling at E13.5. Furthermore, using Cagg-CreER for systemic inducible Cre activity, we show that ATXN10 loss causes sudden death in adult mice and is needed to maintain proper kidney tubule morphology. Kidney tubules in mutant mice assume a more dedifferentiated developmental-like state with colocalization of tubule specific markers and basement membrane abnormalities. Funding: NIH- NICHD and NHLBI

Program Abstract #162

Systematic identification of functional phosphorylation sites during developmental cell proliferation in vivo
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Protein phosphorylation plays a fundamental role in regulating progression through the cell cycle. Numerous studies have been performed in tissue culture to identify cell cycle regulated phosphorylation sites by mass-spectrometry, uncovering tens of thousands of substrates. However, in vivo functionality of these sites is rarely addressed and the potential implications of mitotic phosphorylation for context-specific aspects of epithelial cell division, such as apical cell rounding and planar spindle orientation, remain unclear. Here we report tandem phospho-proteomic and genetic approaches in Drosophila to identify novel, functional, cell cycle dependent phosphorylation sites. Genetic manipulation of mitotic rates in developing wing discs followed by mass-spectrometric quantification of phosphorylation levels led to the identification of over a thousand cell cycle-dependent phosphorylation sites. Validating this approach, we identified expected mitotic and interphase phosphorylation sites on a number of proteins, including Cyclin Dependent Kinase 1. To determine functionality of previously uncharacterized sites, we used a scarless CRISPR genome editing strategy to modify endogenous sites on 10 candidate genes chosen based on protein function and conservation in humans. For each site, we introduced point mutations into the genome that exchanged phosphorylated residues with non-phosphorylatable and phosphomimetic amino acids. We are currently characterizing the precise mitotic requirements for putatively lethal phospho-mutant and phospho-mimetic alleles in four genes (Lpin, 14-3-3 epsilon, CG13994 and Pen). Further characterization of these mutant phenotypes using both mosaic analysis and live imaging will address the role of each site in epithelial proliferation in vivo, with potentially broad implications given the conservation of cognate sites in the relevant human orthologues. Funding NIH - R01GM111733-01A1

Program Abstract #163

The Drosophila Ste20 kinase Slik acts as an effector and a regulator of Rho1 GTPase in tissue growth modulation
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The growth of embryonic tissues is maintained under tight homeostatic control by the combined action and regulation of many signaling pathways. Failure to balance such signals can lead to growth defects in developing organisms. The Drosophila Ste20-family kinase Slik, controls the rate of developmental tissue growth and has distinct catalytic and non-catalytic functions. Slik directly phosphorylates and activates Moesin, favoring cross-linking of the actin cytoskeleton to proteins at the plasma membrane and promoting a stable epithelial phenotype. Slik also regulates tissue growth through a non-catalytic mechanism, stimulating both Slik-expressing cells and neighboring cells to proliferate. How these activities of Slik are regulated was, until recently unclear. A BioID/mass spectrometry screen identified SLK, a mammalian ortholog of Slik, as a new effector of the small GTPase RhoA in mammalian cells. We found that Slik interacts specifically with the active form of Rho1 in Drosophila cells. Using live-cell NanoBiT biosensors, we found that Slik associates with the plasma membrane and dimerizes in response to active Rho1. Consistent with Rho GTPase regulating SLK/Slik catalytic activity, activated Rho1 caused a Slik-dependent increase in Moesin phosphorylation. We also found that Slik feeds back negatively to inhibit Rho1 independently of catalytic activity, and that this mechanism contributes to Slik-dependent tissue growth promotion. Reducing Rho1 activity in the developing fly wing (or wing imaginal disc) induced a non-autonomous proliferation phenotype similar to Slik overexpression and suppressed defects caused by slik depletion in vivo. In fact, Rho1 depletion and Slik overexpression acted synergistically to induce tissue growth in wing discs. Our results suggest that Slik acts both as a conserved effector regulated by Rho1 and, in a negative feedback loop as a Rho1 inhibitor to control localized growth of epithelial tissues. Research supported by NSERC & CIHR.

Program Abstract #164

Regulation of Polarity Establishment in the Developing Zebrafish Epidermis by aPKC and E-cadherin

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The epidermis, a multi-layered epithelium, covers the vertebrate body. Developmentally, it is formed from a bilayered epithelium consisting of the outer periderm and inner basal epidermis. This bilayered and multi-layered epithelium is polarized at the tissue level. How this polarity is established in the developing epidermis remains underexplored. In simple epithelia, the apicobasal polarity is regulated by polarity modules such as the apical Par-aPKC and Crb-Sdt complexes, and basolateral Lgl-Dlg and Yurt-Cora pathways. In simple epithelia, polarity pathways regulate the formation and positioning of the apically localized adherens junctions. Reciprocally, a perturbation in the adherens junctions perturbs the polarity pathways. However, in multi-layered epithelia, the distribution of adherens junctions and their interactions with the polarity pathways remain unknown. We show that adherens junctions are localised in a polarised manner in the developing zebrafish epidermis. The levels of adherens junctions increase along the apicobasal axis in the periderm and decrease in the basal epidermis. We show that aPKC regulates the robustness of the polarized distribution of E-cadherin, a key adherens junction component, in the periderm. Further, levels of E-cadherin in one layer regulate the levels of E-cadherin in the juxtaposing layer in a layer non-autonomous manner. Additionally, perturbing E-cadherin disrupts localization and levels of Lgl in a layer autonomous and layer non-autonomous manner. With this data, we propose that the developing epidermis acquires the tissue polarity in a stepwise manner. Developmentally, periderm forms first wherein aPKC initiates the polarization of E-cadherin. E-cadherin from periderm transduces the polarity cues to the basal epidermis to regulate Lgl and hemidesmosome formation, thus, polarizing the bilayered epidermis.

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Program Abstract #165

Persistent DNA Repair Signaling during Mitosis Facilitates Acentric DNA Segregation

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Polyploid cells (cells with whole genome duplications) are often thought of as terminally differentiated somatic cells. However, during Drosophila hindgut metamorphosis, we discovered that polyploid cells can play an
important role in tissue development. We have established *Drosophila* hindgut rectal papillar cells (hereafter papillar cells) as a model to study the roles and regulation of polyploid organ progenitors. Papillar cells undergo developmentally programmed polyploidization through a cell cycle variation known as endocycling. This process leads to the inactivation of cell cycle checkpoints. Interestingly, these cells enter mitosis several days after checkpoint inactivation. Inactivation of checkpoints is known to lead to broken DNA in mitosis, posing a threat to genome integrity. Indeed, we find that papillar cells accumulate broken DNA in mitosis. Despite lacking kinetochore-spindle attachments, broken papillar DNA can still segregate during mitosis, preventing aberrant chromosome structures known as micronuclei. Although rescue of broken DNA is important for preventing micronuclei, the underlying mechanisms are incompletely understood. We find that despite lacking robust checkpoint inactivation, papillar cells robustly recruit some components of the double-strand break repair machinery. Further, damaged papillar chromosomes retain repair proteins for significantly longer than observed in cells with active checkpoints. These proteins are required for broken DNA segregation and thus micronuclei prevention in papillar cells and proper tissue development following DNA damage. We propose that DNA damage that escapes cell cycle arrest requires initiated, but not complete repair to segregate broken chromosomes. We hypothesize that this mechanism is relevant to cells with inactive cell cycle checkpoints or in cases where DNA damage persists into mitosis. This work is funded by TRISH Grant #NNX16A069A.

Program Abstract #166

**Single-cell transcriptomic analysis reveals unique cell types and patterns of gene expression in the frog corneal epithelium**

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Corneal Epithelial Stem Cells (CESCs) and their proliferative progeny, the Transit Amplifying Cells (TACs) are responsible for homeostasis and maintaining the transparency of the cornea. Due to our limited knowledge of cell fates and gene activity within the cornea, the search for unique markers to identify and isolate these cells is important for ocular surface reconstruction. In a previous study, we used a candidate-based approach to identify molecular markers that characterize the apical and basal corneal epithelial cells, as well as the corneal keratocytes of larval and adult frogs. However, a non-biased characterization of cornea cells has been lacking. Using the 10X Genomics Chromium platform, we performed single-cell RNA sequencing (scRNA-seq) on ~5173 corneal epithelial cells of stage 49-51 *Xenopus* larvae. We identified five main clusters with distinct molecular signatures, which represent apical, basal and keratocyte cell types. Two clusters display strong proliferative characteristic and likely identify early and late stage TACs. Differential gene expression analyses was used to compare transcriptional profiles among the clusters. Our data reveals several novel genes expressed in corneal epithelial cells, and helped validate other putative corneal stem cell markers, previously identified in other vertebrates. Currently, we are examining the localization of these new candidates using fluorescence in situ hybridization and immunofluorescence. Furthermore, we constructed pseudo-temporal ordering of cells to reveal a developmental lineage within the corneal epithelium. Our work provides a comprehensive atlas of single-cell transcriptomes in frog’s corneal epithelium. In future, this work will be useful to understand the function of novel genes in corneal homeostasis, wound healing and cornea regeneration, the latter including regenerating lenses, which arises from basal corneal epithelial cells in frogs. This work is supported by NEI funding (1R01EY023979).

Program Abstract #167

**Interleukin-11 signaling limits scar formation by antagonizing endothelial-to-myofibroblast transdifferentiation during zebrafish heart regeneration**

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In adult mammals, tissue damage after myocardial infarction induces myofibroblast differentiation and the formation of a permanent, functionally inert scar. However, the molecular mechanisms that govern
myofibroblast differentiation and scarring remain poorly understood. Some vertebrates like zebrafish display a remarkable regenerative potential with only limited and transient fibrosis after tissue damage, including in the heart. Here, employing comparative expression profiling coupled with loss-of-function approaches, we identified the canonical Interleukin-11/Stat3 signaling axis as a core component of regeneration in zebrafish. Notably, animals with loss of Interleukin-11 receptor (Il11ra) function reach adulthood without overt developmental defects, but exhibit strongly impaired cardiac regeneration with increased myofibroblast differentiation and the formation of a permanent collagenous scar, similar to what is observed in adult mammals. Using zebrafish fate-mapping approaches, reporter lines and human primary cell culture methods, we provide evidence that Interleukin-11 signaling limits endothelial-to-myofibroblast transdifferentiation and maintains a pro-regenerative niche to promote cardiac regeneration. Altogether, our data reveal a vital role for endothelial Interleukin-11/Stat3 signaling in containing injury-induced cardiac fibrosis. Funding Sources: German Research Foundation (DFG) Project 186133941 and CRC-1213 - Cor Pulmonale and the Max-Planck-Society.

Program Abstract #168
Elucidating the role of cup-2 in the attachment of the C. elegans germline stem cell niche
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The balance between stem cell proliferation and differentiation is imperative for the growth and development of many organisms. This balance hinges on the interactions between the stem cell population and the stem cell niche. The niche is a cell or group of cells that provides an anatomical location and signals to the stem cells to self renew. In the C. elegans germline, the somatic distal tip cell (DTC) acts as the stem cell niche. It regulates the stem cell pool via GLP-1/Notch signaling: cells that are close to the signal remain as stem cells. The DTC typically resides at the distal most end of the gonad. We have identified the degradation in the ER (Derlin) protein DER-1 (CUP-2) as having a role in maintaining the position of the DTC. In cup-2 mutant worms, rather than being at the distal end of the gonad, the DTC becomes displaced and moves proximally down the gonad. The stem cell population also moves proximally with the DTC, suggesting that the niche is functional at the new location. We are exploring cup-2’s two known functions, endoplasmic reticulum associated degradation (ERAD) and endocytosis, to see if they play a role in DTC attachment. In addition to this, we are investigating other cellular components to see if they may play a novel role in DTC placement. We are also determining if the function of cup-2 is required in the DTC, the germline, or both for normal placement. By further investigating CUP-2, we hope to learn more about how the DTC, and potentially other stem cell niches, are held position. Additionally, we hope to uncover how a disruption in the positioning affects the surrounding stem cell pool, which may be relevant for therapeutic purposes. Scadden, David T (2006). The stem-cell niche as an entity of action. Nature 441(7097): 1075–1079. Funding Sources: DH is funded through CIHR

Program Abstract #169
The molecular basis of size regulation during axolotl limb regeneration
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The axolotl (Ambystoma mexicanum) is one of the few tetrapod species capable of regenerating complete limbs. One important yet 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 regulating growth of the tiny limb are not known. We have discovered that size during limb regeneration is regulated through limb extrinsic signals. We have also observed that the abundance of innervation is increased in the tiny limb staged regenerate. Previous evidence also correlates innervations with tissue growth in the regenerate. Thus, nerve signaling is an extrinsic factor that could regulate growth of the tiny limb. We have found that it is required for the elevated cell proliferation and repressed cell death occurring
during regeneration. Furthermore, neurons supplying tiny limbs express elevated levels of BMPs. We are employing molecular, biochemical, and classical embryology techniques to explore the role of BMP signaling in regulating limb size during regeneration. This project was funded by the 1R15HD092180-01A1 awarded to C.M. from NICHD and the Doctoral Dissertation Research Grant awarded to K.W. from UMass Boston.

Program Abstract #170
The Molecular Mechanisms Underlying the Reprogramming of Pattern Information During Limb Regeneration
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Ambystoma mexicanum is a tetrapod species with the capacity to regenerate lost tissues in a scar-free, high-fidelity manner. Following limb amputation, a regenerative organ, the blastema, forms and recreates the pattern of the missing limb structure. While it is unknown how pattern information is reprogrammed within blastema cells, previous studies indicate that chromatin modifications encode patterning information in adult cells in a tissue-specific manner. Our studies demonstrated chromatin is less compacted in the blastema than in stump tissue. This led us to consider whether alterations in chromatin architecture and modifications during regeneration regulates pattern reformation. We discovered chromatin opens significantly in regenerating limbs and wounded dermis, though this euchromatic state only remains in blastemas during regeneration. We investigated changes in both gene expression and the abundance of the inhibitory chromatin mark, H3K27me3, in limb cells that contribute to the regenerate and determined region-specific and global changes in blastemas associated with reprogramming absent in wounds. Using Q-RTPCR, we observed blastemas are reprogrammable using specific signaling molecules, while wounds are reprogramming resistant. Moreover, examination of global H3K27me3 abundance demonstrated significant abundance decrease overtime in blastemas, but not in wounds. We have begun analyzing differences in abundance of H3K27me3 around patterning genes in mature and blastema tissue. We demonstrated that specific alterations to chromatin structure and patterning genes occur in wounds that regenerate structure versus non-regenerative wounds. Future studies will focus on identifying differences in upstream signaling that differentially regulate chromatin in these wounds. This work is supported by NICHD in the NIH, grant number 1R15HD092180-01A1.

Program Abstract #171
NODAL signalling activity is initiated after establishment of the human pluripotent epiblast and is not required for its maintenance prior to implantation.
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Approximately six days post fertilization, the human embryo forms a blastocyst comprised of three distinct cell types. The trophectoderm (TE) and primitive endoderm (PrE) are precursors of extraembryonic tissues, while the epiblast (EPI) will give rise to the embryo proper. In mouse embryos, specification of PrE and EPI is mediated by differential fibroblast growth factor (FGF) signalling between embryonic and extraembryonic cells. However, this mechanism is not conserved in human embryos, and other as yet undefined signalling pathways likely control EPI specification. In human embryonic stem cells (hESCs), ACTIVIN/NODAL signalling is directly required for expression of the pluripotency-associated transcription factor NANOG, the earliest known EPI marker in human blastocysts. Here we report a detailed characterisation of NODAL signalling activity in human embryos, based on expression of the intracellular effector, phosphorylated (p)SMAD2. In early human blastocysts, NODAL signalling activity is first detectable after initiation of NANOG expression. pSMAD2 expression then becomes progressively abundant in the embryonic pole of the blastocyst and is eventually expressed throughout both the PrE and EPI, and in individual polar TE cells. Surprisingly, in contrast to both primed and naïve hESCs, inhibition of NODAL signalling activity in mid- to late- human blastocysts does not lead to loss of NANOG expression. These results suggest that NODAL signalling is not required for the maintenance of the human preimplantation EPI, proposing a fundamental difference in the regulation of a key pluripotency-associated transcription factor, between the human EPI and in vitro models of human pluripotency. This work is supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK, the UK Medical Research Council, and the Wellcome Trust.
Expansion of a pre-existing signaling center underlies the evolution of a morphological novelty in the *Drosophila* genitalia
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How new morphological structures ("morphological novelties") arise during evolution has been a long standing question in biology. Work on morphological novelties has frequently implicated a role for signaling pathways in their origination. In particular, the frequent appearance of signaling pathway ligands in spatial association with novel morphological structures suggests that the evolution of ligand expression is critical to installing the genetic programs underlying novelties. However, we currently lack an understanding of how the regulation of these pathways are altered to establish their roles in the development of novel morphologies. To investigate this problem, I examined the role of Notch signaling during the evolution of a recently evolved structure, the posterior lobe of the fruit fly *Drosophila melanogaster*. This structure is a cuticular outgrowth on the genitalia of males within the *melanogaster* clade. The ligand for the Notch signaling pathway, Delta, is required for posterior lobe development, and its expression has been expanded in lobe-forming species. We’ve identified partially redundant transcriptional enhancers of *Delta*, and show that changes both within the *Delta* locus, as well as upstream of *Delta* have occurred to generate patterns of expression associated with posterior lobe development. While these enhancers regulate *Delta* expression, their activities differ in both time and space. Surprisingly, our analysis demonstrates that these enhancers activate *Delta* over an extended period of days, likely serving multiple roles in patterning genital tissues. This work reveals that a novel role of posterior lobe development was added to an ancient signaling center with different roles that emerge over days of development. This paints a very different picture of how new traits arise: by adding and building upon pre-existing signals. This research is supported by the National Institution of Health.

Mesenchymal Slit2 and Slit3 are required for pancreatic islet morphogenesis
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The embryonic microenvironment of the pancreas is vitally important for the proper development and function of the pancreas in adults. This environment, which includes mesenchymal, neuronal, and endocrine cell types, relies on complex signaling networks to guide the proper development of the pancreas. Alterations to this microenvironment results in aberrant pancreatic islet development, leading to cancer and diabetes. Our lab previously reported that the Roundabout (Robo) receptors are required in the embryonic pancreas for proper islet morphogenesis and function. Robo is a member of the conserved Slit-Robo signaling pathway; in conjunction with its canonical ligand Slit, Slit-Robo signaling mediates axon guidance, cell migration, and cell positioning. The role of Slit ligands in the pancreas has not yet been determined. Due to the requirement of Robo in islet morphogenesis, we hypothesized that pancreatic Slit ligands cooperate with Robo to coordinate islet development. Expression analysis revealed distinct expression patterns between the three Slit ligands. Slit1 is present in a subset of islet β-cells, from late embryonic stages into adulthood. Slit2 and Slit3 are present in the embryonic pancreatic mesenchyme. Here we show that depletion of mesenchymal Slits results in altered islet morphogenesis, while depletion of endocrine Slit1 has no influence on islet architecture. Based on these results, we suggest that mesenchymal Slits interact with endocrine Robo to coordinate islet morphogenesis. This work was funded by NIH T32 GM-7133-42, and the SciMed GRS Fellowship at UW Madison.

Primary Proliferation Pathways are Repressed by Novel Secretory Cell During Intestinal Development in Zebrafish
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The intestine across vertebrate species shares many similarities in structure and function. From mammals to zebrafish, the intestine is organized into column structures called villi (mammal, bird) or folds (zebrafish) wherein
stem cells (SCs) at the base of these columns called crypts (mammal, bird) or interfold space (zebrafish) are constantly renewing epithelial cells lost to apoptosis at column tips. These SC compartments are also home to secretory cells which aid in regulating proliferation and asymmetric divisions of SCs. Less is known about the structure and cell-cell signaling within the immature intestinal SC niche. We have identified a subset of secretory cells that receive Notch signaling as they differentiate (which we call Notch receiving secretory cells-NRSCs). NRSCs associate with proliferative centers during embryogenesis and the immature post-embryonic intestine suggesting that they may play a role in regulation of epithelial proliferation. Here we disrupt development of NRSCs to determine their role in epithelial proliferation during last half of embryogenesis and the first week of post embryogenesis. Since NRSCs appear to use Notch to differentiate, disruption of Notch signaling should disrupt development of NRSCs and interrupt their ability to function. We find that inhibition of Notch signaling leads to increased proliferation along with increases in proliferation promoting signaling pathways such as Wnt, EGF, and IGF. We propose that NRSCs play a role in downregulation of Wnt, EGF, IGF pathways resulting in downregulation of epithelial proliferation. NRSCs may then be part of a system to prevent intestinal epithelial cells from overproliferating. Funding sourced by the NICHD.

Program Abstract #175
Spatial reconstruction of single-cell transcriptomic data enables systematic dissection of Hox dependent tissue segmentation in the starlet sea anemone Nematostella vectensis
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Comparative analysis using cnidarian model systems provides invaluable insights into key evolutionary questions, such as the origin of Hox genes. Previous studies demonstrated that in the cnidarian Nematostella vectensis, a Hox-Gbx code along the directive axis is instructive to the endodermal tissue segmentation process during larval development. However, the molecular machinery downstream of Nematostella Hox genes remains elusive. To address this, we established Endo-atlas, a 3D gene expression atlas of the developing Nematostella endoderm reconstructed using single cell RNA-seq data. We first developed an enzyme/osmolarity-based endoderm isolation method for planula larvae and obtained around 6,500 high quality endodermal cells using 10X scRNA-seq protocol. Using fluorescent in situ hybridization, we then screened through differentially expressed genes under three Hox mutant backgrounds and identified 51 spatial marker genes. Combining the scRNA-seq dataset with marker gene information, we next utilized novoSpaRc, an optimal transport-based algorithm to reconstruct the spatial expression atlas for all transcripts detectable in the endoderm (Endo-atlas). We systematically validated the accuracy of Endo-atlas predictions and identified two homeobox genes, Lbx and Uncx, that exhibited reciprocal expression patterns within each segment pairs. Lbx marks all BMP-side segment boundaries while Uncx marks all Hox-side boundaries. Mutation of different Hot genes resulted in depletion of specific Lbx/Uncx stripes, indicating the presence of Hox-dependent segment polarity in a bi-radially symmetric cnidarian animal. Taken together, our work highlighted the unexpected complexity of the segmentation process in a cnidarian organism and hinted at the possibility that many conserved segmentation genes are subjected to the regulation of Hox genes in the cnidaria-bilatera common ancestor. This work is funded by Stowers Institute for Medical Research.

Program Abstract #176
Alternative Splicing is Differentially Regulated During Limb Development
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How alterations to gene regulation during development contribute to morphological variation is a key question in the field of evo-devo. The developing limb is an excellent model to investigate this question. The key genes involved in limb pattern formation are highly conserved among taxa, and changes in levels of these key genes appear to have a significant evolutionary role. Alterations to cis-regulatory elements and epigenetic modification have already been shown to underlie differences in limb morphology. However, the role of alternative splicing in
limb development remains unexplored. Alternative splicing is a key mechanism used to control the make-up and/or levels of certain proteins. To investigate the role of alternative splicing during limb development we used publicly available RNAseq data generated by sequencing mammalian limbs at the ridge, bud, and paddle stages of development. We employed the rMATS software to perform pairwise comparisons between the three stages and obtain information about splicing events in terms of Percent Spliced In values. Our results show that alternative splicing patterns are similar in the ridge and paddle, but differ in the bud stage. Notably, the majority of splicing events in bud stage limbs consist of mutually exclusive exon (MXE) inclusion. Furthermore, we find that several members of the cell cycle pathway exhibit bud-specific MXE splicing patterns. In silico analysis suggests that unique protein variants are generated during the bud stage. These protein variants are predicted to have disrupted domains that are important for progression through the cell cycle. Given the important contribution of spatial-temporal regulation of proliferation in the bud stage to limb morphological outcomes, these slicing variants may play an as yet unknown role in limb diversification, and may contribute to limb defects observed in some spliceosomopathies. This work was supported by NIH/NIDCR R15 DE026611.

Program Abstract #177
Kinesin light chain 4 contributes to microtubule regulation and neuronal morphogenesis in zebrafish
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The mechanisms by which neurons develop highly elaborate and polarized morphologies are not well understood. Motor proteins like kinesin-1 are key components in cargo trafficking and microtubule (MT) organization, and thus are crucial for proper neural development. Using CRISPR F0 screening in zebrafish, we identified kinesin light chain 4 (klc4), a kinesin-1 subunit, as a candidate gene in axon morphogenesis. Klc4 is highly conserved and implicated in mitochondrial function and the human neurodegenerative disease hereditary spastic paraplegia. We generated a zebrafish Klc4 mutant line and used custom image-analysis tools to quantify and compare sensory axon arborization in Klc4+/+ and Klc4-/- embryos. We found that Klc4-/- embryos displayed multiple defects in sensory axon development, such as reduced axon branching, altered axon directionality, and abnormal fasciculation. To address whether defects in axon morphology were accompanied by MT defects, we imaged MT plus tips in sensory neurons. We found that Klc4 mutants had higher MT comet frequency and velocity. Finally, we asked whether Klc4 loss impacted neuronal circuit function by assessing behavior of the adult Klc4-/- fish in comparison to their Klc4+/+ cousins. We found that Klc4-/- fish showed a mild reduction in feeding ability and a smaller adult body length (83% of control). In a novel tank test, a common assay for anxiety behavior, 43% of Klc4-/- mutants froze upon introduction to a novel tank, indicative of heightened anxiety, with the majority of mutant animals (80%) failing to explore the tank. These data suggest that Klc4 plays a crucial role in neuronal function and morphogenesis across multiple scales, and highlights the diverse roles of Klcs during development. Funding: NINDS R01NS086934 to M. Halloran and F32NS098689 to E. Haynes

Program Abstract #178
4 Steps to Make Every Neuron in the Human Spinal Cord
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The spinal cord contains billions of neurons, with a diversity of subtypes enabling sensory, proprioceptive, and motor function. However, current human stem cell-based in vitro models and prospective cell transplantation therapies fail to reflect the significant regional specificity of spinal cells. Here we recapitulate the full diversity of spinal cell types along both the rostrocaudal (R/C) and dorsoventral (D/V) axes with chemically defined, scalable protocols using human pluripotent stem cells (hPSCs), enabling us to generate the first comprehensive transcriptomic map of the developing human hindbrain and spinal cord. hPSCs were patterned to six region-specific neuromesodermal populations, directed to neural progenitors, then patterned to D/V fates By providing the appropriate concentration and duration of D/V signaling, we generated tunable ratios of motor neurons (MNs) and locomotor interneurons (INs) from the ventral spinal cord, or proprioceptive INs and sensory INs from the dorsal spinal cord. Cultures with over 95% neuronal yield can be generated in as little as 19 days and mature to form functional neural networks. Single-cell RNA-sequencing of 47,628 cells reveals regionally specified
neurons with discrete Hox gene profiles and representation of all major motor and somatosensory spinal cell types, thereby demonstrating the efficacy of our modular approach. Sub-clustering by cardinal phenotype and Hox profile, we have discovered novel region-specific expression differences which are currently being validated in vivo. We anticipate that access to these cells will advance our understanding of spinal development, elucidate human-specific neuronal populations, shed insight into novel region-specific markers and therapeutic targets, and potentially provide novel clinically relevant populations for cell transplantation. Funded by NIH/NINDS F32 NS106740, EPA-G2013-STAR-L1 grant #83573701, NIH/NCATS, R42-TR001270 and UG3-TR003150.

Program Abstract #179
**Somatosensory axon-dependent development of a novel epidermal cell type in zebrafish.**

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Touch is perhaps our most intimate sense and involves the largest organ of the body—the skin. Touch sensation requires interactions between somatosensory axons and many specialized skin cell types. Because of its amenability to imaging and genetic manipulation, zebrafish are an excellent system to study touch system maturation. To date, most studies of zebrafish touch have focused on the larval skin but, at these stages, the touch system lacks many of its mature, specialized features. In a histological survey of adult zebrafish skin, we identified a previously uncharacterized population of somatosensory axon-associated epidermal cells. We found that these epidermal cells show several hallmarks of Merkel cells, including expression of the transcription factor Atoh1a and elaboration of actin-rich microvilli. Merkel cells are touch-sensing cells required for texture discrimination that have been primarily characterized in mouse. The mechanistic basis of how axons and Merkel cells interact during organogenesis remains poorly understood, partly because these events are difficult to visualize during mammalian embryogenesis. Based on the molecular and cellular similarities of the zebrafish epidermal Atoh1a+ cells (epAtoh1a+) to Merkel cells, we hypothesized that they would also exhibit similar developmental properties, such as a tight association with skin appendage formation. Using genetic reagents to track epAtoh1a+ cells during skin appendage (scale) development, we found that epAtoh1a+ cells rapidly populate the zebrafish trunk during scale formation. Intriguingly, mutants lacking trunk (dorsal root ganglion) somatosensory neurons had significantly fewer epAtoh1a+ cells during scale growth, suggesting their development requires axon-derived signals. In summary, we believe that detailed characterization of epAtoh1a+ cell development will yield insights into the dynamics and conservation of touch system maturation. Funding: University of Washington.

Program Abstract #180
**Sight promotes proliferation and survival of stem and progenitor cells in the optic tectum of zebrafish.**

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Zebrafish, like most anamniotes, exhibit lifelong growth in the retina and its target brain tissue, the optic tectum. The retina connects to the optic tectum (OT) through retinal ganglion cells (RGCs), which project from the inner layer of the retina, cross the midline, and ultimately synapse with neurons in the optic tectum, delivering visual information that will be further processed and integrated with other sensory inputs. Both proliferation and cell death of tectal cells appear to be correlated with retinal innervation, especially in adult animals, indicating that RGC axons provide key information to control stem and progenitor cell behaviors. To determine whether this information is transmitted through electrochemical signals or by growth factors transported along RGC axons, we investigated how the loss of visual input alters stem and progenitor cell behaviors in the optic tectum. We first found that lakrtiz (lak) mutant larvae, which lack RGCs, exhibited significantly fewer proliferating cells in the OT and fewer sox2+ stem cells. Next, we examined the tecta of one-eyed larvae, raised in a typical light-dark cycle, and found that the non-innervated tectal lobes exhibited significantly less proliferation than their innervated counterparts and contained fewer sox2+ stem cells. Importantly, when these same enucleation experiments were performed on one-eyed larvae raised in constant darkness, the statistically significant difference between the number of proliferating cells in the innervated versus non-innervated tectal lobes disappeared. These findings
provide preliminary evidence that the cue supplied by RGC axons to tectal stem and progenitor cells arises from synaptic, visually-evoked activity. This project was funded by the Wellcome Trust (HR, MV, and SWW) and Reed College (OH, CR, EK, RB, and KLC).

Program Abstract #181
Multicolor lineage tracing using in vivo time-lapse imaging reveals coordinated death of clonally related cells in the developing vertebrate brain.
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The global mechanisms that regulate and potentially coordinate cell proliferation & death in developing neural regions are not well understood. In particular, it is not clear how or whether clonal relationships between neural progenitor cells and their progeny influence the growing brain. We have developed an approach using Brainbow in the developing zebrafish to visualize and follow multiple clones of related cells in vivo over time. This allows for clear visualization of many dividing clones of cells, deep in proliferating brain regions. As expected, in addition to undergoing interkinetic nuclear migration and cell division, cells also periodically undergo apoptosis. Interestingly, cell death occurs in a non-random manner: clonally related cells are more likely to die in a progressive fashion than cells from different clones. Multiple members of an individual clone die while neighboring clones appear healthy and continue to divide. Our results suggest that clonal relationships can influence cellular fitness and survival in the developing nervous system, perhaps through a competitive mechanism whereby clones of cells are competing with other clones. Clonal cell competition may help regulate neuronal proliferation in the vertebrate brain. This work was supported by the National Science Foundation (Award 1553764), the M.J. Murdock Charitable Trust, and the John S. Rogers Research Program at Lewis & Clark College.

Program Abstract #182
Tangential nucleus subtypes in the larval zebrafish are specified and organized independently of motoneuron-derived signals
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Vertebrates stabilize gaze using central vestibular brainstem neurons that relay head and body destabilization to motoneurons that counter-rotate the eyes. Precise connectivity between directionally-sensitive vestibular neuron subtypes and particular extraocular motoneurons is crucial for behavior. The developmental mechanisms that specify vestibular neuron identity and connectivity are unresolved, though previous work has suggested a role for motoneuron-derived factors. We tested this hypothesis in the tangential nucleus (TAN) of the larval zebrafish, which contains the excitatory vestibular neurons necessary for vertical gaze stabilization. We first defined how TAN neurons develop in space and time. We observed that after an early mediolateral bias, differentiation in the dorsoventral and rostrocaudal axes proceeds from the outside in. To ask whether functional TAN subtypes are organized along a similar anatomical axis, we used swept, confocally-aligned planar excitation (SCAPE) microscopy and two-photon calcium imaging. We discovered functional organization complementary to spatiotemporal development. Our results are the first demonstration of functional sub-organization within a vestibular nucleus. To investigate the requirement of motoneuron-derived signals for TAN identity specification, we are currently using these functional assays to measure changes in TAN identity following constitutive motoneuron knockouts. Preliminary data suggests that TAN function and organization is preserved in the absence of motoneurons, arguing against a role for motoneuron-derived cues. Taken together, we hypothesize that spatiotemporally-linked factors organize TAN subtypes into dorsoventral and rostrocaudal microdomains. We propose that functional identity acquisition and organization occurs in a motoneuron-independent manner. Funding: NIDCD 5R01DC017489-02
Program Abstract #183

Building a planar signaling system that directs actin protrusion and collective migration of epithelial cells

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Collective cell migrations are common, important tools in the animal morphogenesis toolkit. To crawl in a collective, cells must protrude, adhere, and contract as they would individually, but they also need to coordinate these behaviors with their neighbors to achieve efficient directed motion. The Drosophila egg chamber epithelium serves as a model of cell-cell communication during collective migration. The cells of this epithelium undergo a highly coordinated collective migration along an underlying basement membrane extracellular matrix. The tissue lacks leader cells, and tissue-level polarity emerges from cell-cell interactions across the field. We show that Fat2, a protocadherin that localizes to the trailing edge of each cell, promotes lamellipodial protrusion by recruiting the Wave complex to clusters at the leading edge of the cell behind. Acute disruption of Fat2 signaling randomizes protrusion orientation and rapidly halts migration. While Fat2 is required for migration, continuous migration is also required to maintain Fat2 trailing-edge polarization through an unknown mechanism. Using mathematical modeling, we find support for velocity, and not force, as the migration feature that reinforces Fat2 polarization. By following the restoration of Fat2 polarity and migration after Fat2 signaling disruption, we next seek to identify the mechanism by which velocity restricts Fat2 to the trailing edge. Funding: T32 HD055164 and NIH R01-GM126047.

Program Abstract #184

Ccdc103 promotes proliferation and directed migration in embryonic zebrafish myeloid cells

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New genetic and molecular methods have shed increasing light on the pathobiology of ciliopathies, including Primary ciliary dyskinesia (PCD), a rare genetic disease that presents with chronic respiratory infections, situs inversus and infertility. Interestingly, studies have shown that PCD patient neutrophils, which are not ciliated, have functional defects, including difficulty migrating. Mutations in coiled-coiled domain containing protein 103 (CCDC103), a conserved dynein arm assembly factor that can promote microtubule stability, are associated with multiple independent PCD families. Here, we are the first to show that ccdc103 is expressed in embryonic zebrafish and human myeloid cell populations. Utilizing the zebrafish schmalhans (smh) mutant, an established model for PCD resulting from an early truncation in ccdc103, we find that loss of ccdc103 results in fewer myeloid cells at 24 hours post-fertilization (hpf). Furthermore, embryonic neutrophils and macrophages in smh mutants are unable to migrate efficiently towards wound sites, which is consistent with reports that Ccdc103 stabilizes microtubules. A yeast two-hybrid screen for Ccdc103 interacting proteins identified Spag6, which has also been shown to be critical for cell proliferation and migration and is overexpressed in several myeloid malignancies and myeloproliferative diseases, as a potential interactor. Using bioluminescence resonance energy transfer (BRET) based methods, we show that PCD-associated CCDC103 mutations disrupt the interaction with SPAG6. Engineered spag6 mutant zebrafish embryos also have a decreased number of myeloid cells. Therefore, our results suggest that Ccdc103 complexes with proteins that coordinate myeloid cell proliferation and function through affecting microtubule stability independent of its role in motile cilia, which may explain largely overlooked functional defects observed in PCD patient neutrophils. (Funding: NIH)

Program Abstract #185

Claudin-specific Interactions at the Tight Junction Cytoplasmic Plaque

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Tight junctions are essential to regulate paracellular transport as well as to coordinate morphogenesis during development. Our lab showed that claudins, the main components of tight junctions, are essential for the proper elevation and fusion of the neural folds during neural tube closure. In fact, the removal of Cldn3, -4 and -8 through treatment with the C-terminal domain of Clostridium perfringens enterotoxin (C-CPE) generated open
neural tubes and convergent extension defects in 100% of the embryos treated. Claudins are composed of two extracellular, four transmembrane domains and a cytoplasmic tail. They are known to interact with other claudins in the same or apposing cell using the extracellular or transmembrane domains. On the other hand, the cytoplasmic tail has been proposed to interact with different scaffolding proteins and cytoskeleton. However, a direct experiment has not been performed to identify specific interactors for each claudin. In our study, we used the cytoplasmic C-terminal domains from different claudins to identify claudin-specific interaction partners for Cldn1, -3, -4, -8 and -14 during late neurulation and early organogenesis (Day3 chick embryos). We observed different interaction profiles for the individual claudins. We hypothesize that this reflects the claudin-specific functions and dynamic expression patterns during development. Funding Sources: CIHR, NSERC

Program Abstract #186
Elucidation of GDI2 as a regulator of Rab signaling at the primary cilia
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The primary cilia, a microtubule-derived structure found on almost every cell type, is a signaling hub that is required for normal embryonic development. Mutations specifically to Rab GTPases that normally function at the primary cilia, have been associated with ciliopathies or ciliopathy-related disorders. In addition to Rabs, there are many regulators that are required for controlling Rab activity and trafficking. The importance of Rab signaling for normal cilia function has been established; however, the role of most Rab regulators has yet to be elucidated. Through a bioinformatics algorithm, the Rab regulator, GDP dissociation inhibitor 2 (GDI2), was identified to be involved in cilia signaling. GDI2 is ubiquitously expressed throughout mammalian tissues, has enriched localization to the centriolar satellites in many cell types, and interacts with proteins that are required for normal cilia formation and function. Gdi2 KO embryos are embryonic lethal and display severe vascular abnormalities and craniofacial defects that phenocopy mutations associated with receptors that normally traffic to and signal at the primary cilia. Mutant embryos and mouse embryonic fibroblasts (MEFs) have cilia with comparable frequency and length to wild type indicating that GDI2 is not required for ciliogenesis. However, mutant MEFs have a significant increase in the number of cells with supernumerary cilia. Additionally, mutant MEFs have impaired directional migration, which is a property of primary cilia polarization and orientation. These data led to the central hypothesis that GDI2 regulates cilia-associated Rabs that are required for normal cilia signaling and function. The goals of my project are to evaluate the mechanisms by which GDI2 regulates cilia-specific Rabs, and how this regulation affects ciliary biology. This work is funded by the NIH.

Program Abstract #187
Identifying and characterizing novel components in basement membrane adhesion in C. elegans
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Basement membranes (BMs) are dense extracellular matrices that surround and structurally support tissues. BMs of two adjacent tissues can link together and maintain long-term adhesions. Long-term BM-BM adhesions occur in many vertebrate tissues such as the kidney and lung. Usually, these adhesions link a tissue to neighboring vasculature allowing the exchange of nutrients or waste. Disruption of these adhesions can lead to pathologies such as Alport’s syndrome. Despite the importance of BM adhesions, very little is known about their regulation and maintenance. The Sherwood lab has established a model for investigating these adhesions—the basement membrane linkage (B-LINK). The B-LINK is an adhesion complex that mediates BM-BM adhesion between the BMs of the uterine and epidermal tissues in C. elegans. This work is focused on identifying and characterizing new components of the B-LINK. We have completed a visual screen of BM proteins to identify components that are either enriched at the B-LINK or, upon RNAi-mediated knockdown, exhibit the rupture phenotype indicative of B-LINK failure. Ultimately, we have identified four novel B-LINK components (type IV collagen, fibulin, papillinS, and perlecan) and further characterized a previously identified B-LINK component hemicentin. We have determined the functional role of these B-LINK components to generate a working model of B-LINK development. Broadly, we have discovered that these components are key regulators of BM adhesion, with hemicentin facilitating initial adhesion, and the other components maintaining sustained adhesion. This work aims to expand our knowledge of
BM adhesion failure. This work was supported by the NIH training grant (Pharmacological Sciences Training Grant 5T32GM007105-44) to CAG and R35 (GM118049) to DRS.

Program Abstract #188
Adult hematopoietic stem cell (HSC) clonality is determined by embryonic macrophage sensing of calreticulin on HSCs

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In development, local signals induce hematopoietic stem cell (HSC) emergence, trafficking, and division, and dictate the number of HSC clones that support long-term hematopoiesis. Using a brainbow color barcoding system, we previously found that zebrafish produce 20-30 HSCs in the developing aorta. Using spinning disk confocal microscopy, we imaged runx1+23:mCherry+ HSPCs in the fetal niche and found surprising interactions with mpeg1:GFP+ primitive macrophages. From 48-96 hours post fertilization (hpf), macrophages contacted newly lodged HSPCs, scanned their cell surface for 30-45 minutes, and either took a portion of membrane and cytoplasm or engulfed the entire cell. To evaluate any effect on HSC clonality, we depleted embryonic macrophages in our brainbow labeling system and raised fish to adulthood. Embryonic macrophage depletion via the irf8 morpholino or clodronate liposomes significantly reduced the number of HSC clones present in adulthood with an average of 14 clones vs 24.6 in sibling controls (p = 0.0002). To better characterize the interaction between niche macrophages and HSPCs we performed single-cell RNA sequencing and few-cell proteomics. Proteomic analysis identified 166 peptides enriched in macrophages which had recently engaged HSPCs, including three isoforms of calreticulin (calr). Normally ER bound, calr may become surface bound during stress, where it has been reported as a ‘come-eat-me’ signal. Antibody staining confirms surface calr on HSPCs and scRNA-seq identifies the canonical calr receptor in recently-engaged macrophages. Morpholino knockdown of calr reduces prevalence of macrophage-HSPC interaction from 29% to 13% (p = 0.0058). Together, our data support a model in which the environmental stimuli that induce HSCs also result in variable surface display of calr, leading to either macrophage grooming or phagocytosis, which ultimately regulates HSC clonality. This work is supported by an F31 NRSA fellowship from the NHLBI of the NIH.

Program Abstract #189
Understanding the Mechanisms of Cornea Wound Healing using lineage tracing in a model of Stem Cell Deficiency in the frog, Xenopus

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Corneal Epithelial Stem Cells (CESCs) are responsible for cornea homeostasis and wound healing. Damage or loss of these CESCs results in Limbal Stem Cell Deficiency (LSCD), a clinical condition that leads to blindness. Therapies involving epithelial stem cell transplants have been developed to treat LSCD; however, an understanding of how these cells heal wounds or how transplanted CESCs restore vision in LSCD patients is still lacking. To address these gaps, we have developed a CESC deficiency model in the frog Xenopus using Psoralen-UV treatment (Adil et al., 2019, Exp Eye Res, 187, 107767). This frog model recapitulates all the hallmarks of LSCD including increased opacity, neovascularization, and pigment cell invasion from the periphery. To study the response of CESC, we can localize PUV damage by treating only one half of the cornea. These cases initially develop localized stem cell deficiency characteristics on the treated side. Unlike whole treated corneas, the treated half is ultimately able to recover and corneal transparency is restored. To trace the behavior of cornea cells during wound healing and repair, we performed these localized treatments in transgenic frogs carrying a heat-shock inducible H2B-mCherry fluorescent protein. A small group of cells can be labeled using localized heating. We have followed labeled cells as they invade the treated half of the cornea. Labeled cells in the untreated half of the cornea are displaced towards the treated side quickly, within 1 day post treatment; whereas in untreated control corneas, cells labeled near the outer periphery normally move more slowly towards the central region of the cornea. Studying CESC and stem cell deficiency in our Xenopus model provides insights into the response of cornea cells following stem...
cell depletion, and will be valuable in understanding the behavior of transplanted CESCs, leading to progress in LSCD therapeutics and wound healing. (NIH grant: 1R01EY023979)

Program Abstract #190
Skeletal stem cells are maintained in a Wnt-inhibitory environment within the resting zone of the epiphyseal growth plate
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Chondrocytes in the resting zone of the postnatal growth plate are characterized by their slow-cycling nature, including a small population of skeletal stem cells that continue to form columnar chondrocytes, osteoblasts and bone marrow stromal cells. Slow-cycling chondrocytes express parathyroid hormone-related protein (PTHrP), which constitutes the PTHrP-indian hedgehog (Ihh) negative feedback loop essential for maintaining the growth plate. However, the molecular mechanisms underlying how resting chondrocytes differentiate into proliferating, columnar chondrocytes remain unknown. Here, we utilized a genetic pulse-chase approach to isolate slow cycling chondrocytes (LRCs) from the postnatal growth plate resting zone using a chondrocyte-specific doxycycline-controllable Tet-Off system that regulates expression of histone-linked GFP. An RNA sequencing transcriptomic analysis of LRCs identified enrichment of Wnt inhibitors in these cells. To define functional roles of Wnt signaling on LRCs and their differentiation into columnar chondrocytes, we activated Wnt/B-catenin signaling in PTHrP+ resting chondrocytes by conditionally inducing haploinsufficiency of Adenomatous polyposis coli (Apc), a critical component of the B-catenin degradation complex, using a PTHrP-creER line and an Apc-floxed allele in combination with a tdTomato reporter allele allowing cell-lineage analyses. After 3 months of chase at P96, Apc-haploinsufficient PTHrP+ resting chondrocytes established long columnar chondrocytes (composed of >10 cells) less effectively than their controls, indicating that Wnt/B-catenin activation impairs long-term self-renewal of PTHrP+ skeletal stem cells. These findings reveal that slow-cycling chondrocytes are maintained in a canonical Wnt-inhibitory environment within the resting zone, unraveling a novel mechanism regulating self-renewal and differentiation of PTHrP-positive skeletal stem cells of the postnatal growth plate. This work was funded by NIDCR R01DE026666.

Program Abstract #191
Hedgehog Signaling Regulates Cell Dynamics in Zebrafish Scale Regeneration
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Understanding the molecular mechanisms controlling organ and tissue size remains a fundamental open question in developmental biology. To investigate the molecular mechanisms regulating tissue growth, we have developed a system for live imaging regenerating zebrafish scales. Scales are made up of osteoblast monolayers surrounding a bone matrix of collagen and mineral crystals. Zebrafish scales are frequently lost and regrown due to environmental insult (such as bumping into other fish) and can be easily removed by plucking, providing a simple way to prompt regeneration. Using single cell resolution live imaging, we have found that osteoblasts differentiate and proliferate early on in regeneration, but the pathways regulating this remain unknown. Recent studies have shown that the Hedgehog pathway is active in osteoblasts and in the surrounding skin during scale regeneration. We’ve confirmed that Hedgehog signaling is required for scale regeneration and that inhibiting this pathway leads to a reduction in cell proliferation and scale growth. Furthermore, Hedgehog inhibition caused a marked change in scale morphology, including a decrease in radii cells and a loss of proliferative marginal osteoblasts in the posterior of the scale. Our data suggest that Hedgehog signaling is an important regulator of cell dynamics during scale regeneration. This work was supported by a Whitehead Faculty Scholar Award and N.I.H. grant R01-AR076342.

Program Abstract #192
Non-conventional allogeneic response in chimeric planarians causes regeneration defects
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Recent progress has revealed the presence of allogeneic response to nonself tissues in some colonial marine invertebrates. However, it remains unclear how widespread these observations are as this response is mediated by species specific mechanisms and may evolve independently to maintain individual clonality. While unitary animals do not face invasion by another individual, they can encounter threats from within. Many unitary invertebrates reproduce asexually through fragmentation followed by regeneration, often using multipotent stem cells that are capable of continually dividing and differentiating to rebuild any missing body parts. This process is susceptible to accumulation of somatic mutations and emergence of cheating cells, which may over-proliferate and propagate across generations, neglecting their duties in making somatic tissues. Despite this obvious risk, it is unknown whether these animals have mechanisms to sense the presence of genetically altered cells, and if so, whether the regeneration process is affected by allogeneic response. Planarian flatworms provide a powerful model as they are capable of unparalleled regeneration as well as forming chimeric fusions when tissue fragments are surgically stitched together. By creating chimeras comprising distinct Schmidtea mediterranea genotypes, we found that, while the fused tissue fragments integrated anatomically, the two genotypes remained spatially separate, revealing a non-conventional allogeneic response. To evaluate its influence on regeneration, we amputated the chimeras along the anterior-posterior axis and found that the tail piece failed to regenerate a new head. This regeneration defect can be rescued by knocking down activin or beta-catenin. Our work reveals the link between allogeneic response and regeneration program and pinpoints the underlying molecular regulators.

Ectopic kcnh2a slows niche-to-mesenchyme transitions to prolong fin outgrowth in longfin zebrafish
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Adult zebrafish fins regenerate to their original form irrespective of the extent or type of damage, providing a striking example of organ size control. Current models speculate fin cells maintain grid-like “positional identities” that instruct differential outgrowth after injury. We propose an alternative “Transpositional Scaling” model for robust fin size restoration whereby the positional information is stored in skeletal geometry. We show the amount of Wnt produced by a pool of distal “niche” cells determines the extent of fin outgrowth. The niche forms from mesenchyme populating the inside of the eighteen differentially sized and tapered bony rays. The niche, and therefore Wnt production, then gradually depletes by the net re-differentiation of niche cells back into a mesenchymal cell state. Regenerated ray lengths become a function of ray widths at the injury position with the skeletal geometry inherently restored by the depleting niche. Aided by mathematical modeling, we show longfin<sup>12</sup> zebrafish regenerate extraordinarily long fins due to a broken niche “countdown timer”. We determine the longfin<sup>12</sup> phenotype is caused by cis-ectopic expression of the kcnh2a voltage-gated potassium channel. kcnh2a expression is ectopically expressed in longfin<sup>12</sup> intra-ray mesenchyme, where it likely disrupts activity of the Ca<sup>2+</sup>-dependent phosphatase calcineurin. We use blastula transplantations to show longfin<sup>12</sup>-ectopic kcnh2a expression acts autonomously within the intra-ray mesenchyme/niche lineage. We propose skeletal geometry and niche-to-mesenchymal cell state transitions promoted by membrane depolarization and Ca<sup>2+</sup>/ calcineurin signaling restore zebrafish fin size and shape. We acknowledge support from the NIAMS (5R03AR067522, 5F31AR071283), NIGMS (1R01GM127761) and NICHD (5T32HD007348).

Origin, function and evolution of pharyngeal arch signalling centres in jawed vertebrates
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The pharyngeal arches in vertebrate embryos give rise to much of the craniofacial skeleton. Pharyngeal epithelial signalling centres, such as the gill arch epithelial ridge (GAER) of cartilaginous fishes and the posterior ectodermal
margin (PEM) of bony fishes direct the development of pharyngeal arch derivatives. However, the homology and ancestral skeletal patterning function of these signalling centres remain unresolved. Here, we aim to determine the origin and ancestral nature of jawed vertebrate pharyngeal arch signalling centres, using comparative lineage tracing, gene expression and functional analyses in skate (*Leucoraja erinacea*) and chick (*Gallus gallus*) embryos. We find that the GAER and PEM have common pharyngeal endoderm origin and, using multiplexed fluorescent mRNA *in situ* hybridisation, they share co-expression of *Shh* and *Fgf8* in similar patterns through development. We previously discovered that *Shh* signalling from the GAER is required for gill arch skeletal proliferation in skate, and now show, using in ovo pharmacological experiments, a similar requirement for *SHH* signalling in inducing components of the chick hyoid arch endoskeleton. Our findings indicate that the last common ancestor of jawed vertebrates possessed one or more endodermally-derived pharyngeal arch signalling centres, which co-expressed *Shh* and *Fgf8* and functioned as an upstream regulator of skeletogenesis. Funding provided by the Wellcome Trust.

**Program Abstract #195**
The fast and furious hypertrophy of *Xenopus* head cartilages

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Hypertrophic chondrocytes (enlarged cartilage cells) are one of the more obvious morphological features of endochondral ossification, a process by which bone forms indirectly via a cartilage template. Common vertebrate models, including mouse, chick, and zebrafish, basically undergo the same histological and molecular progressions during hypertrophy. Therefore, we hypothesized that amphibian cartilages underwent this standard hypertrophic cascade. To test this, we analyzed histological and molecular markers of developing limb and head cartilages of *Xenopus tropicalis*. Indeed, the standard histological pattern occurred during humeral development, where chondrocytes gradually became hypertrophic prior to ossification. Consistent with the standard molecular cascade, the chondrogenic markers *col2a1* and *sox9* were down-regulated in hypertrophic chondrocytes of the humerus. Interestingly, however, expression of the classical hypertrophic marker *col10a1* was not up-regulated in these chondrocytes. Unexpectedly, many head cartilages, such as the ceratohyal, a homologous element to the laryngeal hyoid bone, underwent rapid histological hypertrophy within mere hours of becoming chondrocytes. In addition, the ceratohyal halted at hypertrophy and persisted as such throughout metamorphosis into adulthood, never ossifying; Meckel’s cartilage and the palatoquadrate displayed similar behavior. Unlike the humerus, hypertrophic chondrocytes in the head cartilages continued to express relatively high levels of *col2a1* and *sox9*, but similarly lacked *col10a1* expression. As such, these unusual discoveries present a unique opportunity to investigate the molecular mechanisms that might explain why frog cartilages deviate from the standard histological and molecular hypertrophic cascade of other vertebrates. Such findings might shed light upon osteoarthritic cartilage, which often has ectopic hypertrophic differentiation. Funding was provided by the College of Medicine at the U of S and NSERC.

**Program Abstract #196**
Clues of multichambered heart evolution are revealed through analysis of zebrafish hearts with adaptive remodeling of the sinus venosus

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The mechanisms underlying the evolutionary transition from the single chambered heart observed in ancestral chordates to the two-chambered pump exhibited by basal vertebrates remain a mystery. Here, we use zebrafish mutants harboring a novel hypomorphic *nr2f1a* allele (*nr2f1aaco*) that can survive to adulthood despite embryonic heart defects. Adult *nr2f1aaco* hearts exhibit enlarged ventricles and overtly malformed “atria.” However, histological and marker analysis showed that *nr2f1aaco* mutants essentially lack a true atrium. Instead, it has been replaced by a chamber with thickened, collagenous tissue that RNA-seq indicated is the sinus venosus (SV), suggesting the SV has undergone adaptive remodeling in the absence of an atrium. Surprisingly, our transcriptomic analysis of all the cardiac chambers coupled with subsequent lineage tracing and reporter analyses of smooth muscle and neural crest also highlighted previously unknown molecular and cellular similarity between
the wild-type (WT) bulbus arteriosus (BA) and SV. Furthermore, principal component analysis indicated that the transcriptional signature of the nr2f1aaco mutant SV becomes even more similar to the thick-walled WT BA. We posited that aberrant blood flow in nr2f1aaco hearts may cause a pressure increase within the SV that elicits the adaptive remodeling. Indeed, echocardiography showed retrograde blood flow in mutant hearts and that prolonged treatment with a vasodilator mitigated this response. Altogether, we find nr2f1aaco hearts come to resemble a single-chambered pump, reminiscent of the bidirectional hearts found in urochordates, except they are flanked by two elastic vessels. Our examination of mechanisms promoting adaptive remodeling of the SV in response to changes in hemodynamic forces highlights unexpected similarity between arterial and venous poles of the zebrafish heart, which hints at anatomical characteristics of the basal heart during the evolution to a multi-chamber heart. Funding: NIH

Program Abstract #197
Comparative transcriptomics reveals the gene regulatory network driving osteoblast differentiation and highlights differences between bones of tetrapods and fish
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Defining the gene regulatory network (GRN) underlying osteoblast development is key to understanding the differentiation and even origins of this skeletal cell type, but current knowledge is limited to a few candidate genes. Here, we use an unbiased approach to reveal for the first time the GRN driving bone formation and make an evolutionary comparison between bones of distinct clades. Previous molecular studies have identified differences in gene expression between tetrapod and fish osteoblasts. For instance, ‘chondrogenic genes’ including sox9, col2a1, and col10a1 are expressed in fish developing bones, but not in tetrapods. To test the hypothesis that fish osteoblasts are more ‘chondrogenic’ than tetrapod osteoblasts, we used laser capture microdissection (LCM) coupled with RNA-seq. Osteoblasts were isolated from the dentary bone, a homologous element between mouse, chick, and gar. Comparative transcriptomics revealed that osteoblasts of mouse and chick shared more genes and had less differentially expressed genes compared to gar. Furthermore, model-based clustering analysis revealed three clusters in which osteoblast gene expression in mouse and chick was similar and statistically different from gar. Importantly, one cluster showed higher expression in the gar osteoblasts and included the hallmark mature chondrocyte gene col10a1. Finally, we focused on conserved osteoblast genes between the three clades. We identified the core set of genes driving osteoblast differentiation and summarized the known regulatory interactions into a GRN. Tetrapod and gar osteoblasts shared 67% of the total number of genes, including the master osteoblast regulator Runx2. Comparative transcriptomics combined with cis-regulatory analysis will contribute in expanding and verifying this GRN in order to have a more comprehensive understanding of the evolutionary origin of the osteoblast. Funding: NSERC

Program Abstract #198
Novel roles of Pax6 transcription factor gene eyeless in the developing Tribolium compound eye
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The Pax6 transcription factor family plays critical upstream roles in the regulation of eye development in bilaterian animals. Key insights into this process have been gained in Drosophila, which possesses two Pax6 genes: eyeless (ey) and twin of eyeless (toy). Initially broadly expressed in the eye-antennal imaginal disc, ey is specifically required for the specification of the Drosophila compound eye precursor cell population. No evidence has been reported that Drosophila Pax6 genes play roles in lens formation or pigment cell development, which, however, is the case in vertebrates. Here we present evidence from staged knockdown experiments in the pupa of the red flour beetle Tribolium castaneum, which reveal that ey is required for the normal development of secondary pigment cells during retinal differentiation by regulating the transcription factor escargot to maintain the competence of the precursor pigment cells. Moreover, we find that late pupal ey expression in the periphery of the retina is essential for the formation of the massive melanized cuticle frame (ocular diaphragm), which
encloses the mature compound eye of *Tribolium*. Given that *ey* is also essential for the specification of the compound eye primordium in *Tribolium* as in *Drosophila*, we hypothesize that *ey* acts as a competence factor during early and late cell fate acquisition events in and around the *Tribolium* compound eye.

Program Abstract #199
Comparative Study of Retinal Development in the Mouse and Thirteen-lined Ground Squirrel

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Many diurnal vertebrate animals have a greater number of cone photoreceptors in the retina, to allow for sufficient visual acuity in the daytime. Nocturnal vertebrates often have fewer cone photoreceptors and more rods (Walls, 1942). Variation in the relative numbers of other cell types such as horizontal cells (HC) or retinal ganglion cells (RGC) have also been observed. Comparative studies of retinal development can be used to determine the role of gene regulation in the varying compositions of vertebrate retinas. Defining gene regulatory mechanisms involved in generating retinae enriched for one neuronal cell type over another may also reveal mechanisms for retinal cell fate specification. Mouse models have been powerful tools for studying retinal cell fate in a rod-dominant model. Genetically manipulated cone-enriched mouse models have been used to study cone fate choice (Mears et al., 2001) but it has been shown that these are insufficient to identify many cone-specific genes during embryonic retinal development (Buenaustrella et al., 2019). The thirteen-lined ground squirrel (GS) retina is an attractive model for studying the development of early-born retinal cell types as it is cone-dominant and abundant in HCs and RGCs. The adult GS retina has been well-characterized and a GS genome assembly is available. Here, we present the first developmental characterization of the thirteen-lined ground squirrel retina and a comparative ATAC-seq analysis between GS and mouse retinae. We use immunohistochemistry to generate a timeline of GS retinal development. This work reveals postnatal migration of HCs and an expanded duration for restricted RPCs biased to producing cones and HC. Our comparative ATAC-seq analysis identified multiple squirrel-enriched and mouse-enriched accessible chromatin regions as strong candidates for regulating the cone and/or HC fates. Funding: NSF CAREER 1453044, Sloan Foundation (Junior Faculty Research Award in Science and Engineering)

Program Abstract #200
Investigating the role of *myc* in clonal fitness in the developing zebrafish hindbrain

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Radial glia are a class of neural stem cells crucial for proper brain development in vertebrates. During embryonic development, radial glial cells divide to produce the majority of neurons that will populate the brain. A recent study (Brockway et al., 2019) observed rare occurrences of coordinated cell death within radial clones, whereby mother cells and their progeny die one after the other over the course of several hours. One possible explanation for this phenomenon is competition, in which clones have different fitness and compete for survival. Cell competition has been widely studied in *Drosophila* as one of the mechanisms to regulate organ size. However, little is known about whether competition exists in the brain. In past studies, it has been shown that one of the determining factors of cellular/clonal fitness is the transcription factor c-myc. This study aimed to test whether c-myc influences clonal fitness and survival. Using a transient expression approach, we induced mosaic expression of c-myc and Brainbow lineage-labeling to test the clonal competition model in radial clones in the zebrafish hindbrain during early embryonic development. My results showed some evidence that clones with higher c-myc expression underwent more rapid proliferation than their wild-type neighbors, suggesting that c-myc promotes clonal fitness and survival. However, decreasing c-myc activity via expression of a dominant-negative c-myc did not affect clonal survival. Further studies on other competition factors and the effects on clonal development are needed to test the clonal competition model in the brain. Funding sources: National Science Foundation, M.J. Murdock Charitable Trust, Lewis & Clark John S. Rogers Science Research Program.
Program Abstract #201
Studying meningeal development using the zebrafish
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The meninges are a complex connective tissue that surrounds the Central Nervous System, protecting it from mechanical shock, supporting brain buoyancy, and maintaining brain homeostasis. Despite its critical roles, the anatomy, molecular identity, developmental origins, and functional properties of this tissue and its resident cell types remain poorly characterized. We are using the zebrafish to carry out a comprehensive anatomical, molecular, and genetic characterization of the meninges and their cellular constituents. Using histology, electron microscopy, and super-resolution confocal imaging, we find that the adult zebrafish meninges are a complex highly vascularized layered tissue containing several unusual cell types. Using single-cell RNA-seq from dissected meninges, we are profiling these cells and correlating our expression and anatomical data to define the morphological and molecular identities and interrelationships between the different meningeal cell populations. Our studies thus far have confirmed that “Fluorescent Granular Perithelial” cells (FGPs), a novel macrophage-like scavenger perivascular cell population, reside within the inner leptomeningeal layer, where they clear waste from the cerebrospinal fluid. In addition to FGPs, we have also identified a meningeal cell population expressing high levels of ependymin (epd), a meningeal cerebrospinal fluid glycoprotein with a poorly understood function, as well as distinct pigment cell populations that reside within the meninges. Our ongoing research uses the powerful tools and methods available in the fish to study these and other meningeal cell populations in detail in intact, living adult animals. Together, our ongoing studies using these tools and methods are facilitating comprehensive understanding of meningeal development and function in health and disease. This work is supported by an NICHD K99 Pathway to Independence Award (to MVG), and the NICHD Intramural Program of the NIH (to BMW).

Program Abstract #202
Identification of a novel neuronal signal that regulates blood-brain barrier development
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The blood-brain barrier (BBB) maintains a tightly controlled homeostatic environment in the brain that is required for proper neuronal function. The BBB is created by the unique properties of the brain vasculature, composed of a single layer of non-fenestrated endothelial cells with specialized tight junction complexes and reduced rates of transcytosis. However, these restrictive BBB properties are not intrinsic to brain endothelial cells, but instead are induced by signals from the embryonic brain microenvironment. What these signals are and which cells produce them remain poorly understood. Zebrafish have recently emerged as a powerful genetic tool to study the BBB in vivo. We recently characterized the spatio-temporal profile of BBB functional development in zebrafish, revealing a posterior to anterior gradient of BBB function due to the suppression of endothelial transcytosis. We also identified a novel mutant with increased barrier permeability throughout the forebrain and midbrain. We mapped this leaky phenotype to a missense mutation in the secreted chimeric proteoglycan Spock1. Using CRISPR mutagenesis, we validated that loss of the Spock1 results in increased BBB permeability, both in larvae and adult fish. Interestingly, spock1 is expressed by developing neurons throughout the central nervous system, but not by endothelial cells, indicating that we have identified the first BBB inducing signal from the brain microenvironment. Using electron microscopy, we then determined that the increased BBB permeability arises predominantly from an increase in endothelial transcytosis of both clathrin-independent small vesicles and macropinosomes. Furthermore, when we assessed BBB function by tracer leakage assays in embryonic Spock1−/− mice, we also observed increased leakage in the brain parenchyma of knockout embryos, suggesting a conserved role for the neuronal signal in establishing vertebrate BBB function during embryogenesis. NO was funded by Damon Runyon.

Program Abstract #203
The role of calcium activity in embryonic neural development
Rithvik Nalamalapu, Sudip Paudel, Margaret Saha
Calcium activity, including calcium spikes and waves, regulate a wide array of physiological processes. Although most intensively studied in adult neurons, calcium activity is also essential in development. While calcium waves have been correlated with cell movement, the dynamics and role of spontaneous calcium spikes, however, are poorly understood. This early spiking activity which occurs in embryos of multiple species is relatively unexplored largely due to the apparently irregular patterns of calcium activity in individual cells and a lack of comprehensive analysis techniques at the cellular level in vivo. Here we attempt to determine if there is a spatially conserved pattern of calcium spikes during early embryonic neural development. To this end, we devised a method to analyze the spatiotemporal patterns of calcium activity through the construction of a composite neural plate. We first imaged calcium activity in *Xenopus laevis* embryos at the neural plate stage with the genetically encoded calcium indicator GCaMP6m using confocal microscopy. We then segmented and tracked the cells' movements and calcium activity over time using multiple thresholds and merged all the thresholds into a single image using a custom-made program in R. This allowed us to obtain higher quality data, with more accurate segmentation and a greater number of tracked cells, than any individual threshold could provide. Next, we constructed a composite neural plate integrating all our time-lapse images using a pan-neural marker gene, Sox2. Our preliminary results suggest that this methodology can be used to study spatiotemporal patterns of a variety of different physiological processes across multiple tissues in vivo.

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Program Abstract #204

**Development of a method to analyze spatiotemporal pattern of calcium activity in vivo in the neural plate of Xenopus**

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Influxes of calcium ions (Ca^{2+}) form the basis of calcium signaling. It regulates various biological processes, including neurotransmission. However, its role during early neural development is less known. For instance, it is not known if there is a stereotypical pattern of spiking activity in embryos during early neural development. This is due to two main reasons. First, unlike mature neurons, early neurons lack a stereotypical periodic activity and second, there is a general lack of comprehensive analysis techniques at cellular level in vivo. Furthermore, while it is a challenge to obtain high throughput activity measurements from a tissue such as early neurons, where cells are undergoing division, intercalation, and migration, tissue imaging is a standard technique to map cellular Ca^{2+} activity. In this technique, an image is segmented into multiple regions of interest (ROIs) often using a threshold (TH) intensity value. This strategy is not optimal especially when detecting early neurons over time. To overcome this issue, and to study the spatiotemporal spiking pattern of early neurons, we devised a method to employ multiple THs when segmenting an image, track all the ROIs, and merge them into a single time series that corresponds to a cell. As a case study, we used six different THs to segment images that were acquired by imaging *Xenopus* neural plate in vivo. We then tracked Ca^{2+} activity of each ROI for at least 5 minutes up to an hour and merged all the THs using biologically relevant parameters. This resulted in more accurate segmentation and a greater number of cells that were tracked for a longer duration of time than any individual TH could provide. This method, in general, can be applied to obtain high-throughput spatiotemporal measurements of various cellular attributes that require tissue imaging and proper identification and tracking of cells over time. Funding: Grants, NSF 1257895, and NIH 1R15NS067566-01, 1R15HD077624-01 and 1R15HD096415-01, to MSS

Program Abstract #205

**Embryonic Alcohol Exposure Impacts the Progenitors of the Precerebellar Mossy Fiber Neurons**

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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 impaired gross motor control. Studies investigating the loss of motor coordination associated with FAS 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 (which 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 precerebellar neurons (PCN) is due to a primary effect of alcohol on PCN development or to a secondary failure of these neurons to properly establish synaptic connections with cells in the cerebellum. Our lab has shown that chronic low dose alcohol exposure results in abnormalities in the mossy fiber (MF) projecting neurons of the precerebellar system that function to relay inputs to cerebellar granule cells. Development of the climbing fiber projecting neurons of the precerebellar system (that function to relay inputs to cerebellar Purkinje cells) appears grossly normal. The observed abnormalities in MF neurons can be traced back to their origins from progenitor cells within the lower rhombic lip (LRL) and correlate with abnormal expression of BMP responsive genes. Non-BMP responsive gene expression remain largely normal. These studies suggest that alcohol affects the development of MF projecting precerebellar neurons through changes in BMP signaling in the LRL.

Program Abstract #206
Elucidating the BMP heterodimer signaling mechanism
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Bone Morphogenetic Protein (BMP) heterodimers signal more efficiently than BMP homodimers in a variety of signaling contexts, including stem cell differentiation, bone regenerative therapies, and the dorsoventral (DV) axial patterning of many animals including Drosophila and zebrafish. When signaling, a dimeric BMP ligand assembles a receptor complex composed of two type I and two type II receptors. Type II receptors phosphorylate and activate type I receptors, which in turn phosphorylate Smad proteins. Phosphorylated Smad then regulates gene expression. The mechanistic differences between heterodimer and homodimer signaling that elicit more efficient heterodimer signaling remain uncharacterized. The zebrafish embryo is an excellent system to investigate BMP heterodimer signaling, as the BMP2/7 heterodimer is the only ligand that signals in DV patterning. We previously found that BMP2/7 owes this competence to its unique ability to recruit two distinct type I receptors, Bmpr1 and Acvr1, into the same signaling complex, as Bmpr1 preferentially binds the BMP2 ligand, and Acvr1 binds BMP7. We have since found that kinase dead Acvr1 cannot rescue acvr1 deficiency, while, surprisingly, kinase dead Bmpr1 can rescue bmpr1 deficiency. To further explore this difference in function, I have developed GFP-tagged versions of both Acvr1 and Bmpr1 and I have observed differential localization within developing zebrafish embryos. Just as the heterodimer recruits two distinct type I receptors, it may also recruit two distinct type II receptors. I have explored the role of the type II receptors Bmpr2 and Acvr2 in zebrafish development, generating the first complete knockout of all four acvr2 receptor genes in the zebrafish. These mutants show disruption of both Nodal and BMP signaling, demonstrating the importance of this receptor class to both signaling pathways. Funded by NIH R35-GM131908 (MCM).

Program Abstract #207
Neuronal ribosomal protein function regulates Drosophila growth and development
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Stimulation of ribosome biogenesis is a conserved mechanism of growth control. Studies, in yeast and cell culture, have shown how ribosome synthesis controls cellular growth. However, less is known about how ribosome synthesis promotes body growth and development. We have been studying this using Drosophila Minutes, a class of dominant ribosomal protein mutants that exhibit a characteristic delay in development – a phenotype classically thought to be a result of reduced ribosome numbers and protein synthesis. However, several Minutes show that this phenotype is not due to a global decrease in either ribosome numbers or reduced translation. We further investigated one Minute, rps13+/+. We looked at ecdysone biosynthesis in these animals, a steroid hormone responsible for coordinating various developmental events including developmental termination. We saw that genes required for its synthesis had delayed expression. Ecdysone feeding partially reversed the delay in development, therefore we postulated that these effects might reflect a specific role for S13 in the
neuroendocrine circuit (prothoracic gland (PG) and CNS) required to synthesize ecdysone. To test this, we used the GAL4/UAS system to see if tissue selective expression of S13 could rescue the delayed development seen in rps13/+ animals. We discovered that expression of S13 in serotonergic (5-HT) neurons lead to a ~35% rescue in timing delay. Three pairs of 5-HT innervate the PG to control ecdysone release, and S13 expression in these neurons also partially rescues developmental delay in rpS13/+ animals. While axonal projections of 5-HT neurons into the PG do not appear to be affected, electrical activation of rpS13/+ animals with TRPA1 channel expression partially rescues adult eclosion delay. Our model suggests that S13 is required for proper synaptic activity in the serotonergic neurons that signal to the PG to produce ecdysone and a reduction in S13 in these neurons leads to delayed development. CIHR

Program Abstract #208
Sp5 acts downstream of Wnt8-Fzl5/8-JNK signaling during positioning of ANE in sea urchin embryos
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A posterior-to-anterior gradient of Wnt signaling plays a fundamental role in anterior-posterior (AP) axis formation in most metazoan species. We have recently discovered that canonical Wnt/β-catenin signaling as well as two non-canonical Wnt signaling pathways (Wnt16-Frizzled1/2/7-PKC and Wnt1/8-Frizzled5/8-JNK) work together as an interconnected Wnt signaling network responsible for specifying and patterning the early AP axis in the sea urchin embryo. Our lab has previously shown that Wnt1/Wnt8-Fzl5/8-JNK signaling plays a critical role in down-regulating the initially broadly expressed anterior neuroectoderm (ANE) gene regulatory network (GRN) in the equatorial ectoderm cells of the embryo. Here, we performed high-throughput whole-transcriptome differential screens to identify Wnt signaling components and transcription factors regulated by Wnt1/Wnt8-Fzl5/8-JNK signaling. From our RNA-seq and qPCR data we observed that several transcription factors including Sp5, a conserved zinc figure transcription factor possibly working downstream of Wnt1/Wnt8-Fzl5/8-JNK signaling, are down-regulated in Fzl5/8, knockdown embryos. Our initial analysis focused on Sp5, an essential transcription factor for early AP neuroectoderm patterning in vertebrate species such as zebrafish. Our results indicate that, similar to vertebrates, Sp5 might work downstream of the Wnt8-Fzl5/8-JNK signaling during AP patterning of the sea urchin embryo. Taken together, our data support that Sp5 is an essential transcription factor for positioning the ANE territory around the anterior pole in deuterostome embryos. This research was supported in part by funding from the Academic Research Enhancement Award (R15) of the National Institute of Health (NICHD) [grant number 1R15HD088272-01], as well as startup funding from the Department of Biological Sciences at Auburn University.

Program Abstract #209
The IL2 sensory neurons regulate C. elegans peroxide resistance in a daf-16-independent manner
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Oxidative stress is associated with a wide variety of age-dependent and chronic human diseases, including diabetes, neurodegenerative diseases and cancers. Cells rely on highly conserved defense mechanisms to prevent and repair that damage induced by reactive oxygen species (ROS). We are using the nematode C. elegans as a tractable model to study how multicellular organisms cope with specific types of ROS. Previously, by screening a collection of neuron-ablation strains, we identified ten classes of sensory neurons that regulate the animal’s resistance to peroxides in the environment. We found that the two ASI sensory neurons send inhibitory signals to target tissues to control the animal’s peroxide resistance, which requires the transcription factors DAF-16/FOXO and SKN-1/NRF. We are interested in understating whether other sensory neurons regulate peroxide resistance via the same mechanisms regulated by ASI. We are systematically knocking down the expression of daf-16 via RNA interference to identify which neurons regulate peroxide resistance via daf-16-dependent and daf-16-independent mechanisms. So far, we have found that genetic ablation of the IL2 sensory neurons increases peroxide resistance in a daf-16-independent manner. These studies will reveal the extent to which sensory neurons cooperate to regulate peroxide resistance. This work was supported by an NSF CAREER award to Javier Apfeld.
Program Abstract #210

Wnt pathway regulation of gastrulation and segmentation in penaeid shrimp

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Penaeid shrimp gastrulation occurs by mesendoblast (ME) ingression followed by naupliar mesoderm (NM) invagination. Segmentation begins with formation of the two antennal and one mandibular segment, with limb formation. Larval growth occurs by segmental addition at the posterior. To study the role of Wnt signaling in penaeid shrimp development, we screened genome and transcriptome databases for Wnt pathway components frizzled (fz), secreted frizzled-related protein (sFRP), and dishevelled (dsh) in Penaeus monodon, P. (Marsupenaeus) japonicus, and P. (Litopenaeus) vannamei. We found that penaeid shrimp had at least four fz genes (fz1/2/3, fz4, fz5/8, fz9/10), two sFRP genes (sfrp1/2/5 and sfrp3/4), and one dsh gene. The splicing structure of these genes was compared to other arthropods. The number of exons and introns of these genes was conserved among shrimp species. From RNA-seq data, we found strong expression of fz5/8 and dsh during gastrulation, indicating a likely function during this stage. The other shrimp fz and sFRP genes were expressed later in embryogenesis or during larval development. Only Fz4 lacked the Dsh binding KTXXXW motif. sFRP3/4 had the Wnt binding N-glycosylation site while sFRP1/2/5 lacked this motif. The requirement of the Wnt pathway during development of the penaeoid shrimp Sicyonia ingentis was studied by small molecule inhibitor treatments through the early larva in membrane stage. Inhibition of the Wnt PCP pathway by TNP-470 had no effect on ME ingression and NM invagination, but blocked segmentation of the embryo. Both inhibition with XAV-939 and overstimulation with LiCl of the canonical Wnt pathway perturbed proper NM invagination and segmentation. These data suggest that the Wnt PCP pathway functions in segmentation but not ME ingression or NM invagination. Furthermore, an optimal level of canonical Wnt signaling appears to be necessary for NM invagination and segmentation.

Supported by FRCE Research funding from CMU.

Program Abstract #211

Mechanisms of Wnt target gene regulation by Hif1aduring Xenopus tail regeneration

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Proper developmental patterning of tissues requires precise spatial and temporal coordination of numerous signaling pathways. These cues must be reestablished in regenerative organisms to correctly replace lost structures. While many embryonic signaling pathways, such as Notch, Wnt, and FGF, are necessary for regeneration, it is not well understood how these pathways are activated in response to injury. Here, we bridge the gap between stress signaling and the regenerative response by addressing how the transcription factor, Hypoxia Inducible Factor 1a (Hif1a), regulates regeneration. Hif1a has been shown to be necessary for full tail regeneration. We find that antagonism of Hif1a results in shorter tail regenerates which lack specific tissue fates, including muscle and axons. These cell fates have previously been associated with Wnt signaling during regeneration, so we sought to determine if Hif1a regulates regeneration through Wnt. We analyzed gene expression of known Wnt target genes and found that Hif1a is necessary for induction of these genes in regenerating tissues. As Hif1a has not previously been associated with regulation of Wnt target genes, we asked how Hif1a could change expression of these genes. Using previously generated ChIP- and ATAC-seq datasets and motif identification software, we asked if Hif1aregulated gene expression by interacting with its canonical hypoxia responsive elements (HRE) or the Wnt responsive element (WRE). We found that Wnt genes sensitive to Hif1anormally have WREs but not HREs, suggesting that Hif1a acts through the WRE. To test this, we utilized a transgenic line to read out WRE mediated transcription and found that induction of WRE response during regeneration is dependent on Hif1a. This work highlights a novel role for Hif1ain tissue regeneration through a non-canonical regulatory program. This work was supported by R01NS099124 from NINDS. JHP was supported by PHS NRSA T32GM007270 from NIGMS and DGE-1762114 from NSF GRFP.

Program Abstract #212

Suspending Cell Death-A Novel Stem Cell Response to Injury in Planarians

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Stem cells are continuously exposed to multiple stresses, including radiation and tissue injury. As central drivers of tissue repair and regeneration, it is necessary to understand how their behavior is influenced by these stressors. Planarians have an abundant population of stem cells that are rapidly eliminated after radiation exposure via apoptosis. Low doses of radiation eliminate the majority of these stem cells, allowing a few to remain. Here, we combine radiation with injury to define how stem cells respond to tissue damage. We find that a variety of injuries induced within a defined window of time surrounding radiation cause stem cells to outlast those in uninjured animals. Injury stimulates localized cell death adjacent to wounds, in the same regions where stem cells persist. Surprisingly, stem cells persist in proportion to dying cells, without proliferating. Instead, they are retained near the wound due to delayed apoptosis, which we quantify by combining fluorescence-activated cell sorting (FACS) with annexin V staining. Pharmacological inhibition of the extracellular signal-regulated kinase (ERK) prevents stem cell persistence after injury, implicating wound-induced ERK activity in this response. By combining radiation with injury, we have identified a novel stem cell response and uncovered a connection between dying cells and stem cells that remain. Future experiments will define mechanisms by which injury-induced cell death influences stem cell persistence. By delineating cellular interactions that govern stem cell function in planarians, we hope to uncover targets that could be used to enhance stem cell activity in more complex organisms. Funding: GRA Fellowship, startup funds-Cornell College of Veterinary Medicine. Seed Grant-Cornell University Stem Cell Program.

Endoglin is an Inhibitor of Cardiomyocyte Proliferation
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Purpose: The adult mammalian heart is incapable of functional regeneration. After injury, such as a myocardial infarction, there is a loss of cardiomyocytes (CMs) and contractile function, without accompanying CM proliferation to sufficiently promote functional recovery. We hypothesize that functional myocardial regeneration can be achieved through reactivation of cardiomyocyte proliferation. Methods: We used lentiviral shRNA and siRNA mediated knock-down of the TGF-β co-receptor Endoglin (Eng) in neonatal CMs, in conjunction with adenoviral dnSMAD2 expression. Cell type specific Eng knockout mice were subjected to apical resection injury. Proliferation was assessed by pulse-labeling with ethynyl deoxyuridine (EdU), and by immunocytochemistry with phosphorylated Histone H3 and Mitotic Kinesin-like Protein 1. Results: Eng mRNA is expressed throughout cardiac development, in fibroblasts, endothelial cells and CMs. We performed shRNA and siRNA mediated Eng knockdown in cultured embryonic and neonatal cardiomyocytes, which resulted in activation of DNA synthesis with significant increases in mitosis (phH3) and cytokinesis (Mklp1) markers. This proliferative phenotype in cultured CMs could be reverted with the addition of dominant negative Smad2. We then used CM-specific Eng knock out mice to assess the role of Eng in regulating CM proliferation in vivo. We injected Tamoxifen at birth and performed apical resection at day 7, followed by assessment of CM cell cycle progression. Our preliminary results suggest that Eng expression inhibits CM proliferation in vivo. Conclusions: Eng knock-down promotes cell cycle progression and proliferation of CMs in a Smad2 dependent manner. We are currently investigating if CM-specific Eng deletion can promote functional heart regeneration. Funding: NIH grants T32GM113846, HL130072, Regenerative Medicine Minnesota and The Hartwell Foundation.

PPARdelta signaling induces metabolic maturation in pluripotent stem cell-derived cardiomyocytes through enhanced fatty acid oxidation.
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Pluripotent stem cell-derived cardiomyocytes (PSC-CMs) provide a powerful approach for regenerative therapies,
drug discovery and disease modeling. One caveat, however, is that PSC-CMs remain functionally and structurally immature, resembling first trimester fetal heart cells. In vivo heart development involves a postnatal metabolic switch from anaerobic glycolysis in the fetal heart to fatty acid oxidation (FAO) in the adult heart, and this switch coincides with an increase in PPAR signaling activity. Here we show that inducing PPAR signaling in vitro in PSC-CMs induces the metabolic switch observed in vivo, resulting in enhanced PSC-CM maturation. PPAR delta (PPARδ) signaling activation resulted in PSC-CM structural maturation through improved myofibril organization, reduced circularity standard deviation and increased binucleation. However, no difference in contractility was observed in either 2D or 3D culture formats. Interestingly, PPARδ activation upregulated genes involved in long chain fatty acid (LCFA) metabolism and inhibition of glycolysis-dependent oxidative phosphorylation. Accordingly, PPARδ-activated PSC-CMs showed increased expression of LCFA membrane transporters (CD36) and FAO enzymes (ACADVL), resulting in increased LCFA uptake and processing. PPARδ induction also increased mitochondrial content, mitochondrial size, maximal respiration rates and FAO flux rates, confirming FAO induction in PSC-CMs. PPARδ-mediated metabolic maturation can be induced in various culture protocols, including 3D Engineered Heart Tissues or after metabolic selection, indicating broad reliability for this mechanism. In summary, we identify a role for PPARδ signaling activation in enhancing PSC-CM metabolic maturation by inducing a metabolic switch to FAO, with no effect on contractility, thus uncoupling a metabolic prerequisite from contractile maturation during early heart development. Funding Sources: NYSTEM-C32561GG.

Program Abstract #215
A model for necrosis in Drosophila reveals regeneration by tissue remodeling
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Cell death is essential for proper regeneration of tissues following injury. Apoptosis surrounding the site of damage can dictate how that tissue responds to such damage, as signals that propagate from dying cells can further induce apoptosis or promote pro-survival signals in neighboring cells. For example, dying cells undergoing apoptosis in the Drosophila wing imaginal disc (larval tissue that forms the adult wing) release TNF-α to adjacent tissue to activate the JNK signaling pathway. High levels of JNK can further induce apoptosis, while low levels of JNK promote regenerative signaling. The crosstalk between apoptotic cells and their neighbors has been extensively characterized. However, much less is understood about the tissue response to non-apoptotic modes of cell death such as necrosis. Necrosis is implicated in many human diseases such as stroke and heart attack, infections and cancer. Despite the obvious need for further study, current approaches rely on the expression of pro-apoptotic genes to kill tissue. To address this issue, we developed a genetic ablation system that drives necrotic cell death in the wing imaginal disc through a caspase-independent mechanism. With this new model we find a similar regenerative response following both apoptotic and necrotic cell death, as ablated discs regenerate at a comparable frequency following both types of cell death, assayed by adult wing size. However, we have also identified phenotypes unique to necrosis, most notably an overall reduction in regenerated wing size, suggesting regeneration via remodeling. Immunofluorescent staining of discs regenerating from necrosis reveals strictly non-autonomous caspase activity as well as weak expression JNK pathway. These experiments reveal key differences in a tissue’s response to necrosis vs. apoptosis, suggesting that as yet unidentified mechanisms of recovery might exist. This work is supported by startup funding from the Harris lab at Arizona State University

Program Abstract #216
Investigating Support Cell Shape Change and Actin Dynamics During Zebrafish Hair Cell Death and Regeneration
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Mammals and nonmammals often differ in their capacity to regenerate tissues after damage. For example, adult mammals are largely unable to produce new sensory hair cells, yet nonmammalian vertebrates can robustly regenerate hair cells throughout life. During hair cell regeneration, nearby glia-like support cells proliferate and differentiate into hair cells. During hair cell death, support cells extrude dying hair cells to maintain epithelial barrier integrity. It is unclear how dying hair cells signal to surrounding support cells to initiate repair and regeneration. We aim to characterize support cell shape change during epithelial repair and regeneration in

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zebrafish lateral line neuromasts, which are highly regenerative and easy to experimentally manipulate. To examine cell shape, we used a stable transgenic zebrafish line that labels the membranes of neuromast cells (Tg:claudinb:lyn-GFP). To study actin dynamics, we used CRISPR-Cas9 to create a transgenic line in which support cells express the F-actin sensor LifeAct-GFP (Tg:atoh1a:LifeAct-GFP). With these transgenic lines, we performed time lapse imaging to visualize support cell responses during hair cell death. Hair cell death was induced by applying neomycin tagged with Texas Red (neo-TR). We also ablate individual cells using transgenic zebrafish that express rat TrpV1 channels in hair cells, which causes specific cell death upon capsaicin addition. We found that neuromast support cells form multicellular F-actin rings that contract around the tops of dying hair cells, similar to structures reported in other animals. The F-actin rings formed around the time when neo-TR uptake spiked in hair cells, indicating that support cells respond quickly to hair cell damage. These support cell cytoskeletal dynamics are the earliest changes that occur after damage, and we hypothesize that they are linked to signals that promote hair cell regeneration. Work was supported by grant # T32 DC005361 from NIH NIDCD.

Program Abstract #217
Dnmt1a is required for the maternal-zygotic transition in the wasp Nasonia
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The regulation of DNA methylation at cytosine residues is necessary for zygotic genome activation (ZGA) and early development in many animal lineages. The most well known role for DNA methylation in vertebrates involves the methylation of CpG islands in cis-regulatory regions to modify the expression of many important developmental genes. However, it is increasingly clear that DNA methylation at gene bodies is widespread and likely ancestral in animals. Despite its broad conservation, the importance of gene-body methylation (GBM) in development is still unclear, due to the difficulty in teasing apart the effects of loss of cis-regulatory methylation from those of GBM in vertebrate model systems. Unlike vertebrate models, the methylation machinery in the jewel wasp Nasonia vitripennis appears to be comprised exclusively of GBM, thus simplifying interpretation of the role of GBM in development. Knockdown of DNMT1 (Nv-dnmt1a) in Nasonia leads to embryonic lethality, which we show is caused by a failure of cellularization and gastrulation. Using RNA-seq we found a subset of maternal transcripts fail to degrade, while roughly a thousand zygotic transcripts are misexpressed at the onset of ZGA after Nv-dnmt1a knockdown. Loss of Nv-dnmt1a also resulted in a global loss of GBM in the embryo, which is strongly correlated with down-regulated gene expression at ZGA. We propose that GBM facilitated by Nv-dnmt1a is required for proper ZGA in the wasp. Funding: NIH 1R03HD087476

Program Abstract #218
Drosophila gametogenesis requires a feedback loop between heterochromatin and the nucleopore complex
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The ability of germ cells to differentiate into gametes is pivotal for all sexual reproduction. While factors that initiate differentiation have been identified, it is not known if or how the genes that encode these factors are silenced upon successful gamete formation. Using Drosophila melanogaster oogenesis, we show that regulated heterochromatin formation and its anchoring to the nuclear periphery is required for silencing of differentiation-promoting genes in the specified gamete. SETDB1, which deposits heterochromatic H3K9me3 marks, has been shown to translocate from the cytoplasm to the nucleus of the germ cells during oocyte specification. We find that SETDB1 and its translocation are required for silencing of genes that initiate differentiation in the specified oocyte by depositing H3K9me3 marks on their gene bodies. These silenced genes are then anchored at the nuclear lamina at regions with a high density of Nucleopore Complexes (NPCs). Intriguingly, we also find that SETDB1 is required for expression of 19 of the 30 NPC members including a germline enriched nucleoporin (Nup) 154. We find that Nup154, like SETDB1, is required for silencing of differentiation-promoting genes in the specified oocyte by anchoring heterochromatin to the nuclear lamina to maintain its integrity. Unlike differentiation-promoting genes, Nups are not direct targets of SETDB1. Instead, Nups that are downregulated upon the loss of SETDB1 are proximate to domains of repressive H3K27me3 marks. Loss of SETDB1 results in
Inability to silence these differentiation-promoting genes due to loss of either SETDB1 or member of the NPC results in loss of oocyte identity and death. We hypothesize that the feedback loop between heterochromatin and NPC is an elegant mechanism that tunes the number of heterochromatin-docking sites to the level of heterochromatin during development.

Program Abstract #219
Closing in on targets: Early HDAC activity is required for appendage regeneration
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In contrast to humans, amphibians have the capability to scarlessly regenerate appendages. Following injury, complex genetic processes must be activated and inactivated with high spatial and temporal resolution to result in a properly patterned appendage. *Xenopus tropicalis* are diploid frogs, whose tadpoles scarlessly regenerate properly patterned tails after amputation, making them an ideal system to query genomic changes that occur during regeneration. Using ATAC-seq, we have found that many differentially accessible regions of chromatin undergo transient periods of inaccessibility. This leads to the question: how is chromatin made transiently inaccessible, and how does this affect downstream gene expression? One major mechanism of chromatin compaction is through the activity of histone deacetylases (HDACs). Treatment with the broad HDAC inhibitor TSA during the first 24 hours post amputation (hpa) causes regeneration failure. To identify regions of the genome that are modified by HDAC activity during the first 24 hpa, I performed ATAC-seq in regenerating tadpoles. We used the regions of the genome identified by ATAC-seq that were differentially accessible over time or between treatment groups as an input for gene ontology (GO) analysis to discern what biological processes may be perturbed by TSA treatment. At 3 hpa with TSA treatment, terms called were related to cell motility, and nervous system development. Many of the genes called in GO analysis as well as regions of differential accessibility such as *foxd3*, *sox10*, *zeb2*, *bmp2*, *bmp4*, and *mitf* are important regulators during neural crest development, migration, and differentiation providing a rich developmental gene regulatory network to scaffold hypotheses. We therefore hypothesize that TSA treatment causes changes in cell migration and premature differentiation resulting in failure to regenerate a properly patterned appendage. Funding Sources: F31HD097910 to HA, 5R03HD091716-02 to AW

Program Abstract #220
Integration of signaling systems within the neural crest gene regulatory network via TFAP2 pioneer factors
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Cell fate commitment involves the progressive restriction of developmental potential, requiring not only shifts in gene expression, but also an extensive remodeling of the epigenomic landscape. To examine how chromatin states are reorganized during cellular specification in an *in vivo* system, we examined the function of pioneer factor TFAP2A at discrete stages of neural crest development. Our results show that TFAP2A activates distinct sets of genomic regions during induction of the neural plate border and specification of neural crest cells. Genomic occupancy analysis revealed that the repertoire of TFAP2A targets depends upon its dimerization with paralogous proteins TFAP2C and TFAP2B. This heterodimeric switch between TFAP2A/C and TFAP2A/B acts to drive the transition from induction to specification, allowing for progressive cell fate commitment within the neural crest lineage. TFAP2B loss-of-function disrupts neural crest formation, and misexpression of TFAP2B in the early neurula results in premature specification. Thus, *TFAP2B* expression is both necessary and sufficient to drive the transition from induction to specification. The dissection of a *cis*-regulatory element controlling *TFAP2B* expression has revealed that SMAD2/3, the nuclear effectors of TGF-beta signaling, are essential drivers of *TFAP2B* expression. Through a number of genomic and biochemical validation strategies, we show that TFAP2B integrates environmental signals into the gene regulatory network to drive the transition from induction to specification within the neural crest lineage. This work reveals a mechanism of how environmental stimuli act to remodel the chromatin landscape within the presumptive neural crest.
Program Abstract #221
Identifying the direct regulatory targets of TBX5 in developing external genitalia
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Abnormalities of the urogenital tract are among the most common human congenital disorders. Studies in both humans and model organisms have shown that disruptions in several genes and signaling pathways can cause morphological defects during development of external genitalia. The TBX5 transcription factor plays a critical role in the growth and development of the vertebrate forelimb and is also known to be expressed in the developing genitalia of several amniote species. However, it remains unknown what role Tbx5 plays during development of the external genitalia. We have found that conditional knockout of Tbx5 in the mouse genital tubercle (GT) causes a reduction of the preputial swellings. To more fully characterize genital tubercle morphogenesis in Tbx5 mutants, we are generating optical sections and three-dimensional renderings of genitalia using light sheet microscopy. In addition, we have used a combination of ChIP-seq and RNA-seq to investigate enhancer regions and target genes directly regulated by TBX5 in the mouse GT. Our results identify a fraction of TBX5 binding sites located near genes that are misregulated in the GTs of Tbx5 conditional knockout mice. To determine the degree to which TBX5 binding events are conserved in amniote external genitalia, we are now performing parallel TBX5 ChIP-seq experiments in embryonic hemiphalluses of the brown anole lizard. Together, our findings will reveal the role of TBX5 in genital formation and identify the direct transcriptional targets this transcription factor regulates in embryonic genitalia. This work was supported by grants awarded to D.B.M. from the NIH (HD081034) and NSF (IOS-1754950).

Program Abstract #222
ISL1 Targets Conserved Appendage Enhancers During Development of the Amniote Phallus
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The development of the genital tubercle (GT) – the embryonic precursor to the penis and clitoris – involves expression of many genes that play a role in limb development. Similarities in early genital budding across amniotes suggest a shared evolutionary origin of the external genitalia from their last common ancestor over 300 million years ago. Prior studies have shown that many limb enhancers are active during phallus development, raising the possibility that limb regulatory elements were coopted during the evolution of the phallus. However, patterns of transcription factor binding during GT development remains largely unexplored. The Isl1 gene encodes a transcription factor required for initiation of hindlimb outgrowth in mice. Conditional knockouts also demonstrate a crucial requirement of Isl1 for GT outgrowth. Using a combination of RNA-seq and ChIP-seq, we have identified putative direct targets of ISL1 during mouse GT development. Limb genes are overrepresented among these targets, and many appear to be activated via known limb enhancers. Comparative ISL1 ChIP-seq analyses between embryonic chick, lizard, and turtle genitalia also reveal deeply conserved ISL1 binding events at some of these enhancers, possibly highlighting targets of functional importance that have been maintained for hundreds of millions of years. A notable example of a conserved target is Tbx4 and its enhancer HLEB, which is also an ISL1 target during the initiation of hindlimb budding. Our results demonstrate that a subset of ISL1 binding events are shared between the developing hindlimbs and genitalia and deeply conserved. This study was supported by grants from NICHD (HD081034) and NSF (IOS-1754950).

Program Abstract #223
Characterizing the role of canonical bone morphogenic protein (BMP) signaling in murine preimplantation lineage specification
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In the mammalian embryo, a single fertilized zygote must divide and differentiate into over 200 known embryonic and extra-embryonic cell types. Strict regulation of the emergence and ratio of these cell types is essential for a successful pregnancy and birth of healthy offspring. The first pluripotent cells arise at the blastocyst stage of development, where the inner cell mass cells undergo a cell fate decision to become either pluripotent epiblast,
which gives rise to the embryo proper, or multipotent primitive endoderm, which will form extra-embryonic structures and direct embryonic patterning. Fibroblast growth factor (FGF) signaling is known to be critical for the formation of primitive endoderm cells in mice, and regulates the ratio of primitive endoderm to epiblast cells within the inner cell mass of the murine blastocyst. A number of groups have suggested that canonical bone morphogenetic protein (BMP) signaling may also regulate the establishment of primitive endoderm and epiblast in preimplantation embryos. Here, our lab has shown that preimplantation lineage specification is apparently normal when BMP signaling is knocked down by deletion of maternal and zygotic Smad4. We do, however, have evidence that exogenous BMP4 may partially inhibit FGF signaling in the mouse embryo, and that this inhibition is dependent on Smad4. Based on these data, we suggest that BMP signaling becomes active in mouse embryos at the time of implantation, and at that time helps to drive primitive endoderm cells into a differentiative state by inhibiting FGF signaling. Funding for this project is provided by NIH T32HD087166 awarded to R.S. and NIH R35GM131759 awarded to A.R.

Program Abstract #224
Fragility in specification; glial cells and the RNA binding protein FMRP
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The specification of multiple cell types from a common progenitor domain is dependent on a variety of factors, including exposure to morphogens, developmental timing, and the regulation of genes driving specification and differentiation. We recently showed dramatic hypomyelination in a zebrafish model of Fragile X syndrome, the most heritable form of autism spectrum disorder, which stems from the absence of the RNA binding protein fragile X mental retardation protein (FMRP). We now describe prominent effects of FMRP on cell specification in the oligodendrocyte lineage. Zebrafish fmr1 mutants generate fewer motoneurons and excess oligodendrocyte precursor cells (OPCs), two cell types sequentially generated from the pMN domain. However, fmr1 larvae produce appropriate numbers of mature oligodendrocytes, resulting in a surplus of OPCs. These excess OPCs are often clustered in both dorsal and ventral tracts of the spinal cord, which suggests that FMRP also plays roles in cell migration and repulsion. Inhibition of neural activity had no effect on OPC specification in both wild-type and fmr1 larvae, which shows that OPCs are generated in an activity-independent mechanism. However, pharmacological blockade of mTOR signaling reduced OPC quantity in both genotypes, which may suggest that FMRP plays a vital role in the inhibition of mTOR during OPC specification. What is the ultimate fate of these surplus OPCs? How does FMRP regulate specification and differentiation? Our ongoing studies seek to nail down the timing of precocious OPC generation in the absence of FMRP and identify the precise sites of FMRP binding to target mRNAs in distinct phases of oligodendrocyte development. Ultimately this work highlights the importance of mRNA regulation during cell specification and further implicates glial cells in the developmental pathogenesis of a major autism spectrum disorder. Funding: NINDS, R21NS110213 (CD,BA); R01NS095679 (BA).

Program Abstract #225
Gastrointestinal transcription factors drive lineage-specific developmental programs in organ specification and cancer
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The adult gut houses a series of organs derived from a naive, embryonic population, the endoderm. During development, the endoderm will specify into unique gastrointestinal (GI) identities through the temporal and spatial control of master transcriptional factors (TFs). Expressed in a tissue-specific manner, these TFs bind to DNA regulatory elements of multiple target genes to activate organ-specific transcriptional programs. These programs drive initial organ specification, growth, lineage determination and maintenance. While accumulating evidence shows global enhancer activation and aberrant expression of developmental TFs in gut cancers, little is known about how master TFs drive gastric development and cancer. Accordingly, we first defined GI organ-specific chromatin accessibility and gene expression during development, using high-throughput sequencing technologies.
Cumulative analysis revealed dynamic epigenetic regulation of SOX TFs during gastric determination. Furthermore, we revealed that Sox2 is not only essential for gastric specification, by maintaining chromatin accessibility at forestomach lineage loci, but is also sufficient to promote gastric/squamous transformation upon Cdx2 deletion in the embryonic intestine. To assess if organ specific developmental programs were activated in adult gut cancers, we compared our GI lineage-specific transcriptomes to human gut cancer data. Here, we found that stomach and intestinal lineage-specific programs are reactivated in Sox2\textsuperscript{high}/Sox9\textsuperscript{high} and Cdx2\textsuperscript{high} cancers, respectively, emphasizing tissue ontogeny as a mechanism for cancer initiation. By analyzing mice deleted for both Sox2 and Sox9, we revealed their potentially redundant roles in both gastric development and cancer, highlighting the significance of developmental lineage programs reactivated by gastrointestinal transcription factors in cancer. This work was funded by CIHR, Medicine by Design, SickKids and the Cancer Research Society.

Program Abstract #226
Ablating Sonic Hedgehog Signaling Regulator Suppressor of Fused Alters Neural Cell Fate
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Background: Early neural cell fates are determined by ventral-dorsal activation of the Sonic Hedgehog (SHH) signaling pathway. Spatial activation of SHH gives rise to motor neurons and interneuron precursors, where temporal activation subsequently gives rise to glial cells. Without SHH ligand, negative regulators of the pathway Patched and Suppressor of Fused (SUFU) promote the conversion of Gli proteins into truncated transcriptional repressors. The SHH ligand inhibits the Patched receptor, causing downstream inhibition of SUFU and subsequent activation of full-length Gli proteins. P19 embryonal carcinoma cells are a robust model for investigating neural differentiation. Treatment with retinoic acid (RA) induces neural lineages where neurons form after 10 days and astrocytes form after 17 days. Hypothesis: If P19 cell differentiation recapitulates the timing of SHH signaling in the developing neural tube, then altering SHH signaling, through genetic ablation of SUFU, will alter neural fate specification. Results: SHH signaling was active 1 day after RA induction and was subsequently re-activated at days 14 and 17. Pathway activation coincided with neural precursor and glial cell differentiation, respectively. Genetic ablation of SUFU induced the expression of SHH target genes Gli1, Ptch1 and Ascl1 in undifferentiated cell cultures. Neuron marker β-III-tubulin signal was attenuated on days 14 and 17 of RA induced differentiation in SUFU\textsuperscript{-/-} cells. This attenuation was accompanied by the appearance of astrocyte marker GFAP at day 14 and an increase in GFAP signal at day 17 with RA induction. SUFU\textsuperscript{-/-} cells also showed GFAP signal on day 17 of differentiation without RA treatment. Conclusion: P19 cells mimic the temporal activation of SHH observed in vivo, where overactivation of the pathway through the ablation of SUFU alters the trajectory of differentiating cells towards a glial cell fate. Funding sources: Natural Sciences and Engineering Research Council of Canada.

Program Abstract #227
Neural crest development is temporally regulated by miR-302
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Cranial neural crest cells are specified from ectoderm during gastrulation and possess a remarkable ability to generate diverse cell types. The mechanisms controlling the timing of neural crest formation are critical for proper development of neural crest and craniofacial structures. Here, we identify two groups of opposing miRNAs that counterpoise the transition from pluripotent ectoderm to multipotent migratory neural crest. MiRNA profiling reveals an early group of miRNAs that are associated with pluripotency and a later group of miRNAs that promote differentiation. Using combined miRNA and mRNA sequencing, we identify a common core set of genes targeted by both groups of prevalent miRNAs. We used an unbiased screening approach to show that these co-targeted genes promote the formation of neural crest in chicken. We identified miR-302 as a stem-associated miRNA that is uniquely maintained in cranial neural crest. Further, we use single cell analysis and genetic gain- and loss- of function to show that miR-302 is critical for preventing precocious neural crest formation in mouse and chick. MiR-302 deletion results in an ectopic accumulation of neural crest cells and disrupts their terminal differentiation. Genetic rescue in mouse revealed that miR-302 coordinates proper timing of neural crest formation by regulating expression of Sox9, a conserved neural crest specifier. Taken together, our findings reveal
a post-transcriptional regulatory network of miRNAs that control the balance between stemness and differentiation by co-targeting genes that control the timing of neural crest formation. This project was supported by CPRIT (RR150106), V Foundation, Andrew McDonough B+ Foundation and the NIH (R01-HD099252, R01-HD098131).

Program Abstract #228
Modular tendon fate specification through nuclear receptor Nr5a2 in lower jaw and middle ear
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Precise connectivity of the jaw skeleton to the underlying musculature through tendons is crucial for jaw movement and force transmission. Compared to skeletal differentiation, we know much less about how tendon fates are specified at distinct regions in the body. The tendons of the face are derived from cranial neural crest cells (CNCCs), as opposed to those of the fins/limbs and trunk that are derived from mesoderm. Do tendons derived from different lineages depend on distinct upstream signals for their specification? By conducting single-cell RNA expression profiling of CNCC derivatives throughout zebrafish facial development, we find that the nr5a2 orphan nuclear receptor is one of the most enriched genes in the early tendon lineage. In situ hybridization reveals that nr5a2 is expressed in the embryonic jaw tendon-forming domain, and time-lapse imaging of a newly generated nr5a2:GFP knock-in line reveals that nr5a2 expression precedes that of the key tenocyte transcription factor scleraxis (scxa). In nr5a2 mutants, loss of lower jaw tendons is accompanied by a corresponding increase in jaw chondrocytes and disorganization of muscles. Reciprocally, misexpression of nr5a2 results in ectopic scxa-expressing tenocytes. Preliminary analysis of conditional deletion of Nr5a2 suggests a conserved role in skeleton and tendon patterning in the mouse mandibular arch. Using single-nuclei ATACseq of purified tenocytes from zebrafish, I have identified putative region-specific enhancers of scxa that contain putative Nr5a2-binding sites. I am currently testing the model that Nr5a2 promotes jaw-specific tendon fate through direct binding to a jaw-specific enhancer of scxa. My findings reveal Nr5a2 as one of the earliest specifiers of tendon fate and suggest modular regulatory control of tendon fate throughout the body. Funding sources: NIDCR R21 DE029656.

Program Abstract #229
Evolution of limb loss in snakes and functional testing of a limb specific shh enhancer in lizards
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The Sonic Hedgehog gene (Shh) is expressed in small group of mesenchymal cells at the posterior margin of the developing limb bud in vertebrates. This region is known as the zone of polarizing activity (ZPA), and the expression of Shh in the ZPA is regulated by a cis-regulatory element known as the ZPA regulatory sequence (ZRS). The ZRS is highly conserved in vertebrates, and point mutations in the ZRS in mammals are associated with ectopic Shh expression and preaxial polydactyly. In contrast, deletion of the ZRS results in a loss of Shh expression and produces severe limb truncations. Recent studies in snakes suggest that the ZRS underwent gradual sequence degradation that contributed to the evolution of limb reduction in this group of squamate reptiles. However, functional tests of snake ZRS elements have only been performed in mice. Ideally, the phenotypic consequences of sequence changes in the snake ZRS should be assessed in limbed reptiles rather than mammals. We recently established the brown anole lizard, Anolis sagrei, as the first reptilian model system where gene editing can be performed. Using the Cas12a system, we have successfully deleted portions of the brown anole ZRS. Our preliminary results demonstrate that deletions within the ZRS are sufficient to produce limb truncations in limbed reptiles. Our results are consistent with the hypothesis that ZRS mutations could have contributed the evolution of limb reduction in snakes. This work was funded by the NSF EDGE Program (#1827647).

Program Abstract #230
Characterization of retinal cell types in the African spiny mouse (Acomys cahirinus)
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Vertebrate retinal development is a complex process that involves the coordinated interaction of multiple
extrinsic signals and intrinsic genetic regulatory networks. Defects in this process are associated with inherited retinal degenerative diseases and blindness. Although retinal structure and cell type composition are generally well conserved across all vertebrates, differences in the precise complement of retinal cell types can reflect functional adaptations to different environments and lifestyles, and there is a need to incorporate additional mammalian models into studies of retinal development, function, and disease. The African spiny mouse (Acomys cahirinus) has been the subject of scientific study for its unique behavioral qualities, precocial development, and more recently for its regenerative abilities. However, the development of the spiny mouse visual system has not been well studied. To provide a foundation for such studies, we have begun characterizing the retinal anatomy of adult Acomys. Here, we present a preliminary comparative analysis of retinal cell type morphology and number in Acomys and Mus adult retinas. Retinal tissue sections were obtained from adult eyes and subjected to histological staining (hematoxylin and eosin) or immunohistochemistry with antibodies for various retinal cell types. Sections were imaged by light and fluorescence microscopy. Overall, cell morphology was comparable between the two species for the majority of the cell types. However, the Acomys bipolar cells were increased in number and displayed altered laminar positioning compared to Mus. Intriguingly, we observed a difference in the abundance and distribution of Acomys photoreceptors, particularly the cones, which could suggest differences in visual acuity between the two species. These results set the stage to investigate the development of retinal cell type diversity in this interesting mammalian model to expand our knowledge of vertebrate retinal development.

Program Abstract #231
Adaptation of proximity-dependent biotinylation approaches to early zebrafish embryos
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The importance of protein-protein interactions to understanding protein function has been well established. However, most studies investigating this do so in cell culture, missing out on potential important context dependent interactions. This is particularly problematic when studying development, as many important developmental processes are absent in cultured cells. We have adapted the Biotin Dependent Proximity Labeling (BioID) method for use in vivo in zebrafish embryos, allowing for global proteomic studies of development in vivo. BioID is used to covalently label and identify proteins proximal to a protein of interest (the bait). This system will allow researchers to investigate global protein dynamics in vivo during early development. To develop and optimize zebrafish BioID we have used the nuclear envelope protein Lamin A (LMNA) as a bait, to allow benchmarking against previous studies. Using the highly efficient enzymes TurboID and MiniTurbo fused to LMNA we identified known proximal interactors of LMNA in vivo in zebrafish embryos. These identified proteins included 14 of 24 components of the nuclear envelope and nuclear pore complex identified in the original BioID publication using LMNA as a bait. We demonstrate that these experiments can be performed effectively and quickly using bait expression via mRNA injection. In this context samples were collected at 12, 24, and 48 hours post-fertilization. We also generated stable lines expressing an inducible bait transgene and demonstrate that labeling in embryos aged 24 - 72 hours post-fertilization for as short as nine hours can achieve effective proximal labeling for identification. To our knowledge this is the first successful application of BioID in zebrafish. By leveraging proteomic discovery methods this technology will greatly complement the existing robust techniques for studying early development in zebrafish embryos. Funding: Canadian Institutes of Health Research (CIHR) and Restracomp (SickKids).

Program Abstract #232
Syntaxin 4 Regulates Cardiac Conduction in Vertebrate Cardiomyocytes
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Arrhythmias are the most common cause of morbidity/mortality in patients with congenital heart diseases and primary arrhythmic disorders. However, the etiologies of these congenital arrhythmogenic disorders (CADs) remain poorly understood. We identified a patient with a spectrum of abnormalities, including dilated cardiomyopathy and a failure to respond to transient cardiac pacing, who ultimately required heart transplantation. Whole-exome sequencing of this proband identified a homozygous non-conservative substitution in the SNARE protein Syntaxin 4 (STX4) locus that previously had not been implicated in playing a role in human disease or cardiac function. To understand this requirement of Stx4, we generated zebrafish stx4 mutants that, in contrast to the early embryonic lethality of murine knockouts, develop a spectrum of defects reminiscent of the patient’s, including pericardial edema, linearized hearts, and bradycardia. Due to the apparent conservation of this phenotype, we first assessed cardiomyocyte (CM) differentiation, but failed to observe a difference in CM number in stx4 mutants. Closer analysis of the developing heart indicated a requirement for Stx4 in cardiac conduction, as mutant hearts exhibited a slower calcium transient velocity, and for vesicle trafficking, as mutant CMs had fewer docked vesicles vs. WTs. Our data also suggest that the conduction defect is autonomously required in CMs, as mutant hearts are competent to respond to sympathetic stimulation, despite the absence of autonomic innervation by 72 hours-post-fertilization (when the cardiac phenotype is fully penetrant). Our data obtained by modeling a previously unreported human disease variant in zebrafish demonstrates an unanticipated/conserved requirement for Stx4 in cardiac function. Because the role of SNAREs in cardiovascular disease is not understood, our investigation of Stx4 in normal cardiac function may elucidate novel therapeutic targets for the treatment of CADs. Funding: NIH.

Program Abstract #233
Myelinating regulatory factor is essential for mouse embryonic development
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Myelin Regulatory Factor (MYRF) is a transcription factor most commonly known to control the expression of myelin-related genes and myelination in the central nervous system (CNS). Even though myelination starts afterbirth, transcriptome data has shown MYRF is expressed in most mouse tissues such as heart, lung, liver and fore limb by mid-gestation (e12.5) during mouse embryonic development. To further understand the transcriptional role of MYRF in embryonic development, we applied a Cre-lox system to generate Myrf null embryos in c57/Blk6 and CD1 stains. Our data indicates that ubiquitous deletion of MYRF in mice is embryonic lethal in both strains. Based on our findings the time of embryonic lethality occurs prior to day 11.5 (~11.5 dpc). To date, no Mendelian disease has been associated with MYRF; however, recent studies have linked de novo mutations in MYRF with heart development where complex congenital heart disease and severe urogenital malformations have been reported. Further examinations are in progress to decipher the cause of lethality by MYRF ablation in mouse embryonic development.

Program Abstract #234
Cellular heterogeneity of the LH receptor and its significance for cyclic GMP signaling in mouse preovulatory follicles
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Mammalian oocytes are maintained in meiotic arrest by an influx of cGMP produced in surrounding granulosa cells by the guanylyl cyclase NPR2. NPR2 is inactivated by a signal from the luteinizing hormone receptor (LHR), resulting in lowered cGMP and meiotic resumption. Although communication between both proteins is important for regulating oocyte maturation, protein localization data is limited, due to the lack of suitable antibodies. To determine where these proteins are located within the follicle, we generated mouse lines with a hemagglutinin (HA) tag on the endogenous N-terminus of the LHR or NPR2, then used a commercially available HA antibody to localize these proteins by immunofluorescence and immunogold microscopy. The LHR was present in outer mural granulosa cells but absent in cumulus cells, consistent with previous studies. Strikingly, expression in the outer mural cells was highly heterogenous, and only 13–48% of total outer mural cells expressed the protein. We
investigated contact sites with the basal lamina and proximity to blood vessels as possible explanations for the heterogeneity, but neither differed between LHR expressing and non-expressing cells. Investigating the NPR2, we determined that the protein was present in both mural granulosa and cumulus cells; however, the concentration was highest in the cumulus cells. We determined that only about 5–20% of the total NPR2 protein is in the same cells that express the LHR, which suggests that a signal is transferred from LHR-expressing cells to inactivate NPR2 in neighboring cells. Consistent with this hypothesis, inhibiting gap junction permeability attenuated the LH-induced cGMP decrease in outer mural cells. These results indicate that LHR signaling generates a second messenger that diffuses through gap junctions to inactivate NPR2 in adjacent cells, lowering cGMP to cause meiotic resumption in the oocyte. Supported by the National Institute of Child Health and Human Development (R37HD014939).

Program Abstract #235
Interaction of the DBL-1/BMP signaling pathway with BLMP-1/BLIMP1 in Caenorhabditis elegans
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Animals use multiple signaling pathways for cell-to-cell communication, which are essential for proper development. One signaling pathway is defined by its ligand family of bone morphogenetic proteins (BMP). In the roundworm C. elegans, BMP member DBL-1 has a well-defined pathway that includes conserved core components and regulators. The DBL-1 signaling pathway is involved in a spectrum of traits, including body size, brood size, male tail morphogenesis, and distal tip cell migration. How does this BMP pathway control target gene expression? We are using the C. elegans system, which has a toolbox of genetic and molecular resources available, to specifically address this question. Previous studies in C. elegans show that transcriptional regulator BLMP-1 affects a similar array of traits as DBL-1. In mammals, both BMPs and BLIMP1 affect overlapping developmental and homeostatic processes and are implicated in cancers. However, the relationship between BMP and BLIMP1 is not clear. We discovered that DBL-1 and DBL-1 signaling are affected by loss of BLMP-1. Notably, we also found that DBL-1 negatively regulates blmp-1 expression in a stage-specific manner. In addition, ChIP-seq data analyses of SMA-3, a DBL-1 pathway transcription factor, and BLMP-1 suggest that these two transcriptional regulators control expression of some common target genes, and may act together. In the future, we will elucidate the molecular mechanism underlying the interaction between this BMP pathway and BLIMP-1 in C. elegans, to gain an understanding of how BMP regulates proper growth and development of animals.
Funding: NIH R01GM097591 and TWU internal funding.

Program Abstract #236
Deep cytoplasmic sorting during Xenopus oocyte-to-embryo transition
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In many species, the zygotic genome is quiescent following fertilization. The genetic program responsible for early embryonic development is executed by RNAs and proteins that are maternally deposited in the oocyte, completely independent of transcription. It is currently unclear how maternal factors are stored in the oocyte in an inactive state, but become activated during the oocyte-to-embryo transition (OET). We recently reported that mRNAs encoding proteasome components are present in a gradient in Xenopus oocyte, with highest levels in the animal pole and the trailing end extending into the vegetal hemisphere. During the OET, proteasome mRNAs undergo a vegetal-to-animal translocation. As a result, the proteasome system becomes highly enriched in the animal hemisphere of mature eggs and early embryos. This creates a permissive environment for embryonic germline development. Here we report that the proteasome dynamics during the OET relies on several cellular and molecular mechanisms, which we collectively refer to it as “deep cytoplasmic sorting”. We show that a significant amount of proteasome mRNAs are associated with the endoplasmic reticulum (ER) in the oocyte. During oocyte maturation, proteasome mRNAs detach from the ER and are actively transported toward the animal hemisphere. Meanwhile, ER is remodeled into a fine tubular network distributed in the entire animal hemisphere, preventing diffusion of proteasome mRNAs into the vegetal hemisphere. Interfering with F-actin, which supports ER, impairs the proteasome dynamics during the OET. We conclude that deep cytoplasmic sorting...
during the OET is critically important for proper localization of maternal factors and the remodeling of the proteasome, ER, and likely other cellular machineries in the oocyte. Our work thus provides novel mechanistic insights into the storage and remodeling/activation of maternal factors during the OET. (This work is supported by R35GM131810).

Program Abstract #237
Morphogenesis of Distinct Lumen Wrinkling Patterns Along the Developing Intestinal Tract
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The avian esophagus, small intestine, and large intestine are separate gut compartments that develop divergent inner epithelial folding patterns—longitudinal ridges, ordered villi, and disordered villi, respectively—despite arising from a simple early embryonic tube. It remains unknown how the intestine regionally diversifies these morphologies during development. In the small intestine, stepwise buckling instabilities driven by differential growth and elasticity between tissue layers molds the lumen into longitudinal ridges, zigzags, and finally villi. Intermediate morphologies correspond to the sequential appearance of oriented muscle layers, which occurs in all three gut compartments. Ultimately, villus formation in the small intestine compartmentalizes the intestinal stem cell niche, thus directly impacting organ function. We predict that distinct folding patterns elsewhere in the gut arise from differences in geometric and physical properties of tissue layers. Using fine dissections, we show that the esophagus maintains longitudinal ridges through dramatic circumferentially-biased growth, despite having the same longitudinal muscle constraints that generate zigzags in the small intestine, while simultaneous longitudinal and circumferential differential growth generates more subtle cross-hatched folds in the large intestine. Next, we used uniaxial tensile testing to show that the stiffness ratio between inner layers, which influences the material buckling mode, increases ten-fold from the large intestine to small intestine, and three-fold from the small intestine to esophagus. This is correlated with buckles of increasing width from posterior to anterior. Our results qualitatively support a model where varying physical properties along the gut shift when and how the lumen buckles in response to muscle constraints. This work is supported by NIH/NICHD R01 grant HD089934 and the NSF GRFP.

Program Abstract #238
TGFß and BMP signaling synchronize the initiation of midgut asymmetry
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The generation of asymmetry is fundamental to embryogenesis. We study the conserved counter-clockwise rotation of the gut as a model to understand left-right (LR) organ asymmetry. A critical aspect of this rotation is initiation of a leftward tilt directed by the conserved Pitx2 transcription factor, the master regulator of LR organ asymmetry. Failure to establish proper gut chirality leads to gut malrotation and catastrophic volvulus. The direction of gut rotation is specified by cellular and extracellular matrix (ECM) asymmetries within the dorsal mesentery (DM), which suspends the gut tube, and is downstream of Pitx2 expressed strictly on the DM left side. Although Pitx2 has been thoroughly studied in development, how asymmetric expression of Pitx2 in the gut is first established is unknown. Here, I uncover that TGFß signaling is restricted to the left side of the DM and drives the timely activation of Pitx2 necessary for gut rotation. On the right side, BMP4 signaling initiates the expression of the enzyme Tsg6 and is the major rival of Pitx2. Tsg6 then triggers the accumulation of hyaluronan (HA) on the right, resulting in a dramatic expansion of the DM ECM, accelerating leftward tilting. Intriguingly, this right-sided expansion is sensed on the left side by the latent TGFß complex, an extracellular feedback mechanosensor, resulting in ECM condensation ensuing deceleration. Surprisingly, Noggin, expressed by the gut tube primordium, antagonizes both the left and right programs, thus restricting morphological LR asymmetries to the DM. These data shed light on the major molecular players that not only break the initial symmetry within the DM but also synchronize the formation of the DM with its LR asymmetric deformation pivotal to gut rotation. This work was supported by the Cornell Center for Vertebrate Genomics (BDS) and NIDDK R01 DK092776 (NAK).
Program Abstract #239
The function of Scribble in neural convergent extension
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Neural tube defects (NTD) occur in 1:500 pregnancies and result in severe birth defects. Neural tube closure (NTC) in vertebrates relies on the elongation, driven by convergent extension (CE), and bending of the epithelium through cell wedging and apical constriction. NTC is dependent on several cellular processes including polarized cell intercalation, cell shape changes, and cytoskeletal dynamics and loss of proteins that regulate these functions results in craniorachischisis (CRN). One such protein is Scribble (Scrib) which is best known for its roles in regulating apical-basal polarity and apical junctional organization. Scrib\textsuperscript{umz} mutants display short body axis phenotypes, axial torsion, and CRN, although how Scrib regulates neural cell behavior during NTC remains unknown. We examined this in Scrib\textsuperscript{umz} mice, using live imaging of cell behavior in the neural plate of E8.0 embryos. We find that the Scrib\textsuperscript{umz} mutation inhibits overall tissue shape changes, leading to decreased CE. Analysis of cell intercalation and cell shape revealed that Scrib\textsuperscript{umz} leads to defects in polarized cell intercalation as well as loss of both apical constriction and cell wedging. Interestingly, we also observed that mutant embryos exhibit a change in the frequency of rosette resolution as a mechanism for cell rearrangement. Scrib\textsuperscript{umz} mutant embryos exhibit mislocalized actin and myosin, and live imaging of embryos expressing GFP-Lifeact shows alterations in the dynamics of apical actin in mutant embryos. In addition, loss of Scrib localization leads to specific effects on tight junctions, with effects on apical localization of the junctional proteins ZO-1 and Par3. This work is supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development-R01HD087093.

Program Abstract #240
The Role Of Hepatoma Derived Growth Factor In Postnatal Neural Stem Cells
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Oligodendrocytes, the myelinating cells of the central nervous system (CNS), perform vital functions in neural protection, communication and efficient information transmission. Moreover, oligodendrocyte lineage cells have been recently implicated in cognitive functions. Thus, it is not surprising that oligodendrocytes and/or myelin are often perturbed in children with neurodevelopmental disorders. Our lab has demonstrated developmental oligodendrocyte formation is regulated by cell-to-cell communication between inhibitory interneurons and neural stem cells (NSCs) (Voronova et al. 2017 Neuron). NSCs form oligodendrocytes via a 2-step process: 1) NSC to oligodendrocyte precursor cell (OPC) commitment; and 2) OPC to oligodendrocyte differentiation. Interneurons affect both steps of this process by secreting over 50 paracrine ligands, including HDGF (Voronova et al. 2017 Neuron). Yet, the role of HDGF in the developing brain or NSCs is not known. To address this, I isolated NSCs from murine postnatal brain subventricular zone (SVZ), an NSC rich area that is known to generate oligodendrocytes throughout life. NSCs were cultured in the presence or absence of HDGF added to minimal media, which allows for oligodendrocyte differentiation and permits the observation of exogenous ligands on this process. My results demonstrate HDGF increases the number of mature oligodendrocytes that express myelin basic protein (MBP) but does not affect the formation of non-myelinating (immature) oligodendrocytes or OPCs. My initial results indicate HDGF may achieve this by increasing proliferation of NSCs, but not OPCs. In summary, my data suggest a novel role of HDGF signalling in NSCs. Future studies will address the in vivo effect of HDGF on NSCs in the developing murine brain. This study was supported by NSERC Discovery Grant awarded to A.V., NSERC USRA awarded to Y.L. as well as AGES and University of Alberta scholarships awarded to A.W.

Program Abstract #241
Impaired cortical cytoarchitecture and reduced excitability of deep layer neurons in the offspring of diabetic rats
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The laminar structure of the cerebral cortex is originated during embryo development by the interaction of multiple factors that regulates cell proliferation, differentiation and migration. High glucose during development promotes altered cortical proliferation, migration and differentiation. However, the postnatal consequences on cortical cytoarchitecture and function have been scarcely addressed. The aim of this study was to evaluate the effect of maternal hyperglycemia on postnatal cortical development. Pregnant rats (5 days after matting) were intraperitoneal injected with streptozotocin (diabetic group) or the vehicle (citrate buffered; control group). Offspring was sacrificed at postnatal days 0 and 21. The neonatal frontal cortex was obtained to evaluate SATB2, FOXP2, TBR1, REELIN and MAP2 through immunofluorescence, qRT-PCR and Western blot, while in 21-day old pups the cytoarchitecture and neuron morphology was assessed using and SATB2, FOXP2 and TBR1 immunofluorescence and Golgi-Cox staining, respectively. Furthermore, neuron functionality was evaluated by electrophysiology. Neonates from diabetic rats presented significant increases of the deep cortical markers FoxP2 and Tbr1, and altered SATB2, FOXP2, TBR1, REELIN and MAP2 laminar distribution. Later in P21 pups from diabetic rats showed altered laminar marker distribution, decreased dendritic complexity, impaired cell polarity, and lower deep layer neurons excitability. Overall, our results suggest that maternal diabetes promotes complex changes in cell neurogenesis, migration, polarity establishment and dendritic arborization, which in turn lead to reduced neuronal excitability.

Program Abstract #242
Transcriptional regulation of MGE progenitor proliferation by PRDM16 controls cortical GABAergic interneuron production
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The mammalian cortex is populated by neurons derived from neural progenitors located throughout the embryonic telencephalon. Excitatory pyramidal neurons are derived from progenitors located in the dorsal telencephalon, while inhibitory interneurons are generated by ventral telencephalic progenitors. The transcriptional regulator PRDM16 is expressed by radial glia, highly self-renewing neural progenitors present in both regions; however, its mechanisms of action are still not fully understood. It is unclear if PRDM16 plays a role in neurogenesis in both dorsal and ventral progenitor lineages, and if so, whether it does so by regulating common or unique networks of genes. Here, we use a mouse model in which Prdm16 is knocked out in the medial ganglionic eminence (MGE), the main source of cortical interneurons. Loss of Prdm16 in MGE progenitors results in defects in their proliferative capacity, leading to a decrease in the number of pallial GABAergic interneurons and diminished inhibitory input onto pyramidal neurons. Using RNA-Seq and ChIP-Seq, we find that PRDM16 controls the transcriptional programs involved in cortical interneuron production through its binding to cis-regulatory elements, which represses the expression of region-specific neuronal differentiation genes, thereby controlling the timing of neuronal maturation. Our results highlight the importance of PRDM16 for the development of both excitatory and inhibitory cortical circuits. We propose the existence of convergent developmental transcriptional programs regulated by Prdm16 that utilize both common and region-specific sets of genes in the cortex and the MGE in order to control the proliferative capacity of neural progenitors, ensuring the generation of correct numbers of cortical neurons.

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Program Abstract #243
The tyrosine phosphatase PTPRD regulates embryonic neurogenesis during cortical brain development
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Neurodevelopmental disorders are due to an abnormal brain development and are commonly associated to cognitive impairment, motor disorders and communication problems. Recent work suggests that neural precursor cells (NPCs) are important cellular substrates for neurodevelopmental disorders, since disruption of genes associated to neurodevelopmental disorders in NPCs perturb the numbers and types of neurons that are generated, resulting in aberrant brain formation and cognitive impairments. We have focused on the receptor tyrosine phosphatase PTPRD because is genetically associated with several neurodevelopmental disorders such as autism spectrum disorder, attention deficit hyperactivity disorder and restless leg syndrome, and because is highly expressed in neurogenic brain areas during the development of the CNS, suggesting a role for PTPRD in brain development. Therefore, we asked whether PTPRD mutations could cause aberrant cortical development by perturbing embryonic neurogenesis. We used primary cultures of E12.5 cortical precursors cells and embryonic brains at different stages obtained from PTPRD−/− mice. Then, we evaluated whether PTPRD regulates self-renewal, proliferation, and differentiation of NPCs during embryonic neurogenesis. We showed that loss of PTPRD caused aberrantly increased neurogenesis. PTPRD mediated these effects by dephosphorylating TrkB and PDGFRβ, two known receptor tyrosine kinases associated to neurogenesis, and loss of PTPRD caused hyperactivation of TrkB, PDGFRβ and their downstream MEK-ERK signaling pathway in NPCs. Moreover, inhibition of aberrant MEK activation by specific pharmacological inhibitors or shRNAs rescued the increased neurogenesis caused by loss of PTPRD in NPCs. These results suggest that PTPRD regulates RTKs to ensure appropriate developmental neurogenesis, providing a mechanism for its genetic association with neurodevelopmental disorders.

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Program Abstract #244
Let-7 mediates progenitor cell cycle length during S/G2 in the embryonic retina and cerebral cortex
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Neural progenitors in the developing central nervous system (CNS) must generate mature cell populations in a specific proportion that ensures correct synaptic connectivity and tissue function. In neural progenitors, cell proliferation is tightly regulated and coordinated with cell fate decisions. In many regions of the CNS, cell divisions are very rapid during early development, with cell cycle length progressively increasing as more cells exit the cell cycle to differentiate when development advances. Unfortunately, our understanding of the mechanisms and molecules linking cell cycle progression to developmental time and cell fate is incomplete. Here, we show by in situ hybridization that let-7 is up-regulated in neural progenitors as development proceeds. Interestingly, our in situ hybridization, flow cytometry, and fluorescent miRNA sensor analyses suggest that the level and activity of let-7 oscillates as neural progenitors progress through the cell cycle. We also show that let-7 mediates cell cycle dynamics: increasing the level of let-7 in a human derived retinal cell line (HER10 cells) and mouse cortical stem cells leads to a significant increase in cell cycle length and promotes cell cycle exit while let-7 knock down in these systems shortens the cell cycle. Furthermore, live imaging in HER10 cells using a cell cycle reporter indicates that let-7 specifically regulates S/G2. Together, our findings suggest that let-7 links cell proliferation to developmental time and regulates the progressive cell cycle lengthening that occurs during development. In our ongoing investigation, we used RNA-sequencing in HER10 cells to identify putative let-7 targets. Our preliminary results suggest that let-7 may regulate multiple components of the cell cycle machinery and Activin signaling pathway. Study funded by R01:EY026942 to A.L.T and F32:EY030349 to C.L.F.

Program Abstract #245
Investigating the emergence of vertebrate cranial cartilage diversity
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Cartilage is a specialized connective tissue with structural roles in many parts of the vertebrate body. Depending on location and developmental stage, cartilaginous tissues in the body exist in a range of phenotypic forms with different physiological properties. In mammals, this diversity is most striking in the head, where the more abundant hyaline cartilage coexists with the histologically distinct elastic cartilage in the external ear and larynx. Yet in studies of cartilage development, this diversity is often overlooked. Our single-cell RNA sequencing of cranial neural crest derivatives in zebrafish reveals unexpected diversity of cartilage subtypes in the larval and adult head. Through in situ validation, I find a cartilage sub-cluster that localizes to the gills and displays histological properties reminiscent of mammalian elastic cartilage. Through single-nuclei ATACseq analysis of chromatin accessibility, we have further identified putative enhancers specific for hyaline versus elastic cartilage and have validated an enhancer associated with ucmab enhancer as being hyaline-specific in zebrafish transgenics. I will discuss ongoing efforts to use motif enrichment analysis of differentially accessible chromatin regions to identify key transcription factors that specify elastic versus hyaline cartilage. These studies will ultimately uncover the regulatory nodes involved in cartilage subtype diversification. In addition to addressing the development and evolution of vertebrate cartilage subtypes, my research will have implications for repairing elastic cartilage defects, such as those resulting from external ear malformations in craniofacial syndromes. This project is being supported by the NIH T32 HD060549 training grant.

Program Abstract #246
hoxb5b enhances neural crest cell production along the vagal axial region in a temporally-defined manner during zebrafish embryogenesis
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Neural Crest Cells (NCCs) contribute to numerous cell types throughout the vertebrate body during development; notably pigment cells, glia, and certain neuronal populations, such as enteric neurons. Remarkable research efforts have illuminated many of the genetic regulatory networks that underpin NCC specification and migration. Much still remains to be described, however, with respect to the spatiotemporal and genetic mechanisms that govern NCC localization patterns throughout the developing embryo, especially in caudal NCC populations such as the Vagal NCCs. Using the vertebrate model zebrafish (Danio rerio), we provide evidence for a developmentally restricted window in which hoxb5b controls NCC production and spatial localization along the embryo. hoxb5b is expressed along the vagal neural tube and within subsets of NCCs during NCC specification phases. Using an inducible fish line, we discovered that when hoxb5b expression was elevated during discrete times between 21-24 hours post fertilization (hpf), the NCC markers crestin and foxd3 were expanded within vagal axial regions, but not the trunk, between 24-26 hpf. In particular, we found that an ectopic pulse of hoxb5b expression at 21 hpf was sufficient to elicit significantly more enteric neurons when compared with control larvae by 5 days post fertilization (dpf). Intriguingly, this result implies that a hoxb5b-mediated increase in NCC localization along the vagal level embryo later enhances the production of NCC-derived enteric neurons. Together, these data highlight that hoxb5b is sufficient to influence NCC production during early embryogenesis and that it affects the subsequent NCC-derivative populations. We have shown that hoxb5b plays an essential role in driving NCC behavior, increasing our understanding of the molecular mechanisms that regulate NCC development. Funding provided by Cancer Prevention & Research Institute of Texas (CPRIT) Recruitment of First-Time, Tenure-Track Faculty RR170062

Program Abstract #247
Clonal analysis reveals multipotency of vagal neural crest cells toward cardiac and enteric fates
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Neural crest stem cells arising within the post-otic neural tube migrate long distances to form diverse cell types, ranging from heart muscle to enteric ganglia. Mistakes in their development lead to some of the most common
congenital birth defects. A long-standing question is whether these neural crest precursors are multipotent or predetermined toward neuronal versus mesenchymal fates. Here, we tackle this question by performing lineage tracing of chick neural crest cells at single-cell resolution using two complementary approaches: retrovirally mediated multiplex clonal analysis and a single-cell photoconversion assay. Both methods show that the majority of precursor cells are multipotent, with clones frequently producing both mesenchymal and neuronal derivatives in the heart and gut. Time-lapse imaging further shows that stochastic cell migration of sister cells leads to distinct destinations. Taken together, the results suggest that environmental influences rather than intrinsic information govern cell fate choice of vagal neural crest stem cells. This work is supported by NIHR01DE027568 and NIHO1HL14058 to M.E.B.

**Program Abstract #248**  
**Reprogramming Axial Level Identity to Rescue Neural-Crest-Related Congenital Heart Defects**  
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The cardiac neural crest arises in the hindbrain, then migrates to the heart and contributes to critical structures, including the outflow tract septum. Chick cardiac crest ablation results in failure of this septation, phenocopying the human heart defect persistent truncus arteriosus (PTA), one of the most common birth defects in newborns. Trunk neural crest, when implanted in place of cardiac neural crest, fails to rescue its loss. Here, we probe the molecular mechanisms underlying the cardiac crest’s unique developmental potential to give rise to structures in the developing heart, with a focus on outflow tract morphogenesis. Transcriptional profiling identified cardiac-crest-specific transcription factors, with single-cell RNA sequencing revealing surprising heterogeneity, including a previously overlooked ectomesenchymal subpopulation within the early migrating population. Loss-of-function analyses uncovered a transcriptional subcircuit, comprised of Tgif1, Ets1, and Sox8, critical for cardiac neural crest and heart development. Importantly, ectopic expression of this subcircuit changed the transcriptional signature of resident trunk neural crest cells and was sufficient to imbue them with the ability to rescue PTA after cardiac crest ablation. Together, our results reveal a transcriptional program sufficient to confer cardiac potential onto trunk neural crest cells, thus implicating new genes in cardiovascular birth defects. Funding sources - National Institutes of Health, American Heart Association, and the Company of Biologists.

**Program Abstract #249**  
**Maintaining neural crest multipotency with canonical Wnt signaling**  
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The neural crest (NC) cells are a temporary population of multipotent stem cells that generate a diverse array of cell types. Defective NC development can cause severe structural birth defects, such as craniofacial anomalies and congenital heart disease. However, the mechanisms maintaining the stemness of the NC cells remain poorly understood. We used single-cell RNA sequencing and Cre/loxP conditional gene-targeting approaches to address the molecular mechanisms maintaining the stemness of NC cells in mouse embryos. The single-cell transcriptome atlas shows that NC cells start to differentiate as early as they emigrate from the dorsal neural tube. Lrp5 and Lrp6 are single-span transmembrane coreceptors in the canonical Wnt signaling pathway. Conditional inactivation of Lrp6/Lrp5 in the NC cells results in craniofacial and cardiovascular defects. Mechanistic studies suggest that Lrp6/Lrp5-mediated Wnt signaling is essential for NC cell expansion and maintenance of their multipotency in order to generate diverse of NC derivatives. This project is supported by the National Institutes of Health (R01DE026737 & R01NS102261 to C.Z.) and Shriners Hospitals for Children (research grant 85105 to C.Z.).

**Program Abstract #250**  
**A two-step mechanism for foveolar cone patterning in human retinal organoids**  
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Johns Hopkins University, USA

The mechanisms that generate patterns of cell fates unique to humans are poorly understood. A region of particular interest is the high-acuity foveola, the central region of the macula. The central retina becomes
impaired during macular degeneration, a leading cause of vision loss. The foveola is characterized by the dense packing of green and red cones and the absence of blue cones, suggesting regionalized regulation of cone subtype specification. The current model proposes that migration of cones determines this distinct pattern. Our data support an alternative mechanism in which blue cones are born and then transdifferentiate into green/red cones in the foveola. We observed extensive co-expression of blue and green/red opsin in cones at the presumptive foveola and surrounding areas in fetal retinas, a phenomenon rarely observed in adults. scRNA-seq revealed that Cyp26a1, which decreases retinoic acid (RA) signaling, is expressed in the foveola early, and Dio2, which increases thyroid hormone (TH) signaling, is expressed later. Testing the roles of these pathways in human retinal organoids, we found that low RA signaling promotes green/red cones and high signaling promotes blue cones early in development. Increasing TH signaling late in organoid development, after the specification of blue cones, converts blue cones to green/red fate. Our data support a two-step mechanism for foveola development in which low RA signaling promotes green/red cones and suppresses blue cones early and high TH signaling converts the remaining blue cones to green/red cone fate late in development. Our work suggests that human cone fates are plastic during development and provides a mechanism for cone patterning in the foveola.

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Program Abstract #251
Basal epidermis collective migration and local Sonic hedgehog signaling promote fin skeletal branching morphogenesis
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Highly conserved signaling pathways direct appendage patterning, underscoring the deep evolutionary homology of fins, limbs, rays, and digits. Zebrafish fin appendages robustly develop and regenerate elaborately branched bony ray skeletons. We recently showed Sonic hedgehog (Shh) specifically promotes ray branching during adult caudal fin regeneration by basal epidermal-initiated signaling that directs progenitor osteoblasts (pOb) into split pools. We investigated if and how Shh signaling similarly functions during development. shha is expressed by small basal epidermal domains overlying pOb pools at the distal aspect of outgrowing fins. Basal epidermal cells continuously migrate distally, upregulating shha only when passing over pOb. Lateral splitting of each shha+ domain followed by Hh-responsive pOb precedes ray branching. Kaede photoconversion of TgBAC(ptch2:Kaede) fish, which report on Hh pathway receptor and activity indicator Ptch2, revealed Hedgehog/Smoothened (Hh/Smo) signaling is restricted to shha+ basal epidermis and neighboring pOb. Hh/Smo-inhibition using the small molecule BMS-833923 prevented branching in all fins, paired and unpaired, with minimal effects on outgrowth. Phased Hh/Smo inhibition indicates Hh/Smo signaling acts throughout the branching process. shha+ basal epidermal cells and pOb form extensive surface contacts in distal developing rays. We used live time-lapse imaging and cell tracking to find Hh/Smo signaling restrains basal epidermal cell distal migration by apparent tethering to pOb. We conclude short-range Shh/Smo signaling enables ray branching by positioning pOb during development and regeneration. We propose instructive basal epidermal movements and Shh/Smo-promoted heterotypic cell adhesion directs zone of polarizing activity (ZPA)-independent branching morphogenesis to pattern fin skeletons. This work was supported by a T32 Genetics Training Grant 5T32GM007413-42 funded by the University of Oregon and NIH/NIGMS.

Program Abstract #252
Hedgehog-activated Fat4 and Planar Cell Polarity Pathways Mediate Mesenchymal Cell Clustering and Villus Formation in Gut Development
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The mammalian intestine is lined with millions of finger-like projections, termed villi. These villi are critical for maximizing nutrient absorption, digesting food and serving as a barrier from the harsh luminal environment. As such, compromised villi can lead to serious diseases including malabsorption, short bowel syndrome, celiac and others. Therefore, understanding how intestinal morphogenesis occurs is essential for regenerative therapies for these diseases. Although villi are precisely patterned by a network of signalling pathways during embryogenesis, most notably Hedgehog (Hh), it remains unclear as to how these signals translate into distinct morphogenetic transformations. Using the mouse model, our RNA-seq analyses coupled with GLI2 (Hh-transcriptional activator) ChIP-seq reveal that planar cell polarity (PCP) genes such as \textit{Fat4}, \textit{Dchs1} and \textit{Vangl1}/2 are direct targets of Hh in the gut mesenchyme. Notably, mice deleted and/or mutated for these genes exhibit severe villus fusions and fail to form mesenchymal clusters, demonstrating for the first time the importance of PCP in villus formation. Furthermore, genetic interaction studies reveal that the core-PCP axis (\textit{Vangl2}) acts in parallel to the atypical cadherin axis (\textit{Fat4}, \textit{Dchs1}) in maintaining PCP. By utilizing live light-sheet fluorescence microscopy, we have visualized and tracked stromal cell behavior in the embryonic intestine ex vivo and have identified perturbed cell migration and orientation upon \textit{Fat4} knockout. Additionally, we have been able to model this stromal behavior in vitro using 3D-culture based approaches. Together, we introduce Hh-activated stromal PCP as novel mechanisms required for morphogenetic cell behavior, critical for villus formation. Funding sources: This work was supported by SickKids Foundation start-up, the NSERC discovery grant, the March of Dimes Basil O’Connor Starter Scholar Research award, the SickKids Restracco and Ontario Graduate Scholarship awards.

Program Abstract #253

Progestosterone-WNT5A signaling mediated uterine luminal folding is essential for embryo-uterine axis alignment
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In preparation for embryo implantation, the uterine lumen in mice undergoes architectural changes to form structures known as uterine crypts. Once the embryo attaches, the site of attachment develops into a structure known as implantation chamber. Histologically defined two-dimensional (2D) crypts overlap with three-dimensional (3D) uterine luminal folds as seen by confocal microscopy and 3D image reconstruction. Here we use a finer time course of mouse pregnancy to show that on gestational day (GD) 3 of pregnancy, the pre-implantation folds (crypts) display an organized pattern along the uterine mesometrial-antimesometrial (M-AM) axis. Post attachment, on GD4.5, the embryo homes specifically at the AM pole with its embryonic axis along the uterine M-AM axis, leading to the formation of a post-implantation chamber. Using a uterine WNT5A-deficient mutant, we observe that aberrant pre-implantation folds on GD3.5 prevent the alignment of the embryo’s embryonic-abembryonic axis along the uterine M-AM axis on GD4.5. Poor alignment of the embryo in turn results in abnormal post-implantation chamber formation and mis-oriented growth of egg cylinder on GD5.5. Our data provides new insights into the permissive role of WNT5A mediated organized luminal folding in aligning the embryonic axis with the uterine M-AM axis during embryo attachment to allow formation of a functional post-implantation chamber. We also observe that an interaction between progesterone signaling and WNT5A regulates organized uterine folding. Our mouse models with disrupted folding provide a system to explore the role of endometrial folds described in the human uterus. Our data suggests that progesterone-WNT5A signaling regulates pre-implantation 3D uterine luminal folding and disrupting the organized folding pattern affects post-implantation chamber formation and embryo orientation, leading to poor pregnancy outcomes. This project was funded by startup funds provided by Michigan State University.

Program Abstract #254

ALAn: A tool for automated analysis of epithelial layer architecture
Nicole Dawney, Christian Cammarota, Dan Bergstralh

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Epithelial tissues are typically comprised of layers; sheets of cells that are one-cell thick. During tissue development, the integrity of these layers must be maintained despite the potentially disruptive single-cell behaviors (e.g. divisions and rearrangements) that participate in morphogenesis. We are addressing the question of how integrity is maintained using Madin Darby Canine Kidney (MDCK) cells, which can form a polarized epithelial layer in culture. We and others have observed that these layers are frequently characterized by extralayer cells, positioned above (apical to) the layer. One possible origin for extralayer cells is an initial failure in monolayer formation, meaning that cells “clumped” on top of each other during plating. Another possibility is that newly born division products fail to integrate into the extant layer. To investigate the aetiology of misplaced cells, we have developed an analysis pipeline called Automated Layer Analysis (ALAn) to quantify aspects of MDCK cell layer architecture. Using this tool we can automate quantification of layer characteristics, including density, layer height and the number of extralayer cells. We are now using ALAn to investigate how cell division and cell-cell adhesion contribute to layer development. Research supported by NIGMS R01GM125839.

Program Abstract #255
Dunk interacts with anillin and regulates its cortical localization during Drosophila cellularization
Jiayang Chen, Melissa Wang, Bing He
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Cleavage is a common step of early embryonic development, generating a monolayer of epithelial cells at the surface of the embryo called “blastoderm”. In Drosophila, this process is achieved through cellularization, a special form of cytokinesis that partition the peripheral syncytial nuclei into individual cells. Similar to typical animal cytokinesis, cellularization is initiated by recruiting non-muscle myosin II (“myosin”) to the cleavage furrows, which involves a cortical flow of myosin towards the leading edge of the newly formed furrows. We have previously identified a novel gene dunk that is required for cortical retention of myosin as myosin flow occurs, but the underlying mechanism is unclear. Through a genome-wide yeast two-hybrid screen, we identified anillin (Scraps in Drosophila), a conserved scaffolding protein involved in cytokinesis, as the primary binding partner of Dunk. Dunk binds to the highly conserved C-terminal domain of anillin, which also contains binding sites for several important regulators for anillin, including Rho1 and PI(4,5)P2. Live imaging analysis indicates that myosin and anillin colocalize at nascent furrows at the onset of cellularization. Dunk does not appear to affect the rate of nuclear import of anillin during cellularization, but the localization of anillin to the cleavage furrows is severely disrupted in the absence of Dunk. anillin mutants and dunk mutants showed comparable cortical myosin loss phenotype during early cellularization. Furthermore, both mutants showed similar synthetic effects when combined with the loss of Bottleneck, an actin cross-linking protein controlling actomyosin organization during cellularization. We propose that Dunk facilitates cortical myosin retention during early cellularization by regulating the localization of anillin through direct interaction. This research is supported by NIGMS ESI-MIRA R35GM128745 and the start-up fund to B.H.

Program Abstract #256
Investigating growth regulation within synchronously developing epithelia
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Animal development often requires multiple epithelia to grow in coordination with each other to produce functional multilayered organs. The Drosophila wing disc, the precursor to the adult wing and thorax, consists of two associated epithelia- the disc proper (DP) and the peripodial epithelium (PE)- which grow synchronously during larval development, providing an ideal model to study how multiple epithelia coordinate growth. To determine which signals might pass between the layers to coordinate growth, we investigated whether the layers came into contact sufficiently to mediate juxtacrine signaling, using a synthetic juxtacrine signaling system (He et al 2017). We found close contact between the layers was restricted to specific regions of the disc. Two conserved morphogens, Hedgehog (Hh) and Decapentaplegic (Dpp/TGF-B), are key regulators of DP growth; we investigated their role in the growth of the PE with the goal of understanding coordinated epithelial growth. We showed that PE Dpp is Hh-dependent by raising larvae with a temperature-sensitive Hh allele to a high temperature and observing loss of Dpp signaling. By knocking down expression of the Hh receptor in the PE, using previously
unpublished PE-specific genetic tools which we identified, we determined that Hh signaling is required for PE growth overall, but is dispensable at late growth stages. This is in contrast to the DP. Similarly, by knocking down expression of a Dpp receptor in the PE, we found that Dpp signaling is surprisingly dispensable for PE cell survival. We confirmed this result by making mitotic clones in the PE that lack the Dpp receptor, observing that these clones survive and proliferate. These data demonstrate that growth regulation within the PE does not operate as a simple parallel to canonical DP growth, and suggests that alternative regulatory pathways may be more important. This work was funded by NIH grants R35 GM122490 and T32 GM132022.

Program Abstract #257
Transgenic sensors to measure nuclear mechanotransduction in vivo
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In addition to shaping tissues during development, emerging research reveals mechanical forces have a role in the regulation of gene expression. A traditional view of mechanotransduction is that extracellular and intracellular forces elicit a biochemical response that transmits information to the nucleus. Intriguingly, there is also evidence in vitro that nuclear strain can directly influence transcription. Forces are transmitted from the cytoskeleton to the nuclear lamina by the LINC complex in a direct mechanical fashion which has the potential to efficiently couple morphogenesis with gene expression. To facilitate investigation of nuclear mechanotransduction, we generated two transgenic FRET-based tension sensors to measure nuclear force transmission in vivo. The proteins into which we integrated tension sensor modules are Nesprin 2G which links actin to SUN proteins, and the inner nuclear membrane protein NEMP1. The transgenes were knocked-into the mouse Rosa26 locus and are expressed conditionally. For each sensor strain, we generated control strains that either lacked the head or tail region of the protein, the FRET donor or acceptor. We tested the responsiveness of each sensor to physical and chemical manipulation in the mouse embryo and developed software (FLIMvivo) to resolve challenges unique to fluorescence lifetime imaging analysis in vivo. These tools have the potential to more precisely test hypotheses concerning mechanisms of mechanotransduction. We thank CIHR for funding.

Program Abstract #258
A Precision Stretcher for Developmental Mechanobiology
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The mechanical microenvironment can play a key role in developmental processes, such as cell proliferation, differentiation, and tissue morphogenesis. To carry out a systematic analysis of these roles requires effective manipulation of mechanical stimuli in living tissues, combined with high-resolution live imaging. Ideally, experimental approaches should strive to produce tissue deformations and rates that are analogous to endogenous mechanical cues. However, current approaches are generally limited to a maximum of 30% strain in living embryonic tissues. Live imaging is also compromised in these systems, typically limited to low numerical aperture 20x objectives. Here we introduce a custom-designed tissue stretcher capable producing tensile strain > 100% in Xenopus laevis organotypic explants, that mimics developmental strains observed during morphogenesis. This device has been designed and fabricated for a motorized confocal microscope stage to allow simultaneous collection of high-resolution live images with high numerical aperture objectives. Using this tool, we have been able to visualize gastrula and neurula stage non-neural ectoderm subjected to high strain for more than 1 hour. Unlabeled explants can be tracked with brightfield illumination or after expressing mem-RFP with spinning disk confocal microscopy. Surprisingly, we observed few cell rearrangement events in these highly stretched samples, instead, we observed heterogeneous cell-to-cell deformation and a range of behaviors, including cell division during the relaxation phase. Tools such as ours can also yield quantitative data on the effect of mechanical stimuli on protein transport and localization, cytoskeletal dynamics, and cell signaling that are central to patterning and morphogenesis. Acknowledgements: This work has been supported by a grant from the NIH to LAD (R01 HD044750).
Program Abstract #259
A feedback mechanism mediated by myosin-dependent apical accumulation of Rab11 vesicles in apical constriction
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During tissue morphogenesis, cell shape changes driven by mechanical forces often require active regulation of intracellular trafficking. It is not fully understood how mechanical stimuli influence intracellular trafficking and how such regulations impact tissue mechanics. To address these questions, we investigated the behavior of Rab GTPases, the master regulators of intracellular trafficking, during apical constriction-mediated mesoderm invagination in Drosophila. We found that during apical constriction, vesicle-like structures marked by Rab11, a recycling endosome marker, become enriched in the vicinity of apical myosin. The apical accumulation of Rab11 vesicles does not require endocytosis. Instead, it depends on vesicle transport along microtubules and is sensitive to disruption of myosin activity. Our quantifications further reveal that the transport is substantially biased towards the apical direction. Acute inhibition of myosin rapidly eliminates the directional bias of transport and inhibits apical accumulation of the vesicles, suggesting that myosin activity may promote apical enrichment of Rab11 vesicles by influencing the directionality of the transport. At the apical domain, Rab11 vesicles are moderately enriched around the adherens junctions, suggesting a role of these vesicles in regulating adherens junctions. Injection of dominant negative Rab11 proteins can acutely inhibit Rab11 vesicle accumulation, which does not obviously affect apical myosin accumulation but instead causes fragmented apical adherens junctions, altered spatial organization of apical myosin, frequent myosin breaks and a reduction in apical constriction rate. We propose that apical myosin-induced biased transport of Rab11 vesicles serves as a feedback mechanism to promote proper organization of the contractile machineries and thereby facilitate apical constriction. Funding: This research is supported by NIGMS ESI-MIRA R35GM128745 & ACS #IRG-82-003-33.

Program Abstract #260
Regulation of cell contact and tissue organization by Eph/ephrin signaling
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Cellular self-organization by cell segregation, leads to boundary formation and is critical for the organization of morphogenetic movement and tissue patterning. Signaling between membrane-bound EPHRINS and EPH receptor tyrosine kinases is essential in boundary formation, driving segregation between EPHRIN-expressing and EPH-expressing cells. Here we examine how EPH/EPHRIN signaling modulates interfacial tension to regulate cellular contacts and drive cellular self-organization. Using a cell culture system to model EPH/EPHRIN cell segregation we analyzed the contact angle of cells to estimate interfacial tension between EPHB2- and EPHRIN-B1-expressing cells and observe an increase in interfacial tension relative to homotypic cell pairs. Inhibitors of actomyosin contractility significantly diminish this increase, suggesting that actomyosin contractility drives heterotypic interfacial tension. Cell segregation assays reveal that EPH/EPHRIN driven segregation is actomyosin contractility dependent. Further, atomic force microscopy shows that EPH/EPHRIN signaling results in increased cortical tension during cell segregation. Interestingly, actomyosin contractility also drives increased EPHB2:EPHB2 homotypic contacts through an increase in tension away from the cell contact. Using a mouse model involving mosaicism for EPHRIN-B1, resulting in cell segregation between EPHRIN-B1 expressing and non-expressing cells, we are able to demonstrate that actomyosin contractility is critical for cell segregation in vivo. Finally, we demonstrate that tissue-wide changes in cellular organization and tissue shape are driven by minimization of heterotypic contact. These data suggest a model for cell segregation and tissue organization in which EPH/EPHRIN signaling results in a cortical actin differential that prevents cells from making stable contacts and drives cell segregation, and ultimately affects tissue morphology by modulating interfacial tension. NIH/NIDCR F31DE028175 to A.K.

Program Abstract #261
Exploring the passive mechanics of early neurulation through computational modeling
Sommer Anjum, Lance Davidson
Convergent extension (CE) shapes embryos and organs by narrowing a tissue in one direction and lengthening it in the orthogonal direction. For instance, the vertebrate neural epithelium elongates via directed cell intercalation as it forms the neural tube, the precursor of the spinal cord. Failure of this process leads to birth defects such as spina bifida. CE is proposed to be the result of a coordination between biochemical patterning and mechanical responses from passive and active elements. However, it has been difficult to dissect contributors with traditional experimental design, so we have adopted a computational modeling approach inspired by ongoing studies of *Xenopus* neural CE. To isolate the passive responses to tissue field elongation from contributions from active cellular processes, we simulate epithelial responses to external CE forces. In the model, cells are represented as interacting particles in a rectangular bounding box. Boundaries are moved such that the field of cells undergoes area-conserving CE. We present a new method of tessellation of cell centroids to better reflect the physics of the system and represent radial cell intercalation and extrusion. Time series of tessellated polygonal cell networks are analyzed to quantify deformation and strain both globally at the tissue scale and locally by considering the domain of a cell and its immediate neighbors. We also quantify cell neighbor exchanges and cell area fluctuations. Recapitulating endogenous rates of *Xenopus* neural CE reveal neighbor exchanges and directional remodeling that mimic *in vivo* observations. Extending our simple simulations by incorporating active cell elements such as actomyosin contractility and junctional remodeling will enable quantitative assessment of specific biochemical and mechanical drivers of tissue shape change. We thank NIH for our funding, particularly NICHD for R01 HD044750 and NIBIB for the Biomechanics in Regenerative Medicine T32 Training Grant EB003392.

Program Abstract #262

**Myocardial Afterload is A Key Biomechanical Regulator of Valve Development**

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The incidence of congenital heart disease (CHD) is estimated to be 1% of all human births. CHD of the heart valves occurs in over 50% of CHD cases. Despite significant clinical interest, the molecular mechanisms that govern valve development remain poorly elucidated. As the heart develops, blood flow and blood pressure increase rapidly to support the growing demands of the embryo. Our group has previously shown that pressure at the developing atrioventricular valve dramatically increases through development. Consequently, we hypothesized that afterload—defined as the pressure the ventricle must overcome in order to pump blood through the body—may be a cue that cardiac valve cells read and respond to build a valve leaflet. Here, we present a zebrafish model in which afterload has been increased by vasopressin, a vasoconstrictive drug. We first show that application of vasopressin reliably produces an increase in afterload without directly acting on cardiac tissue in zebrafish embryos. To evaluate cardiac function and valve leaflet dynamics, we took a quantitative live-imaging approach. Consistent with pathology seen in adult human patients with clinically high afterload, we see defects in both form and function of the valve leaflets. To identify the cause of this functional defect, we utilized *in situ* hybridization to evaluate makers of cell differentiation for both valve leaflet cells and the adjacent myocardial cells. Surprisingly, our results suggest that this valve defect is due to changes in atrioventricular myocyte differentiation and signaling, rather than pressure directly acting on the valve leaflet cells. We next took a transcriptomics approach to identify regulators of atrioventricular myocyte differentiation and identified a subset of differentially-expressed transcription factors that are putatively responsible for sensing afterload. Funding from NSF [grant number 1235305], the AHA [grant number 17GRNT33460256], and the Eppley Foundation for Research.

Program Abstract #263

**Ccer2: A Novel Gene Upregulated in the Early Stages of Mammalian Sensory Hair Cell Differentiation**

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Hearing loss affects people of all ages, and so far, there are no therapies for this disorder. *Atoh1*, a helix loop helix transcription factor, is a key regulator in sensory hair cell formation and differentiation in inner ear development. Here, we characterized the transcriptome of sensory hair cells in the early stages of development by identifying
genes upregulated by Atoh1 24 hrs after overexpression. We electroporated embryonic day 13 mouse cochlear explants with an Atoh1 GFP reporter construct or with an empty GFP vector as a control. At this stage of development, overexpression of Atoh1 results in 100% conversion of electroporated cells into hair cells. To identify the immediate genes upregulated by Atoh1 we used fluorescence-activated cell sorting (FACS) to capture cells overexpressing GFP 24hrs after cochlear explants were electroporated. RNA was extracted from both Atoh1 and control cells and RNA-sequencing was performed. We found ~700 genes upregulated, and our differential expression analysis detected several known hair cells genes in the Atoh1 expressing cells. We also identified Ccer2, a novel gene that was significantly upregulated. We confirmed expression of Ccer2 in endogenous cochlear and vestibular hair cells across several embryonic and postnatal stages. To investigate the role of Ccer2 in sensory hair cells, we generated Ccer2 mutant mice, using CRISPR/Cas9 technology and performed ABR and DPOAE to examine hearing and outer hair cell function respectively, as well as balance tests. Our transcriptomic analysis is the first RNA-seq study that characterizes Atoh1 downstream targets activated in the early stages of hair cell differentiation. The discovery of Ccer2 would give insights into both Atoh1 and other signalling pathway(s) where Ccer2 is involved, advancing our understanding of inner ear development and potential hearing loss treatments. Funding: Barberian Scholarship, Dept. of Otolaryngology, UofT.

Program Abstract #264
A gradient of Wnt activity regulates the spatial patterning of inner ear sensory organs
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The inner ear contains distinct auditory and vestibular organs sensitive to sound and head movements. These sensory organs originate from neurosensory-competent domains specified at early stages of otic development, but the molecular signals controlling their formation remain unclear. The transcription factor Sox2 is required for prosensory specification since its absence abolishes sensory organ differentiation. Sox2 is initially present throughout the otic vesicle, then it becomes progressively restricted to two prosensory domains. We demonstrate that changes in the spatial pattern of Sox2 expression are regulated by Wnt/B-catenin signalling. We found that a dorso-ventral (high-to-low) gradient of Wnt activity is present in the otic vesicle. The elevation of Wnt activity in the dorsal vesicle coincides with the loss of dorsal Sox2 expression. Blocking Wnt activity in dorsal otocyst induces ectopic neurosensory territories, whilst in the ventral domains represses Sox2 expression. This suggests that low levels of Wnt activity are required for prosensory specification. Using pharmacological treatments of explanted otocysts, we confirmed that Wnt activity regulates in a dose-dependent manner the size and position of the Sox2-expressing domain. Altogether, these results suggest that a Wnt activity gradient controls the spatial pattern of neurosensory specification along the dorso-ventral axis of the inner ear. The work was funded by Medical Research Council, UK and Action on Hearing Loss, UK

Program Abstract #265
Identifying signals downstream of canonical Wnt signalling during prosensory specification in the inner ear.
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The inner ear is composed of distinct sensory organs crucial for the detection of sound and perception of angular and linear head movement. The molecular mechanisms controlling their development remain unclear. Our ongoing work suggest that Wnt signalling regulates the positioning and size of the inner ear sensory organs. Wnt signalling is a cell communication system operating through secreted Wnt ligands binding to transmembrane receptors to activate expression of target genes, regulating various developmental events. To better understand the molecular cues by which Wnt signalling controls sensory organ patterning, we performed a transcriptome profiling of chicken inner ears after loss of Wnt function. Our results identified a number of candidate target genes. Further expression and functional studies suggest that some transcription factors from this list could directly regulate prosensory specification of the inner ear sensory organs. The work is funded by the MRC.
Program Abstract #266
Investigation of RNA Polymerase II Elongation Factor Ell2 Regulation By the Cataract-Linked RNA-Binding Protein Celf1 in Mouse Lens Development
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The ocular lens, a transparent tissue, enables high-resolution vision by focusing light on the retina. Lens opacity is termed cataract, the leading cause of blindness. We applied a novel bioinformatics approach called iSyTE (integrated Systems Tool for Eye gene discovery) to identify a new cataract-linked gene, Celf1 (CUGBP Elav-like family member 1), which encodes an RNA-binding protein that controls post-transcriptional gene expression. Celf1 lens-specific conditional knockout mice (Celf1loxP/loxP) exhibit severe lens defects starting at embryonic development that culminate into cataract at birth. To gain insight into the molecular basis of the cataract pathology, we performed high-throughput RNA-seq and identified a new factor, Ell2 (Elongation factor for RNA Polymerase II 2), to be abnormally upregulated in Celf1loxP/loxP mouse lenses. Ell2 encodes a “transcription elongation” factor that interacts with RNA polymerase II and increases its elongation rate along template DNA, and also participates in mRNA splicing. Further, Ell2 exhibits high iSyTE lens-enriched expression scores, further supporting it as a new key regulator of lens development. Indeed, by performing Celf1-antibody based RNA-immunoprecipitation (RIP) on wild-type (WT) mouse lens, we find that Celf1 protein directly associates with Ell2 mRNA. To further investigate Ell2 function in the lens, I generated lens-specific conditional Ell2 knockout mice (Ell2loxP/loxP) by crossing mice carrying Ell2loxP/loxP conditional knockout alleles with mice carrying lens-specific promoter-driven Cre recombinase (Pax6GFPCre). Phenotypic analysis demonstrates that Ell2loxP/loxP mice have abnormally smaller lenses compared to age-matched WT and heterozygous littermate control. Further characterization indicates abnormal lens fiber cell morphology in Ell2loxP/loxP mice, suggesting its key function in lens development. Thus, this research identifies a new regulatory protein, Ell2, to be linked to lens defects.

Program Abstract #267
Elucidating the mechanism by which OCT4 and GATA6 direct primitive endoderm cell fate in pre-implantation mouse embryos via repression of TE genes
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A few cell divisions into their life, the cells of the embryo must make a choice, what do I become and how do I get there? These cells can become outside trophectoderm (TE) which go on to be the placenta, or one of the two cell types in the inner cell mass (ICM), the epiblast (EPI) or primitive endoderm (PE). Becoming the ICM is a particular challenge for the developing embryo as both EPI and PE cell fates have been shown to be driven by the same factor, POU5f1 (OCT4). The goal of our study is to learn how ICM cells know what to become when both EPI and PE fates are driven by the same factor, POU5f1 (OCT4). The goal of our study is to learn how ICM cells know what to become when both EPI and PE fates are driven by the same transcription factor, at the same stage of development.
OCT4 is known to pair with EPI specific factor SOX2 to drive EPI development; however, it is not known how OCT4 drives PE development. I hypothesize that OCT4 is also working with a PE specific factor to drive PE development. The factor I have been investigating is GATA6. GATA6 expression is specific to PE cells within the ICM, and loss of GATA6 has previously been shown to phenocopy loss of OCT4, resulting in loss of expression of PE specific factors SOX17 and GATA4. To further explore the loss of GATA6 and OCT4, we use RNA-seq on OCT4-/- and OCT4-/- mouse embryos. Results of our RNA-seq analysis suggest a role for OCT4 and GATA6 in the repression of TE specific factors. TE specific factor FABP3 shows a 5-fold and 4-fold increase in OCT4-/- and GATA6-/- embryos, respectively. While TAGLN2, another TE specific factor, shows a 2-fold and 4-fold increase in OCT4-/- and GATA6-/- embryos, respectively. This up regulation of TE specific factors when OCT4 and GATA6 are lost may indicate these factors work together to indirectly drive PE development by repressing TE gene expression.
Funding: Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health T32HD087166

Program Abstract #268
Chromatin topology and the timing of enhancer function at the HoxD locus
Eddie Rodriguez Carballo1, Lucille Lopez-Delisle2, Andréa Willemin1, Leonardo Beccari1, Sandra Gitto1, Bénédicte...
In tetrapods, the HoxD gene cluster is critical for proper limb formation. In the emerging limb buds, different sub-groups of Hoxd genes respond first to a proximal regulatory signal, then to a distal signal that organizes digits. These two regulations emanate from the two TADs flanking HoxD, both containing a range of appropriate enhancer sequences. The telomeric TAD (T-DOM) contains several regulatory elements controlling Hoxd genes, initially in a temporal manner and then in the proximal presumptive forearm. T-DOM is divided into two sub-TADs separated by a CTCF-rich boundary defining two regulatory modules with most limb enhancers concentrated in the most distant module. In order to understand the importance of this regulatory topology to elicit a precise Hoxd gene transcription in time and space, we both deleted or inverted this sub-TAD boundary and eliminated the CTCF binding sites. These perturbations caused a time delay in gene activation, which was subsequently resumed. We then inverted the entire T-DOM to change the respective position of the two sub-TADs, which concomitantly introduced a TAD boundary between HoxD and the inverted T-DOM. This re-arrangement had a stronger impact on the early expression and flattened the Hoxd mRNAs levels. The latter effect was rescued by re-granting access to the enhancers upon deletion of the ectopic boundary. These results highlight the importance of regulatory topologies in the temporal control of gene expression. We also show that, along with time, the affinity of enhancers to find their natural target genes can overcome the presence of a strong TAD border and an unfavourable orientation of CTCFs. This project was funded by the SNF and the ERC.

Program Abstract #269
Retinoic acid differentially affects first and second heart field progenitor development within the anterior lateral plate mesoderm
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Tight regulation of retinoic acid (RA) levels is critical for normal heart development in all vertebrates. Thus, a better understanding of how RA signaling patterns cardiac progenitors will provide insights into congenital heart defects. Despite studies showing that RA signaling has a conserved requirement restricting the size of the cardiac progenitor field within the anterior lateral plate mesoderm (ALPM), how RA signaling affects the populations of earlier-differentiating first heart field (FHF) and later-differentiating second heart field (SHF) progenitors remains poorly understood. Here, to elucidate how loss of RA signaling affects these different cardiac progenitors within the ALPM, we first quantified the number of cardiac progenitors and earlier differentiating cardiomyocytes in DEAB-treated zebrafish embryos using immunostaining for the pan-cardiac marker Nkx2.5 and sarcomeric myosin (MHC), respectively, at the 20 somite (s) stage and 24 hours post-fertilization (hpf). We found that while the overall number of Nkx2.5+ cells was increased, there was a significant expansion in the relative proportion of differentiating FHF cardiomyocytes (CMs) compared to SHF progenitors in DEAB-treated embryos within these cells. Similar results were seen using aldhlα2 zebrafish mutants. To examine the effect on SHF-derived CMs, we next used a temporal differentiation assay where differentiated CMs were photoconverted from green-to-red using a KikGR protein in 24 hpf zebrafish embryos. Consistent with the reduced number of SHF progenitors, we found there is a decrease in the number of green-only later-differentiating SHF-derived CMs in the outflow tract of DEAB-treated embryos. Together, our data show that loss of RA signaling in zebrafish embryos results in an increase in the FHF at the expense of the SHF within the ALPM, suggesting RA signaling determines the relative ratios of these cardiac progenitors within an enlarged cardiac progenitor pool. (Funding: NIH)

Program Abstract #270
Profiling Atrial-Ventricular Specification During Early Mouse Development Through Single Cell RNA Sequencing
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The molecular mechanisms driving atrial and ventricular fate acquisition in vivo are incompletely understood. Others have sought to understand this fate specification by profiling transcriptional differences between the
chambers through RNA sequencing, but much of this work is at stages after lineage specification. We have previously identified a cardiac progenitor population that transiently expresses Foxa2 during gastrulation and gives rise to progeny that form ventricular, but not atrial myocytes. Foxa2 lineage tracing thus allows us to track ventricular progenitors prior to and during the morphogenetic events that drive chamber formation. In this study, we sub-dissected the cardiac tissues and surrounding areas of Foxa2-Cre; mTmG embryos at the cardiac crescent (E8.25), primitive heart tube (E8.75), and late heart tube (E9.25) stages and performed single-cell RNA sequencing (scRNAseq) to profile the heterogeneity of cardiac precursors and identify mechanisms driving atrial and ventricular specification. We performed clustering and differential gene expression analysis on >10,000 cells at each stage. We identified several cardiac subpopulations corresponding to early progenitor populations, as well as other non-cardiac cell populations known to provide important signaling cues directing cardiac development. We find that Foxa2 lineage-traced cells can be identified on the basis of EGFP expression without the need for cell sorting, and that distribution of EGFP expression within cardiac lineages corresponds with clusters forming the developing ventricle. Next we will use computational lineage trajectory tools to track these progenitors over time to understand lineage hierarchies governing atrial/ventricular specification. In summary, combining genetic tools with scRNAseq provides resolution of atrial and ventricular precursors and highlights the sequential specification and differentiation trajectories in early heart development. Funding NIH/NHLBI R01HL134956-03

Program Abstract #271

Nr2f1a maintains nkx2.5 expression to repress sinoatrial node identity within venous atrial cardiomyocytes
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Nr2f transcription factors are conserved regulators of vertebrate atrial development, with NR2F2 mutations in humans being associated with congenital heart defects (CHDs). However, the mechanisms by which Nr2fs function within atrial cardiomyocytes (ACs) remain poorly understood. Here, we performed transcriptomic analysis of isolated ACs from WT and nr2f1a mutant zebrafish at 48 hours post-fertilization (hpf), as zebrafish Nr2f1a is the functional equivalent of mammalian Nr2f2 with respect to heart development. Surprisingly, our results showed increased and decreased expression of core genes that respectively promote and repress sinoatrial node (SAN) differentiation, including tbx3a and decreased nkx2.5. Quantification of SAN cells from 48 to 96 hpf revealed a progressive expansion of SAN identity from the venous pole throughout the atrium of nr2f1a mutant hearts. Furthermore, high-speed time lapse imaging showed that at 72 and 96 hpf nr2f1a mutants have slower calcium conduction across the atria and reduced heart rates compared to WT embryos, consistent with an acquisition of pacemaker identity within nr2f1a mutant ACs. Genetic epistasis using a heat-shock inducible nkx2.5 transgene showed that restoration of Nkx2.5 can repress the SAN expansion in nr2f1a mutant hearts. Furthermore, analysis of open chromatin in isolated ACs identified a putative nkx2.5 enhancer harboring an Nr2f-binding site that is sufficient to promote expression in ACs of transgenic embryos, suggesting that Nr2f1a may directly maintain atrial nkx2.5 expression. Altogether, our results reveal a novel requirement for Nr2f transcription factors in concurrently maintaining AC identity and repressing SAN identity through directly maintaining nkx2.5 expression in vertebrate atria, which may provide insights into the etiology of arrhythmias and CHDs associated with NR2F2 mutations found in humans. Funding: NIH

Program Abstract #272

in vivo epigenetics roadmap of cell fate regulation by the lung lineage transcription factor NK Homeobox 2-1
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Lineage transcription factors mark, promote, and maintain multiple distinct cell types originating from a common progenitor. Despite their essential role, how such factors bind genome wide to orchestrate the epigenetic changes necessary to form and maintain these identities in vivo is unclear. NK Homeobox 2-1 (NKX2-1) is an optimal lineage transcription factor in the lung to investigate in vivo in that it is required in two well characterized cell types, the alveolar type 1 (AT1) cell and alveolar type 2 (AT2) cell, that arise from a common progenitor and are
mobilized in lung injury-repair. Complementing cell-type specific ChIP-seq with single-cell epigenomics and precise genetic knockouts, we demonstrate the genome-wide functions of NKX2-1 in binary cell fate decision and maintenance of stem cell potential in the murine lung. NKX2-1 binding unique to AT1 or AT2 cells required cell-type specific transcription factors TEADs and CEBPa, respectively, to selectively trim NKX2-1 bound sites found in the progenitor and form NKX2-1 de novo binding sites. Common NKX2-1 binding between AT1 and AT2 cells consisted of sites accessible across the epithelial lineage cell types and housekeeping locations accessible across lineages. Interestingly, a subset of common NKX2-1 binding sites were H3K27ac positive exclusively in AT1 cells. Such sites were near AT1 genes first to be expressed developmentally and during AT2 to AT1 differentiation in lung injury repair—suggesting a role for NKX2-1 in maintaining AT2 stem cell potential. Our study not only demonstrates how the lung lineage transcription factor NKX2-1 regulates the development and maintenance of distinct cell epigenomes, but also establishes an experimental paradigm to investigate lineage transcription factors in vivo. This work was supported by the MDACC institutional research grant and start-up funds, NIH R01HL130129, F31 Diversity Fellowship F31HL139095, and Gigli City Family Endowed Scholarship.

Program Abstract #273
Characterization of a New Epithelial Progenitor Cell in the Developing Human Lung
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The human lung begins developing at 4 weeks of gestation when two epithelial buds emerge from the ventral anterior foregut endoderm1,2. The lung buds undergo repeated bifurcations, a process known as branching morphogenesis, to establish the complex, arborized network of the lung3,4. During branching, a specialized population of lung progenitors at the tips of these branches, bud tip progenitors (BTPs), give rise to all epithelial cell types of the lung including proximal airway cells - basal, secretory, ciliated, and neuroendocrine cells - and distal alveolar cells5; they fully differentiate before birth. To better understand how BTPs give rise to a diverse array of cell types that compose the human lung, we recently performed single-cell RNA sequencing (scRNAseq) on the human fetal lung from 10 to 21 weeks gestation6 and identified a novel epithelial cell population characterized by a unique gene expression profile: SCGB3A2hi/SFTPB hi/CFTR hi which we refer to as Fetal Airway Secretory (FAS) cells. FAS cells are localized to small cartilaginous and large non-cartilaginous airways and are not present in healthy adult lung. Trajectory analysis suggests that FAS cells are a previously uncharacterized progenitor cell that gives rise to proximal airway cells. We tested this hypothesis using a BTP organoid model7 which confirmed BTPs give rise to FAS cells through directed differentiation protocols. Furthermore, FAS cell abundance is dependent on NOTCH signaling, specifically through the downstream effector HES1, suggesting NOTCH signaling regulates the cell’s maintenance or differentiation potential. Despite strong evidence for a central role of FAS cells during lung development, the cell types that FAS cells give rise to remain unknown. We propose to evaluate the developmental potential of FAS cells in a human lung organoid model using a novel cell barcoding lineage tracing technique8,9. Funding: NIH MSTP T32 GM007863

Program Abstract #274
Skin-derived ionocytes contribute to hair cell-containing mechanosensory organs in a salinity-dependent manner
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Zebrafish sense water movements via hair cells in neuromast organs of the lateral line. Aquatic vertebrates regenerate these hair cells after damage and extensive studies have defined neuromast cellular composition to identify regeneration competent cells. Using live imaging of fluorescent reporters, we have unexpectedly found a new cell population that contributes to neuromasts. Here we report the discovery and characterization of this novel cell type in the zebrafish neuromast. Using lineage tracing and time lapse microscopy, we show that these cells do not have the same embryonic origin as the other neuromast cell types but are derived from basal keratinocytes of the skin adjacent to the neuromast. They are highly motile cells and enter mature neuromasts as pairs, rearrange before anchoring in specific positions and extend basal and apical cellular protrusions.
Importantly, these invading cells do not express neuromast-specific markers, confirming they do not originate from the lateral line placode and do not transdifferentiate later into lateral line cell types. The cells express Na⁺/K⁺ ATPases, and one cell in the pair is enriched for mitochondria. The same features are also found in mitochondria-rich basal keratinocyte-derived skin ionocytes that are often partnered with an accessory cell, suggesting these cells are ionocytes. Indeed, we discovered that the number of neuromast-associated ionocytes correlates with the water salinity, suggesting that they are involved in controlling the ionic composition of the hair cell environment.

To molecularly characterize the neuromast ionocytes we performed single cell RNA sequencing on sorted cells from zebrafish epidermis and validated candidate genes using in situ hybridization. To our knowledge, this is the first description of an epidermal cell contributing to a mechanosensory organ. This work was funded by the Stowers Institute for Medical Research.

Program Abstract #275
Cell-level analysis of fusion during avian lung development
Michael Palmer, Celeste Nelson
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As it develops, the avian lung transforms from an initially terminally branched epithelial tree to a continuous circuit of airways. This occurs via a large-scale epithelial fusion event, wherein airways that originate proximally collide with those that originate distally, and then generate a single continuous lumen that joins them. We investigated airway fusion in the domestic chicken, Gallus gallus, and found that the process is not stereotyped, instead occurring between airways that are stochastically located adjacent to each other. Prior to fusion, the proximal and distal airways bend away from each other, then initiate and extend new branches which contain the cells that form the first contact. These changes in epithelial shape coincide with the differentiation of smooth muscle cells that wrap the airways, suggesting a possible physical role for smooth muscle in shaping the pre-fusion epithelium. From the resulting nascent branches, individual epithelial cells extend cytoskeletal protrusions that reach toward and form a bridge with their target airway. Additional cells then join the fusion site and build a bilayered epithelium between the two airways. During this process, the basement membrane around the pre-fusion airway epithelium degrades, and then reforms after the airways have fully collided. Par3, however, remains localized to the apical membranes of the fusion cells throughout this process, suggesting that apical polarity is maintained even while cellular extensions and basement membrane remodeling are occurring at the basal surface. The epithelial bilayer then undergoes apoptosis, which clears the path between the two lumens. This process of airway epithelial fusion in the developing chicken lung constitutes a novel mechanism for the generation of complex multicellular epithelial tubes. Funding provided by the HHMI and the Schmidt Fund.

Program Abstract #276
Rab35 regulates skeletogenesis and gastrulation by facilitating actin remodeling and vesicular trafficking
Carolyn Remsburg, Jia Song
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Rab35 is a small GTPase that regulates plasma membrane to early endosome vesicular trafficking and mediates actin remodeling to form actin-rich cellular structures. While the function of Rab35 in the cellular context has been examined, its role during development has not been well studied. In this study, we take advantage of the sea urchin’s high fecundity, external fertilization, and transparent embryos to determine the function of Rab35 during development. We found that loss of function of Rab35 results in defects in skeletogenesis and gastrulation, which were rescued by co-injection of sea urchin Rab35. The loss of Rab35’s function results in decreased endocytosis and impaired exocytosis, which may be important for skeletogenesis and gastrulation. Skeletal spicules of Rab35 knockdown embryos lack actin compared to the control, supporting the notion that Rab35 regulates actin dynamics. In addition, the skeletal and gastrulation defects induced by Rab35 knockdown were rescued by co-injection with Fascin, an actin-bundling protein, indicating that proper actin dynamics plays a critical role for both skeletogenesis and gastrulation. Overall, results indicate that through its role in mediating vesicular trafficking and actin remodeling, Rab35 is an important regulator of embryonic structure formation in early development. This work is funded by NSF CAREER (IOS 1553338) to JLS, NIH NIGMS P20GM103446, and Townsend Biotechnology Fellowship to CR.
**Program Abstract #277**

**Cell polarity determinant Dlg1 facilitates epithelial invagination by regulating tissue-scale mechanical coordination**

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Apical constriction mediated epithelial folding serves as a fundamental mechanism to convert epithelial sheets into multilayered tissues. In *Drosophila* gastrulation, ventrally localized prospective mesoderm cells constrict apically and subsequently invaginate to form a ventral furrow (VF). While the role of apical constriction in VF formation has been well demonstrated, it remains elusive whether additional molecular and mechanical inputs, other than apical constriction, play any role in folding. We found that depletion of the apical-basal polarity determinant Dlg1 results in a delay in VF invagination and a reduction in invagination depth without affecting the rate of apical constriction. The invagination defect is associated with an altered behavior in the neighboring non-constricting cells that is detectable soon after the onset of apical constriction. In the *dlg1* RNAi mutant, non-constricting cells exhibit an irregular, overextended apical morphology when they are pulled by the constricting cells, and this seems to slow down the overall tissue movement towards the ventral midline. Using an in vivo magnetic tweezers-based approach, we found that the mutant tissue is less elastic and more prone to irreversible deformation than wild type. The impaired mechanical properties of *dlg1* RNAi embryos are associated with a basal dispersion of the normally subapical actin cytoskeleton in the non-constricting cells. To directly test the role of non-constricting cells in facilitating VF ingestion, we disrupted the non-constricting cells by performing spatially confined laser microdissection or optogenetic depletion of cortical actin. Both treatments impaired VF ingestion. Our findings demonstrate that robust VF formation requires tissue-level cooperation between constricting and non-constricting cells, which is contingent on the mechanical integrity of the non-constricting cells. This research is supported by NIGMS ESI-MIRA R35GM128745 & ACS #IRG-16-191-33.

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**Program Abstract #278**

**PR/SET Domain 5: A Critical Transcriptional Regulator of Craniofacial Development**

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Development of the craniofacial skeleton is dependent on complex cellular processes involving cellular migration, differentiation and morphogenesis. Disruption of these processes can result in craniofacial defects which have a significant impact on quality of life. To identify the genes and molecular pathways involved in craniofacial development, an N-ethyl-N-nitrosourea mutagenesis forward genetic screen was performed in zebrafish. This screen uncovered a novel zebrafish line with morphological defects in multiple lower jaw cartilages stemming from a mutation in the *PR/SET Domain 5 gene (PRDM5)*. Interestingly, *PRDM5* mutations have previously been identified in humans with Brittle Cornea syndrome; a condition with multiple clinical presentations including extreme corneal thinning and spontaneous corneal ruptures, joint hypermobility and associated hearing loss. Alcian blue staining of this unique zebrafish line revealed chondrocyte stacking defects and a morphogenic change in Meckel’s and palatoquadrate cartilages of the lower jaw. As chondrocytes are a Neural Crest derivative, these cartilage defects suggest a role for *PRDM5* in Neural Crest cell development. Comparative expression analysis revealed no significant changes in Neural Crest induction and migration, however a reduction in *Collagen Type 2 alpha 1 (Col2a1)* was identified in homozygous mutants, suggesting that PRDM5 normally acts as a positive regulator of *Col2a1* expression in cartilage precursors. Given the homology between the proximal portion of Meckel’s cartilage and the palatoquadrate cartilage of zebrafish with the incus and malleus of the middle ear in mammals, this data provides new insight into the mechanisms by which *PRDM5* mutations contribute to the etiology of Brittle Cornea syndrome.

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**Program Abstract #279**

**Decreased function of the Kir2.1 potassium channel sensitizes mice to craniofacial defects due to nicotine-vaping**

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Decreased function of the Kir2.1 potassium channel sensitizes mice to craniofacial defects due to nicotine-vaping. This work was supported by NIGMS ESI-MIRA R35GM128745 & ACS #IRG-16-191-33.
Nicotine vapes and electronic-cigarettes (e-cigs) have rapidly gained popularity over the last decade among both adult and adolescent populations. Both consumers and manufacturers often make claims about the safety of these products compared to their cigarette counterparts. However, the specific consequences of prenatal exposure to these substances is still unknown. Nicotine binds and blocks the Kir2.1 potassium channel. Our lab has previously shown that Kir2.1 has a crucial role in craniofacial development. Mutations that disrupt the Kir2.1 potassium channel in humans cause craniofacial defects. Kir2.1 homozygous knock out mice have hypoplastic mandibles, maxilla, tympanic rings, frontal bones, and components of the zygomatic arch, as well as a cleft of the secondary palate. Kir2.1 heterozygous mice are indistinguishable from their wild type littermates. We hypothesize that Kir2.1 heterozygous mice will be sensitized to craniofacial defects under nicotine exposure. Under low dose e-cig exposure, we see reductions in birth weights and mandibular ramus height, increased fontanelle area, and disrupted palatogenesis of Kir2.1(KO/+), but not WT or Kir2.1(KO/KO) when compared to unexposed controls. Nicotine-vaped Kir2.1(KO/+) and Kir2.1(KO/KO) mice showed reductions in length and total area of the premaxilla. Kir2.1 also has a role in long bone formation. Nicotine-vaped Kir2.1(KO/+) mice have decreases in femur and humerus lengths. We show that vaping nicotine, similar to genetic perturbation of Kir2.1, can cause disruptions in bone development. Funding for this project is provided by grants from the NSF and NIDCR.

Program Abstract #280
The novel ECM protein SNED1 regulates neural crest cells for proper craniofacial development
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The extracellular matrix (ECM) is a complex meshwork of proteins providing structural and biochemical support to cells. We previously reported the identification of a novel ECM protein, SNED1 (Sushi, Nidogen And EGF Like Domains 1) as a promoter of breast cancer metastasis (Naba et al., eLife, 2014). To determine the role(s) of SNED1 in physiology, we generated a knockout (KO) mouse. We observed that Sned1 KO resulted in neonatal lethality. Interestingly, a few KO mice survived but were smaller and exhibited craniofacial malformations (Naba et al., bioRxiv, 2018). Morphological assessment, μCT scans examination, and quantitative geometric morphometrics of the skulls revealed that Sned1 KO mice have shorter cranial sutures, wider snouts, nasal occlusion, and under-developed mandibles. All these phenotypes can lead to impaired breathing and suckling thus compromising mouse survival. Using the lacZ reporter gene and in-situ hybridization, we observed expression of Sned1 in the frontonasal process and pharyngeal arches. Since cranial neural crest cells (NCCs) form the bone and cartilage of the head, we hypothesized that Sned1 regulates NCC phenotype. To test this, we generated an NCC-specific Sned1 KO mouse. While Sned1NCC-/NCC- mice survive, they present facial anomalies resembling malformations observed in Sned1 KO mice. To form craniofacial structures, NCC first undergo epithelial-to-mesenchymal transition, then migrate, proliferate and finally differentiate into bone and cartilage. Our goals are now to 1) determine precisely which cellular mechanisms along the NCC path is under the control of SNED1 and 2) identify the signaling pathways activated downstream of SNED1 and controlling NCC phenotype. Funding: Start-up fund from the Department of Physiology and Biophysics (AN), a Provost’s Graduate Research Award and an Award for Graduate Research from UIC’s Graduate College (AB), a LASURI award and Honors College grants (KJ), and a L@S GANAS fellowship (EDLF) from UIC.

Program Abstract #281
Shifts in chromatin accessibility define the developmental trajectories of neural crest cells
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The neural crest is a multipotent stem cell population that migrates throughout the embryo to give rise to a variety of derivatives that include the craniofacial skeleton, the peripheral nervous system, and melanocytes. To examine changes in chromatin accessibility during cell diversification, we performed single-cell ATAC-Seq on neural crest cells. Cranial neural crest cells from chicken embryos were labeled using a specific neural crest enhancer driving GFP and FACS-sorted from seven timepoints across their induction, specification, migration, and
differentiation. Dynamic chromatin accessibility allowed us to construct a lineage segregation tree that describes the split of the neural crest into four major derivatives: craniofacial mesenchyme, sensory ganglia and neurons, autonomic neurons, and melanocytes. Each lineage was characterized by a distinct profile of chromatin accessibility that gradually emerges from common progenitor cells. Testing of dozens of lineage-restricted regulatory elements with transient transgenesis revealed that dynamic accessibility predicts enhancer activity and specificity, allowing us to define the position of clusters of progenitor cells in developing embryos. By analyzing thousands of differentially enriched regulatory elements, we identified key transcription factor motifs with lineage-specific enrichment. Finally, by observing co-accessibility between individual regulatory elements and nearby promoters, we can also predict which genes these elements activate. This enables us to reconstruct gene circuits for each lineage. Interestingly, co-accessibility is dynamic over time and between lineages, allowing for refinement of these circuits for each differentiation lineage. This work was supported by the following grants: DP2 HD102043 - to M.S.C., T32HD057854 to A.S.H., Cornell Stem Cell Program Seed Grant to M.S.C., A.S.H., and M.R.

Program Abstract #282
Single cell transcriptome and open chromatin region analysis of development of cranial neural crest-derived cell populations in zebrafish.
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The vertebrate neural crest gives rise to a multitude of differentiated cell types. How neural crest-derived cells are directed to make such a diversity of cell fates, and the extent to which they give rise to multipotent progenitors required for adult homeostasis and repair, remain incompletely understood. To characterize the full repertoire of cranial neural crest-derived populations throughout the life of a vertebrate, we conducted single-cell RNA and single-nucleus ATAC sequencing at 7 stages from 36 hours to 7 months of age using zebrafish (Danio rerio) as the model. I have developed methods to align all the scRNA and snATAC libraries and have found common trajectories of cartilage, bone, and other connective tissue cells, as well as considerable diversification of mesenchyme. In situ validation of mRNA expression and transgenic testing of cluster-specific enhancers have revealed novel neural crest-derived populations, including a previously unappreciated elastic cartilage type and a potential periosteal niche cell type. I have also found that chromatin accessibility patterns can be maintained through later stages of development, thus allowing me to use snATAC data to connect early regional patterning with later cell type specification. In the future, we aim to integrate normal development with craniofacial regeneration models to understand how cranial neural crest cells alter their transcriptomes and chromatin accessibility to repair adult connective tissues. The project is supported by NIH R35 DE027550.

Program Abstract #283
Heterogeneous effects of Hoxa2 in mouse cranial neural crest cells establish axial identity
Irina Pushel, Robb Krumlauf
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Cranial neural crest cells (NCCs) are multipotent cells in the developing vertebrate embryo that are initially specified at the neural plate border, delaminate, and migrate into the frontonasal prominence and pharyngeal arches (PAs) to ultimately differentiate into bone, cartilage, neural, glial, and connective tissues of the head. Hoxa2 acts as a selector gene for PA2 fate, including formation of the hyoid bone, by modifying the Hox-free NCC ground state of PA1, which ordinarily gives rise to the jaws. However, the molecular mechanism through which Hoxa2 establishes this PA2 fate remains unknown. In this work, we aimed to identify targets of Hoxa2 that contribute to the establishment of NCC axial identity, as well as determine whether Hoxa2 activity modulates differential responses across subpopulations of NCCs during mouse embryonic development. To do this, we performed transcriptomic analysis using both bulk and single-cell RNAseq in PA1 and PA2 of wildtype and Hoxa2/- embryos at the start of NCC differentiation at embryonic day 10.5. Through differential expression analysis across these datasets, we identified both previously characterized and novel targets of Hoxa2 important for the establishment of NCC axial identity. Moreover, we observed high levels of heterogeneity in the expression patterns and response to Hoxa2 activity for many of these genes across subsets of NCCs. This suggests that Hoxa2 is acting through distinct downstream pathways to impart a PA2 fate on specific NCC derivatives. In this work, we
not only identify potential effectors of Hoxa2 activity important for the establishment of PA2 identity in cranial NCCs, but also characterize the heterogeneity of their response to Hoxa2 and the underlying complexity of NCC differentiation, leading to an enhanced understanding of the molecular mechanisms of NCC axial identity specification. We thank our funding sources, including the Stowers Institute (IP and RK) and NIH F31DE028469 (IP).

Program Abstract #284
Single-cell transcriptomics reveals early emergence of liver parenchymal and non-parenchymal cell lineages
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The cellular complexity and scale of the early liver have constrained analyses examining its emergence during organogenesis. To circumvent these issues, we analyzed 45,334 single-cell transcriptomes from embryonic day (E)7.5, when endoderm progenitors are specified, to E10.5 liver, when liver parenchymal and non-parenchymal cell lineages emerge. Our data detail divergence of vascular and sinusoidal endothelia, including a distinct transcriptional profile for sinusoidal endothelial specification by E9.5. We characterize two distinct mesothelial cell types as well as early hepatic stellate cells, and reveal distinct spatiotemporal distributions for these populations. We capture transcriptional profiles for hepatoblast specification and migration, including the emergence of a hepatomesenchymal cell type and evidence for hepatoblast collective cell migration. Further, we identify cell-cell interactions during the organization of the primitive sinusoid. This study provides a comprehensive atlas of liver lineage establishment from the endoderm and mesoderm through to the organization of the primitive sinusoid at single-cell resolution. We acknowledge funding from the Natural Sciences and Research Council of Canada, the Canadian Institutes of Health Research, the Canadian Foundation for Innovation, and the National Institutes of Health.

Program Abstract #285
New insights into gonadal sex differentiation provided by single cell transcriptomics in the chicken embryo.
Martín Estermann, Sarah Williams, Craig Smith
Monash University, AU
Gonadal sex differentiation, testis versus ovary formation, is a fundamental process required for reproduction and evolution. Reflecting this importance, the embryonic gonads of vertebrate species comprise the same major cell types; germ cells, supporting cells and interstitial steroidogenic cells. Remarkably, the genetic triggers for gonadal sex determination vary across species (the SRY gene in mammals, DMRT1 in birds and some turtles, temperature in reptiles, AMH and various other genes in fishes). Despite this variation, the cell biology of gonadal development was long thought to be largely conserved. Here, we present a comprehensive analysis of gonadal sex differentiation, using single-cell sequencing in the embryonic chicken gonad during sexual differentiation. We sampled over 40,000 cells across several developmental stages, prior, during and after the onset of gonadal sex differentiation. The data show that chicken embryonic-supporting cells do not derive from the coelomic epithelium, in contrast to other vertebrates studied. Instead, they derive from a DMRT1+/PAX2+/WNT4+/OSR1+ mesenchymal cell population. We find a greater complexity of gonadal cell types than previously thought, including the identification of two distinct sub-populations of Sertoli cells in developing testes and derivation of embryonic steroidogenic cells from a differentiated supporting-cell lineage. Altogether, these results indicate that, just as the genetic trigger for sex differs across vertebrate groups, cell lineage specification in the gonad may also vary substantially. This work was funded by an Australian Research Council Discovery Project grant.

Program Abstract #286
Investigating the relationship between genome organization of paired chromosomes and genome function at the single cell level in Drosophila
Jumana AlHaj Abed1, Jelena Erceg1, Anton Goloborodko2, Son C. Nguyen1, Ruth B. McCole1, Wren Saylor1, Antonios
Trans homologous interactions are unique in *Drosophila*, where homologous chromosomes are paired in somatic cells, and pairing-dependent gene regulation, or transvection, is prevalent. Although the relationship between homolog pairing and gene function has been extensively investigated in a locus-specific manner, only recently have we elucidated the architecture of homolog pairing using haplotype-resolved Hi-C. Using this approach and a hybrid *Drosophila melanogaster* cell line or early embryos, we discovered that homologs pair extensively and precisely. We found pairing to be highly structured, including trans-homolog domains, loops and compartments. We also found a significant variation in pairing and reveal different forms of pairing: tight and loose. Additionally, we find that tight pairing correlates with gene expression, compartments, and active epigenetic marks. In embryos, homolog pairing is related to early chromatin accessibility and pioneer factor Zelda. We were motivated by these results, and the variation of pairing, to study the implications of these findings at the single cell level. For example, what is the relationship between tight or loose pairing and gene expression or epigenetic state? For that reason, we turned to single-molecule localization microscopy, or OligoSTORM, to visualize pairing, understand the properties of tightly and loosely paired regions and relate these structures to epigenetic signatures and gene expression. Additionally, using Homolog Specific Oligopaints (HOPs) which target single nucleotide differences between homologs in our hybrid cell line, we distinguished paternal and maternal chromosomes and found evidence for extensive intermingling between homologs with the maximum physical separation detected between them, often, approaching 100nm. Funding: NIH/NIGMS: R01HD091797, R01GM123289, DP1GM106412 and HMS to C.-t.W., NIH: R01 HG003143 to J.D., NIH/NIGMS: R01GM114190 and 4D Nucleome Grant US4 DK107980 to L.A.M., J.D.

**Program Abstract #287**

**The connectome of neural crest enhancers reveals regulatory features of signaling systems**

Ana Azambuja, Marcos Simoes-Costa

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Cell fate commitment is controlled by cis-regulatory elements that are often located in remote regions of the genome. To examine the role of long-range DNA interactions in early development, we generated a high-resolution contact map of active enhancers in the neural crest, a stem cell population unique to vertebrate embryos. This analysis revealed that the gene regulatory network that controls neural crest specification is regulated primarily by cis-regulatory elements that contact promoters in a cell-type-specific manner. Neural crest genes are associated with long-range Wnt-responsive enhancers, which contain degenerate TCF/LEF binding sites and act as sensors for extracellular signaling pathways. Canonical Wnts drive cell state transitions by simultaneously regulating multiple network nodes. We propose that, rather than acting as upstream activators, signaling systems connect regulatory sub-circuits into a hub-and-spoke architecture. These results shed light into the tridimensional organization of the neural crest genome and define how signaling systems provide progenitor cells with spatial cues that transform their molecular identity. This work was supported by NIH grants R00DE024232 and R01DE028576 to Marcos Simoes-Costa.

**Program Abstract #288**

**Zygotic Arrest Proteins 1 and 2 Interact with Other Proteins Associated with Germ Granules In *Xenopus laevis***

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In early development there is no transcription of the zygotic genome. Despite this, there is a significant amount of gene regulation achieved by activation or repression of translation of maternally provided mRNAs. One of the earliest described examples of factors that promote this regulation are members of the Zygotic Arrest (Zar) family of proteins. There are two isoforms of Zar, Zar1 and Zar2. Their significance in early development was shown
through gene knockout experiments, which showed that when Zar1 is knocked out in mice, development of the fertilized egg halts at the one-cell stage, and when a dominant negative Zar2 is introduced to mice embryos the development of the fertilized egg halts at the two-cell stage. Despite their significant role in developmental pathways, little is currently known about their molecular mechanism of action or the interactions necessary to fulfill their role. In this study, co-purification followed by mass spectrometry performed on extracts of oocytes expressing GST tagged Zar1 and Zar2 provided likely candidates for the protein interactions of endogenous Zar1 and Zar2 in stage VI oocytes. Co-immunoprecipitations of endogenous Zar1 and Zar2 followed by western blot analysis showed that eukaryotic translation initiation factor E1b (eIF4E1b), eIF4E transporter (4E-T) and embryonic poly(A) binding protein (ePAB) all associated with Zar1 and Zar2. These associated proteins have been found in germ granules, areas where RNA and proteins clump together to repress RNA translation. The association of Zar1 and Zar2 with these proteins has strong implications that they may also play an important role in these granules. Funding for this project comes from the National Institute of Child Health and Human Development and the University of Colorado Denver.

Program Abstract #289
Poly(A) tail dynamics during early development in *Xenopus laevis*
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The maternal to zygotic transition is a unique switch of control during development. Oocytes and early embryos are controlled by maternal factors, then, by poorly understood mechanisms, the zygotic genome is activated and takes over control of embryogenesis. One hypothesized mechanism of activation is the limited machinery model, that states the embryo initially lacks sufficient concentrations of transcription factors, like Pou5 and Nanog, needed for genome activation. Maternal transcripts are translationally repressed by a short poly(A) tail and translationally activated by poly(A) tail elongation. We are interested in elucidating when and how Pou5 accumulates during early *Xenopus laevis* development. In this study, we investigated maternal poly(A) tail dynamics of Pou5 mRNA, along with the established mRNAs of Mos, cyclin and Wee1, as controls, from oogenesis through embryogenesis. All maternal transcripts tested had moderate length poly(A) tails in stage I-III oocytes, and then surprisingly, they deadenylated to short poly(A) tails in stage IV oocytes. As expected, polyadenylation of Cyclin 1A, Cyclin 1B, Mos, and Wee1 were elongated during oocyte maturation. Curiously, the poly(A) tails of Pou5f3.2 and Pou5f3.3 mRNA did not change during oocyte maturation but they were elongated immediately before zygotic genome activation. Consistent with maximum elongation, maternal Pou5f3.2 and Pou5f3.3 protein accumulation also increased. This study suggests a new general mechanism of mRNA processing during oogenesis. It also suggests that Pou5f3.2 and Pou5f3.3 are limited machinery candidates. This research is funded by the National institutes of Health. Travel is funded by the Department of Integrative Biology, the Graduate School and the College of Liberal Arts of Science at University of Colorado-Denver.

Program Abstract #290
Determining the gene regulatory network for hair cell regeneration in the zebrafish adult inner ear at single-cell resolution
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Hair cells are the mechanosensory cells used in auditory and vestibular organs of all vertebrates. In zebrafish and other non-mammalian vertebrates, hair cells turn over during homeostasis and regenerate completely after being destroyed or damaged by acoustic or chemical exposure, while in mammals, destroying or damaging hair cells results in permanent impairments to hearing/balance. We hypothesize that appropriate gene regulation by enhancers is key to how genes respond during regeneration. Our goal is to identify enhancers involved in repairing a vertebrate inner ear. To identify enhancers in response to hair cell damage, we developed a transgenic zebrafish to permit conditional and selective ablation of hair cells in the adult inner ear. This model expresses the human diphtheria toxin receptor (hDTR) gene under the control of the myo6b promoter, resulting in hDTR expressed only in hair cells. Cell ablation is achieved by intraperitoneal injection of diphtheria toxin. On adult
zebrafish that have undergone hair cell ablation, we investigated the epigenome and transcriptome of single-cells from the inner ear at consecutive time-points following hair cell ablation. Because physical accessibility of genomic DNA is used as a proxy for the “active” genome, we identified open chromatin locations using single-cell Assay for Transposase Accessible Chromatin with high-throughput sequencing (scATAC-seq). Machine learning on regeneration induced open chromatin revealed unique cell-specific transcription factor (TF) motif patterns. We correlated enhancer activation with transcription (using scRNA-seq) to identify gene regulatory networks. We detected a clear pattern of overlapping Sox- and Six-family TFs, suggesting a combinatorial program of TFs determining cell fate. By correlating cell-type, enhancer activation, and TF expression, we are beginning to understand the combinatorial “code” of TFs that initiate regeneration and instruct hair cell differentiation.

Program Abstract #291
Dynamic microRNAs (miRNAs) and their target mRNAs in zebrafish heart development
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A growing body of evidence suggests that congenital heart disease (CHD) can result from posttranscriptional or epigenetic regulation, including regulatory events such microRNA directed mRNA degradation. In order to better understand the RNA regulation events that drive heart development, we conducted an RNA-seq time course in zebrafish where RNA was extracted from isolated hearts at five time points and each sample was split to simultaneously produce small RNA and total RNA libraries for sequencing. We have identified several temporal patterns of regulation corresponding to specific developmental processes and over ten thousand significantly changed transcripts over the time course. In order to share this dataset with the scientific community, we built a web-based application in R that allows the on-line user to investigate their genes of interest rapidly and easily. In addition, we developed a pipeline that identifies multiple miRNA families that are dynamically expressed in heart development and inferred mRNA targets through inverse correlation analysis and predictive algorithms. From this pipeline we selected candidate miRNA/mRNA combinations for functional analyses and in vivo validation. One of the highly expressed and dynamic miRNA families, mir-24, has around 20 probable cardiac mRNA targets. We found that mir-24 quadruple mutants display cardiac development phenotypes. In a collaboration between this Cardiovascular Development Consortium project and the Pediatric Cardiac Genomics Consortium, we used human WGS datasets to identify 3’UTR’s of candidate miRNA targets that contain rare variants in CHD patients. These candidates are prioritized for functional analyses using CRISPR/Cas9 technology and detailed evaluation of cardiovascular phenotypes. This dataset provides an important resource for studies of RNA regulatory events implicated in cardiac development and CHD. Supported in part by UM1 HL098160 to HJY and CIHR Postdoctoral Fellowship to CH.

Program Abstract #292
Rauber’s Layer Disappearance and Proamniotic Cavity Formation: two strategies for the same end?
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All eutherian mammals can be divided into one of two categories: they either maintain the trophoblast overlying the epiblast or they lose this polar trophoblast tissue. In the latter case, such polar trophoblast is known as Rauber’s layer (RL). It has never been clear why two such distinct modes of early development exist, in particular as polar trophoblast can be important for epiblast patterning as shown in the mouse. We propose that there is a unifying principle that explains these different developmental modes, namely that all mammals need to shield the central epiblast from signals emanating from the overlying trophoblast. In RL-mammals, such shielding is achieved by the active removal of the polar trophoblast. We tested this idea by maintaining the polar trophoblast for longer than normal in cattle, a typical RL-mammal. When overexpressing the anti-apoptotic protein BCL2, RL disappearance was delayed by a day. While this had no effect on the induction and maintenance of the anterior visceral hypoblast, it resulted in embryos exhibiting erroneous patterning as displayed by ectopic BRACHYURY
expression, in several cases reminiscent of supernumary axis induction. This suggests that the disappearance of RL is a necessity, presumably to avoid excessive signalling interactions between this layer and the subjacent epiblast. So how do non-RL mammals achieve the central epiblast shielding? To answer this, we analysed over a century’s worth of data on all described mammalian orders and discovered a remarkable correlation: all mammalian species that maintain the polar trophoblast form a proamniotic cavity, whereas all RL- species do not! This remarkable correlation, in combination with our new data using transgenic cattle embryos, leads us to the interpretation that proamniotic cavity formation is predominantly an adaptation for preventing inappropriate polar trophoblast signalling in animals that have a need for maintaining the polar trophoblast. Funded by MBIE NZ.

Program Abstract #293
A versatile depigmentation, clearing, and labeling strategy for advancing the exploration of organs and nervous systems across animal phyla
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A small set of well-established “molecular model species” has driven biological discovery and technological development for the past decades. However, it is increasingly recognized that these model species only cover a limited spectrum of ecological and evolutionary diversity, calling for a more systematic effort in exploring novel model systems. The establishment of versatile technologies are keys in this effort. Tissue clearing combined with deep imaging has emerged as a powerful alternative to classical histological techniques, bypassing the need of resource-intensive and slow sectioning and reconstruction. Whereas current techniques have been optimized for imaging selected non-pigmented organs such as the mammalian brain, natural pigmentation remains challenging for most other biological specimens of larger volume. We have developed a fast DEpigmEntation-Plus-Clearing method (DEEP-Clear) that is easily incorporated in existing workflows and combines whole system labeling with a spectrum of detection techniques, ranging from immunohistochemistry to RNA in situ hybridization, labeling of proliferative cells (EdU labeling) and visualization of transgenic markers. With light-sheet imaging of whole animals and detailed confocal studies on pigmented organs, we provide unprecedented insight into eyes, whole nervous systems, and subcellular structures in animal models ranging from worms and squids to axolotls and zebrafish. DEEP-Clear thus paves the way for the exploration of species-rich clades and developmental stages that are largely inaccessible by regular imaging approaches. This study was supported by the Austrian Science Fund (FWF), projects P30035, I2972, F78.

Program Abstract #294
Sex-specific yeast interfering RNA larvicides for effective sex sorting in the human disease vector mosquito Aedes aegypti
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Although several emerging mosquito control technologies rely on effective means of sex sorting prior to the mass release of male mosquitoes, practical and affordable sex-sorting methods that can be implemented in resource-limited locations have not been established. To address this, our laboratory pursued an siRNA screen that identified numerous female-specific larval lethal genes in Aedes aegypti, the primary mosquito vector of dengue and Zika viruses, and an emerging model for the study of mosquito development. siRNA-546, one of the larvicides identified in the screen, corresponds to a protein-encoding gene of unknown function that is located at the sex-determining M locus region in A. aegypti. In recent years, we have established a Saccharomyces cerevisiae (baker’s yeast) system for effective and affordable expression and delivery of interfering RNA to mosquito larvae. S. cerevisiae was engineered to express short hairpin RNA (shRNA) corresponding to siRNA-546. The resulting yeast strain 546 permitted functional assessment of the hypothesis that yeast-mediated RNAi silencing can be utilized for sex-sorting of male mosquitoes. Consumption of yeast strain 546 by A. aegypti during larval development resulted in female death beginning in the second larval instar. However, consumption of the yeast
by male larvae did not impact male survival or fitness, which is critical for effective male release mosquito control strategies. The results of these studies reveal that yeast-based RNAi larvicides may represent an efficient and affordable means of facilitating the mass rearing of A. aegypti males. Future studies will aim to scale production of shelf-stable formulations of this yeast for global distribution, to understand the functions of the gene targeted by the yeast, and to identify and characterize orthologs of this gene in additional insect species. Funding source: NIH-NIAID 1R21 AI144256 to MDS.

Program Abstract #295
Live Imaging of Intracranial Lymphatics in the Zebrafish
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The recent discovery of meningeal lymphatics in mammals is reshaping our understanding of fluid homeostasis and cellular waste management in the brain, but visualization and experimental analysis of these vessels is challenging in mammals. Although the optical clarity and experimental advantages of zebrafish have made this an essential model organism for studying lymphatic development, the existence of meningeal lymphatics has not yet been reported in this species. Using high-resolution optical imaging of the meninges in living animals, we show that zebrafish possess a meningeal lymphatic network comparable to that found in mammals. We confirm that this network is separate from the blood vascular network and that it drains interstitial fluid from the brain. We document the developmental origins and growth of these vessels into a distinct network separated from the external lymphatics. Finally we show that these vessels contain immune cells and perform live imaging of immune cell trafficking and transmigration in meningeal lymphatics. This discovery opens up new avenues for experimental analysis of meningeal lymphatic development and meningeal lymphatic function in health and disease. This work was supported by NICHD, NIH intramural funds.

Program Abstract #296
Identifying novel molecular mechanisms underlying lateral line sense organ development using an unbiased, comparative approach
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Electroreception is an ancient division of the lateral line sensory system of fishes and aquatic-stage amphibians, enabling the detection of weak electric fields in water. It was lost in the ancestors of frogs and teleost ray-finned fishes and evolved independently at least twice within teleosts. In all fishes and aquatic-stage amphibians, the lateral line has a mechanosensory division that detects local water movement. In non-teleost jawed vertebrates, electroreceptor cells reside within 'ampullary organs' in fields on the head that flank lines of 'neuromasts' containing mechanosensory hair cells. These lateral line organs originate from placodes that elongate over the head to form sensory ridges. To identify genes involved in ampullary organ vs. neuromast development, the lab previously used differential RNA-seq in late-larval stages of a chondrostean ray-finned fish, the Mississippi paddlefish (Polyodon spathula), to generate a lateral line organ-enriched gene-set (Modrell et al., 2017, eLife 6: e24197). This contains 45 genes encoding transmembrane receptors and ligands. I cloned cDNA fragments of these genes, plus 19 others of interest, in an experimentally tractable chondrostean, the sterlet (Acipenser ruthenus, a sturgeon). I performed in situ hybridisation to determine which are expressed within developing ampullary organs versus neuromasts. During the 2019 spawning season, I targeted some of these genes for CRISPR/Cas9-mediated mutagenesis, and also chemically inhibited the Notch and Bmp signalling pathways, generating some interesting lateral line phenotypes. I also plan to compare the expression and function of validated genes in zebrafish, whose lateral line placodes only form neuromasts. Overall, this unbiased, cross-species approach should identify novel signalling pathways important for the development of hair cells and/or electroreceptors, and determine whether such mechanisms are conserved or lineage-specific. Funding: the Anatomical Society.
Program Abstract #297
Investigating the Specification of Hair Cells Versus Electroreceptors in the Lateral Line Using CRISPR/Cas9 in a Sturgeon
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The mechanosensory lateral line system of fishes and aquatic-stage amphibians comprises lines of neuromasts containing hair cells (like those of the vestibular inner ear), which detect local water displacement. In all lineages except neopterygian fishes (gars, bowfin, teleosts) and frogs, neuromast lines are flanked by ampullary organs containing electroreceptors, which detect weak, low-frequency electric fields around other animals. Both mechanosensory and electroreceptive organs, and their afferent neurons, develop from lateral line placodes. The formation of different but related sense organs from the same placode provides a unique opportunity for studying sensory cell-type specification. As both zebrafish and Xenopus lack ampullary organs, we previously used differential RNA-seq in late-larval paddlefish (Polyodon spathula) to generate a lateral line organ-enriched gene-set, showing that developing ampullary organs express all the transcription factor genes known to be essential for hair cell formation, including Atoh1, Pou4f3 (Modrell et al., 2017, eLife 6: e24197) and Gfi1. Validation of other genes from the paddlefish gene-set has identified a few ampullary organ-specific transcription factor genes and one mechanosensory-restricted transcription factor gene, Foxg1. To overcome the limited availability of paddlefish embryos, we have optimized CRISPR/Cas9-mediated mutagenesis in another chondrostean, the sturgeon Acipenser ruthenus (sterlet). Our results suggest that the 'hair-cell' transcription factor Atoh1 is required for Pou4f3 and Gfi1 expression in ampullary organs and neuromasts, and for the differentiation of electroreceptors as well as hair cells. Conversely, Foxg1 seems to prevent neuromasts from differentiating as ampullary organs. Overall, CRISPR/Cas9-mediated mutagenesis in sterlet will help us reconstruct the transcription factor hierarchies underlying formation of hair cells versus electroreceptors in the lateral line system. Funding: BBSRC

Program Abstract #298
A mechano-molecular insight into the process of forebrain roof plate invagination
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The forebrain roof plate (RP) of the vertebrates acts as a signaling center and itself undergoes the process of invagination to split the embryonic forebrain vesicle. Absence of RP invagination results in the holoprosencephaly. The factors responsible to influence the mechanics of the invagination process remains to be understood. The evidence of such factors comes in the form of differential thickness (DT), differential proliferation (DP) and outer mesenchymal covering. We employed in-silico computational modelling which predicted that a dorsal perturbation along with the proliferating neuroepithelium is sufficient for RP invagination and the DT and DP contributes to the rate of invagination. Experimentally perturbing the DP of the RP midline resulted in a V shaped rather than normal W shaped invagination but did not affect the invagination depth. Previous study from our group showed that the RP has active RA signalling which regulates the proliferation and the expression of BMP ligands. Anti-pSmad1/5/8 staining revealed that the domain of lower canonical BMP signalling overlaps with the domain of active RA signalling in the W shaped RP midline. Ectopically activating canonical BMP signalling resulted in the thickening of the RP midline, perturbing the DT and resulting in the shallow V/U shaped invagination without affecting the DP. We further found presence of p-Cofilin1 overlapping with the thin region of the invaginating forebrain RP neuroepithelium. Our investigations revealed that the changes in cell morphology are regulated through reciprocal interactions between RA and BMP signaling, which in turn modulate Cofilin-1, a factor that modifies the actin cytoskeleton. Ectopically expressing the constitutively active form of Cofilin protein in the RP midline, was sufficient to perturb the DT and recapitulated the V/U shaped phenotype of the invaginating RP. Funding sources: DBT-Gov. of India and MHRD-Gov. of India.
**Program Abstract #299**

**The Hedgehog effector Netrin regulates optic fissure and stalk morphogenesis**

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Developmental defects in eye structure commonly account for visual impairment in newborns. One such defect, uveal coloboma, results from failed development of transient structures in the embryonic eye: the optic fissure and optic stalk. Mutations in the Hedgehog (Hh) receptor Patched (Ptch) lead to overactive Hh signaling and can cause coloboma in humans and zebrafish. Using zebrafish multidimensional imaging and computational analyses, we previously pinpointed the specific cell movements disrupted in *ptch2* mutants. We also found that overactive Hh signaling disrupts optic fissure and stalk development in a Gli-dependent manner, through both cell- and non-cell-autonomous mechanisms. Our model is that overactive Hh signaling induces overexpression of multiple factors that directly disrupt cell movements and morphogenesis. To identify the relevant Hh transcriptional targets, I have initially focused on a candidate: the Netrin family of secreted ligands. I found that multiple Netrin ligands are upregulated in the *ptch2* mutant during optic fissure and stalk development. To test if upregulation of Netrin is sufficient to cause coloboma, I have taken gain-of-function approaches to overexpress *netrin1* in a spatiotemporally-specific manner. To determine whether Netrin is necessary for the *ptch2* mutant phenotype, I have utilized loss-of-function alleles of *netrin1* to test if loss of Netrin can rescue coloboma. Our results suggest that *netrin1* overexpression is sufficient to disrupt optic fissure and stalk morphogenesis, and additional Netrin ligands will be tested. Beyond Netrin, our model suggests that multiple Hh effectors are responsible for disrupting optic fissure and stalk development in the *ptch2* mutant, thus I am carrying out single-cell RNA-sequencing to identify additional factors. Together, this work will uncover molecular mechanisms controlling optic fissure morphogenesis, and in turn, coloboma. This work was supported by the NIH (F31 EY030758, R01 EY025378).

**Program Abstract #300**

**Retinoic acid controls endothelial progenitor migration and trunk vasculogenesis via somite maturation**

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Maternal vitamin A deficiency is known to cause cardiovascular defects in vertebrate embryos. Vitamin A functions as a precursor for retinoids; and previous studies implicate impaired retinoic acid (RA) signaling in heart defects. However, its role in the formation of the blood vessels is not well understood. Using the zebrafish embryo, we have determined that RA is required for timely migration of angioblasts to the midline. In addition, the definitive vasculature of RA depleted embryos contain morphological defects, with large common cardinal veins developing at the expense of the dorsal aorta. Interestingly, RA seems to be required in non-autonomous fashion for angioblast migration. Analysis of RA indicates RA signaling occurs in non-endothelial tissues. Since RA depletion causes numerous changes in an embryo, we performed morphological analysis to determine any secondary effects that could account for this defect. We discovered that angioblasts could not effectively migrate to the midline in RA depletion conditions, as the angioblasts are occluded from the midline. We observed a midline cavity that forms as somites adopt their more mature morphology. Angioblasts migrate into this cavity in order to coalesce into the vascular cord. RA loss significantly impairs the development of this cavity, restricting angioblast movement and preventing migration. This research presents a novel observation that angioblasts require RA mediated somitic maturation to migrate properly to the midline. This work was supported by American Heart Association [13SDG14360032] and National Science Foundation [IOS1452928] grants to B.L.M.

**Program Abstract #301**

**The EMT transcriptional regulator Snai1 promotes myocardial integrity by regulating intermediate filament organization in zebrafish**

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The transcription factor Snai1, a key regulator of epithelial-to-mesenchymal transition (EMT), regulates early stages of cardiac development including looping and endocardial cushion formation. However, little is known
about its role in cardiomyocyte differentiation and morphogenesis. Taking advantage of zebrafish as a model system, we used high-resolution in vivo imaging and genetic manipulations to study the role of Snai1 in cardiac development at the single-cell level. Of the two zebrafish snai1 paralogues, snai1b is expressed throughout the myocardial layer. Strikingly, snai1b loss-of-function leads to disorganized sarcomeres and cardiomyocyte extrusion in a cell-autonomous manner, resulting in a reduced heartbeat and impaired circulation. snai1b mutant heart transcriptome and proteome analyses revealed dysregulation of cytoskeletal factor genes, including desminb, which encodes the main intermediate filament component in muscle tissues. We found that dysregulation of desminb expression in cardiomyocytes leads to defects in sarcomeric arrangement, indicating a Snai1b-regulated crosstalk between the actin and intermediate filament cytoskeletons. Altogether, our results reveal a previously unsuspected role of Snai1 in maintaining myocardial integrity by regulating the crosstalk between actin filaments and intermediate filaments. Funding Sources: the Max-Planck-Society.

Program Abstract #302
Zebrafish notochord elongation requires the activation of matrix metalloproteinase 2 mediated by serine proteases.
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Zebrafish require Mmp2 (matrix metalloproteinase 2, also called gelatinase A) for normal embryonic development. When Mmp2 expression is inhibited by anti-sense morpholino probes, embryos develop a truncated body axis. Mmp2 is a zinc dependent endopeptidase that is initially translated as a zymogen and requires the proteolytic removal of its pro-peptide in order to be enzymatically active. Much is known about when and where MMP-2 is expressed and to which disease contexts it correlates, but less work studies its activation state and the activation mechanisms that render it biologically relevant. Historically, cell culture models have been used to describe several activation mechanisms for MMP-2. The more commonly referenced mechanism requires the activity and dimerization of transmembrane MMP-14 to activate the complex formed between MMP-2 and TIMP-2, a tissue inhibitor of metalloproteinases. Other more contentious mechanisms require the activity of serine proteases thrombin or plasmin. Here we use the Epitope Mediated MMP Activation (EMMA) assay in zebrafish to visually track activation by a comparison of N- and C-terminal signals. Our activation maps show endogenous activation of Mmp2 in the outer epithelium, somite boundaries, neural tube, and notochord of young embryos. During the second and third days of development, Mmp2 is activated more strongly in the neural tube and most strikingly in podosomes associated with the collagenous actinotrichia in the developing fin folds. Consistent with the most common mechanism for Mmp2 activation, the inhibitor phenanthroline reduces activation in the notochord, but surprisingly not as strongly as AEBSF, indicating that serine protease activation predominates in the notochord activation of Mmp2 during notochord elongation. When Mmp2 activation in the notochord is inhibited, the tail develops kinks and twists depending on the severity of the treatment. Work was funded by NSERC, NBIF and the O’Brien Foundation.

Program Abstract #303
A new genetic mutant uncovers a novel step of vertebrate eye development: dynamic tissue de-adhesion
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Morphogenesis requires dynamic coordination between cells, tissues, and their environment to generate precise, stereotyped 3D structures. Because a comprehensive understanding of the molecular and cellular basis of morphogenesis remains elusive, we undertook a zebrafish haploid mutagenesis screen to uncover novel factors governing embryonic eye development. We isolated shutdown corner (sco), a novel mutant with eye, cardiovascular and locomotion defects. In the eye, the prospective retina and lens initially develop normally, making close contact and undergoing invagination; however, the retina and lens maintain close contact and fail to separate, or de-adhere. Although lens induction has long been studied, sco uncovers a previously unappreciated step of eye morphogenesis: retina-lens de-adhesion. To understand the basis for the sco phenotype, we first considered cell movements and extracellular matrix (ECM) deposition. Using 4D cell tracking, we found that movements of neighboring retina and lens cells are unaffected, and these cells move independently in sco. We
find, however, that deposition of the ECM factor laminin is disrupted, and preliminary transmission electron microscopy also suggests aberrant features at the retina-lens boundary. To elucidate the genetic basis for this loss of de-adhesion phenotype, we mapped sco and identified a ~10 Mb deletion (~100 genes) on Chromosome 5. To determine the causative gene, we performed a candidate-based CRISPR mutagenesis screen. Our preliminary results indicate that the chondroitin sulfate proteoglycan versican (vcana) is required for retina-lens de-adhesion, suggesting a crucial role for ECM in this process. We are now working to determine how vicana regulates tissue de-adhesion, and if other developing tissues utilize this mechanism. Our work shows for the first time that tissue de-adhesion in the eye is genetically regulated. This work was supported by the NIH (R01 EY025780, R01 EY015128, T32 GM007464).

Program Abstract #304
Investigating the role for motile ciliated cell lineages in spine morphogenesis
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The goal of this project is to further understand the connection between motile cilia defects and adolescent idiopathic scoliosis (AIS). AIS is a highly prevalent disease characterized by an abnormal three-dimensional curvature of the spine. Unlike congenital or neuromuscular scoliosis it has no known underlying cause, arising in the absence of obvious congenital or physiological defects. Genetic heterogeneity in patients plus a historical lack of suitable animal models have confounded our understanding of the disease. Surprisingly, spinal curvatures rank among the most common deformities observed in teleost fish, and the Ciruna lab defined the first genetic model of idiopathic scoliosis in zygotic ptk7 mutant zebrafish (Hayes et. al., 2013; 2014). Work in the lab has established a requirement for ptk7 in motile ciliated cell lineages, specifically in the brain (Grimes et. al., 2017 and Van Gennip et. al., 2018), although the role for motile cilia in scoliosis pathogenesis remains unclear. To gain better insight, I am investigating the cause of IS in zebrafish katatin p80 subunit (katnb1) mutants, which also develop spinal curvatures during adolescence. Katnb1 is a microtubule severing protein that has roles at the cilia and basal body in single celled organisms, and is important in vertebrate neurogenesis, early embryonic patterning and ciliary signaling. We have demonstrated that zebrafish Katnb1 is also required in Foxj1a positive cell lineages for normal spine development, that Katnb1 is not required for primary or nodal cilia formation and, strikingly, that multi-cilia formation is affected in the brain of katnb1 mutants at juvenile stages. Here I report on our progress in identifying specific cilia subpopulations that contribute to IS pathogenesis, and determining the molecular genetic mechanisms underlying spinal curve formation in our zebrafish models. Funding provided by CIHR and Ontario Trillium Scholarship.

Program Abstract #305
Pathogenic human variants in KIAA0753 eliminate primary ciliogenesis and Sonic Hedgehog signaling in vitro
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Compound heterozygous variants in the gene KIAA0753 (OFIP) were identified in four patients with Joubert syndrome. This is a primary ciliopathy disorder which causes various brain development abnormalities characterized by a molar tooth sign on brain MRI and severe developmental delay or early demise. The KIAA0753 protein has previously been implicated in formation of a trimeric complex with OFD1 and FOPNL within the basal body of the primary cilium. We hypothesized that these new variants in KIAA0753 disrupt primary ciliogenesis and are the causes of the Joubert syndrome. Fibroblasts from one patient indeed demonstrate a deficiency in ciliation compared to control, yet KIAA0753 protein is stably produced. CRISPR-Cas9 mediated deletion of KIAA0753 from NIH3T3 fibroblasts showed that loss of KIAA0753 ablates the formation of primary cilia. As cilia are known to mediate the Sonic hedgehog pathway, KIAA0753 knockout cells do not activate Shh response genes when the pathway is activated. Overexpressing wild-type KIAA0753 via plasmid transfection in these cells significantly rescues ciliogenesis. However, overexpression of any of six patient variants in KIAA0753 knockout NIH3T3 cells does not rescue ciliogenesis, suggesting that these variants are indeed pathogenic. Initial experiments show localization of FOPNL to the basal body does not appear to be disrupted in the absence of KIAA0753, but OFD1
largely becomes sequestered in the nucleus. This research is supported by Cincinnati Children’s Research Foundation funding to Dr. Rolf Stottmann.

Program Abstract #306
Systematic analysis of ciliary Hedgehog signaling in immortal cell lines and human fibroblasts
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The primary cilium plays a vital role in development and tissue homeostasis. This organelle protrudes from the surface of most vertebrate cells, transduces extracellular signals into cells, and mediates multiple signaling pathways, notably Hedgehog (Hh). Ciliary dysfunction causes human ciliopathy conditions, with outcomes ranging from embryonic lethality to organ degeneration over decades. In Joubert syndrome (JS), a genetically heterogeneous ciliopathy, a variety of Hh defects are documented across multiple model systems; however, it is not known whether Hh dysregulation is an obligate feature of JS. To determine if specific Hh defects are required to cause JS, we plan to assay Hh response across JS genetic causes in engineered cell lines. Here, we evaluate the suitability of five immortal cell lines (ARPE-19, HEK293T, hTERT RPE-1, NIH/3T3, ReNcell CX) and eight unaffected human fibroblast lines for modeling Hh signaling based on assays of key pathway activation events: ciliary SMO/GPR161 relocalization by quantitative immunofluorescence, GLI3 transcription factor processing by western blot, and GLI1/PTCH1 induction by RT-qPCR. With pathway stimulation, hTERT RPE-1 and NIH/3T3 cells appropriately relocalize SMO/GPR161, shift to GLI3 activation, and upregulate GLI1/PTCH1. HEK293T cells relocalize SMO/GPR161 without robust GLI1/PTCH1 induction. ARPE-19 and ReNcell CX cells weakly respond to pathway stimulation, while primary human fibroblasts respond variably. These data indicate that hTERT RPE-1 and NIH/3T3 cells are suitable for modeling Hh pathway defects and human fibroblast data should be cautiously interpreted. Based on our results, we will genetically engineer hTERT RPE-1 cells to determine whether Hh dysregulation is an obligate feature of JS across genetic causes, enabling identification of targets for precision therapies and JS-related developmental mechanisms. Funded by NIH NICHD U54HD083091 Genetics Core and sub-project 6849 and private donations to DD.

Program Abstract #307
A Novel Role for DYRK1A in Kidney Development
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Congenital anomalies of the kidney and urinary tract (CAKUT) are a leading cause of pediatric renal failure resulting from defects in morphogenesis. Approximately 14% of CAKUT cases have a known genetic component. This low causality suggests that there are underlying genes and mechanisms which lead to CAKUT that have not been identified. Our collaborators made a novel observation that a cohort of patients with DYRK1A-intellectual disability syndrome has a higher prevalence of CAKUT (73% of those assessed), including kidney defects. DYRK1A-related intellectual disability syndrome is caused by a de novo mutation in a single DYRK1A (dual-specificity tyrosine phosphorylation regulated kinase 1A) allele, leading to haploinsufficiency. DYRK1A functions as a kinase that maintains an active conformation after autophosphorylation. Importantly, the function of DYRK1A in kidney development is not known. Our model is the embryonic kidney of Xenopus laevis, which consists of a single nephron that has many characteristic features of a mature mammalian nephron, making it a simple model for human kidney disease. To assess Dyrk1a’s role in nephrogenesis, we first demonstrated that Dyrk1a is expressed in the Xenopus kidney by in situ. My data using morpholino (MO) knockdown demonstrate that Dyrk1a loss leads
to abnormal kidney formation in *Xenopus* embryos that can be rescued by co-injecting human *DYRK1A* RNA. Additionally, injecting Dyrlka MO into 2 of 4 cells results in edema, suggesting a disruption in renal function. Furthermore, we have generated animal models of catalytically inactive nonsense and missense *DYRK1A* patient mutations that fail to rescue the kidney phenotype, suggesting that these mutations are responsible for kidney defects and that *DYRK1A*’s kinase activity is important for nephrogenesis. Funding Sources: Schissler Foundation Fellowship, NIDDK (K01DK092320, R03DK118771, R01DK115655), and startup funding from the Pediatric Research Center, McGovern Medical School.

**Program Abstract #308**

Odd-Skipped Related 1 (Osr1) Regulates Extracellular Matrix Deposition During Bladder Development and Disease

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Collagens form a network of interwoven fibers in the bladder wall which mediate biomechanical properties that maintain low pressures during emptying and prevent over-distension during storage. When the bladder is obstructed, there is a marked increase in collagen that impairs its function. This raises the question: how is collagen deposition regulated during development and in disease? The transcription factor Odd-skipped related 1 (Osr1) regulates ECM formation and maintains mesenchymal cell progenitors in several organs. We hypothesize that Osr1 regulates mesenchymal cell progenitors that mediate the deposition of ECM in the bladder during development and in injury. Osr1 mRNA is initially expressed in epithelial and mesenchymal cells at the onset of bladder formation, but then it becomes restricted to a subset of mesenchymally-derived cells in the lamina propria and muscle layers of the adult bladder. Osr1 1/2 newborn mice have a decreased number of fibroblasts in the lamina propria accompanied by decreased collagen I and III. To understand the consequences of these ECM changes, bladder function was assessed. Adult Osr1 1/2 mice had lower bladder capacities, and they voided more frequently. Interestingly, adult Osr1 1/2 mice had an increase in collagen in the bladder suggesting injury. To determine if Osr1 regulates collagen deposition during bladder injury, we induced bladder obstruction in wild-type mice from spinal cord injury. Injured mice exhibited increased mRNA expression of Osr1 and collagen III. The results show that haploinsufficiency of Osr1 results in decreased collagen deposition. During bladder injury, there is an increase in collagen deposition and Osr1 is upregulated. These findings suggest that Osr1 demarcates a fibrogenic progenitor cell population during bladder development and disease. This work has been funded by CIHR.

**Program Abstract #309**

Grhl3 regulates lamellipodia in the non-neural ectoderm during neural tube closure

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During the final step of neural tube closure (NTC), two tissues: the continuous neural ectoderm (NE) and non-neural ectoderm (NNE) must separate from each other and fuse with their respective opposing fold to form the closed neural tube (NT) with an overlying epithelial layer (NNE). These contacts involve the use of small cellular projections induced by rearrangements of the actin cytoskeleton. The two main types of cellular projections are long thin projections known as filopodia, as well as broader and branched-actin based lamellipodia. Intriguingly, the composition of cellular projections that NNE cells use to facilitate closure changes depending on where closure is occurring along the rostral-caudal axis. Furthermore conditional deletion of Rac1 in the NNE, a RhoGTPase which controls lamellipodia dynamics, yields an axially biased NTD phenotype (89% Spina bifida vs. 25% anencephaly) (PMC4846376). Another protein whose loss of function yields similarly biased NTDs with regards to axial position is the NNE expressed transcription factor Grainyhead-like 3 (GRHL3). Grhl3 mutants exhibit spina bifida with 100% penetrance and only rarely exhibit exencephaly. GRHL3 has also been shown to be an important regulator of the actin cytoskeleton (PMC6170465) during NTC which, combined with the similar phenotype to Rac1 conditional knock-outs, presents a model where Grhl3 is also necessary for proper induction.
and regulation of lamellipodia during caudal NTC. Our study examines the effect that loss of GRHL3 has on the regulation of cellular protrusions, and more particularly, lamellipodia during caudal NTC. We demonstrate using E8.5-E10.5 mouse embryos from a Grhl3 knockout strain that loss of GRHL3 during caudal NTC results in altered cellular protrusions in the NNE. Grhl3 knockouts display less lamellipodia, defects in organization of lamellipodia and increased filopodia in the NNE compared to WT littermates during caudal NTC. Funding provided by NICHD R01 HD81117.

Program Abstract #310
Deciphering the regulatory code driving neural crest evolution and development
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Neural crest (NC) cells are a unique feature of the vertebrate lineage. This embryonic cell population is uniquely multipotent and gives rise to many structures including peripheral neurons and the craniofacial anatomy, the latter which contributes to the diversity and success of modern vertebrates. Defects in the NC are implicated in developmental disorders and knowledge of the essential elements of NC regulation could reveal therapeutic targets. The sea lamprey, Petromyzon marinus, is at the base of the vertebrate lineage and offers an ideal model for the ancestral NC. Comparisons to the modern NC gene regulatory network (GRN) such as that of the chicken, Gallus gallus, can elucidate essential conserved regions of the network. A draft of the NC GRN has been assembled, but many of the cis-regulatory elements and direct interactions between genes are unknown. Previous ATAC-seq studies in P. marinus revealed enhancer regions for the SoxE1 and Tfap2 genes which are active specifically in the lamprey NC. Additionally, the lamprey SoxE1 enhancer showed conserved activity in both the chicken and zebrafish NC. Here, we subdivide the SoxE1 and Tfap2 enhancers to find the core regions responsible for their activity. Preliminary findings show that the central 610 bp of the SoxE1 enhancer, which encompasses the original ATAC peak, retains its activity in both the lamprey NC and chicken NC. Mutation of a putative Sox10 binding site within this region does not result in a loss of enhancer activity in the chicken NC. Future assays will mutate additional transcription factor binding sites in the core region of the SoxE1 enhancer and resulting constructs will be tested for activity in both the chicken and lamprey NC. NC specific-reporter activity will be confirmed by HCR staining on lamprey and chicken embryo sections. This data provides insight into the core regulatory mechanisms underpinning the NC as well as its conservation across the vertebrate lineage. Funded by the NRF.

Program Abstract #311
Gene regulatory network evolution during Drosophila melanogaster and Aedes aegypti nervous system development
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Development proceeds through coordinated gene expression programs governed by gene regulatory networks (GRNs). Understanding how GRNs evolve over a large evolutionary range is challenging due to a lack of known transcription factor interactions, cis-regulatory modules (CRMs), and gene expression patterns for related organisms. We are studying GRN evolution during development of the central nervous systems (CNS) of Drosophila melanogaster and its ~250 Mya diverged relative the disease vector mosquito Aedes aegypti. Despite similar CNS morphology, expression of a set of genes co-expressed in the ventral midline of the D. melanogaster CNS, including the master regulator single minded (sim), has diverged in the two species. Midline expression of these genes is absent or severely diminished in A. aegypti; instead the genes are co-expressed laterally. This suggests that the D. melanogaster “midline GRN” has been redeployed laterally in A. aegypti. To characterize the responsible mechanisms, we identified relevant CRMs in both species. Analysis of these regulatory sequences in transgenic D. melanogaster suggests that the altered gene expression observed in A. aegypti is primarily a trans-dependent redeployment of the GRN. Potentially, this stems from a cis-mediated change in the expression of sim and other upstream regulators. We are continuing to evaluate midline CRMs for both species using reciprocal reporter gene assays in both transgenic D. melanogaster and A. aegypti. To elucidate the trans-acting factors
contributing to the altered gene expression, we are ectopically expressing combinations of candidate transcription factors in the lateral *D. melanogaster* CNS with the goal of phenocopying the *A. aegypti* lateral expression pattern. Our results illuminate a novel mode of evolution, “repeal, replace, and redeploy”, in which a conserved GRN functions at a new site while its original function is co-opted by a different GRN. Funded by the National Science Foundation.

**Program Abstract #312**

**Cartilage proteoglycans inhibit BMP-mediated bone formation**

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The involvement of the extracellular matrix (ECM) in cell signalling is not fully understood, but it may hold secrets relating to human pathologies of the skeleton. During endochondral ossification, which produces most bones in the human body, chondrocytes secrete a proteoglycan (PG)-rich ECM in which growth factors such as Indian Hedgehog (IHH) can travel to induce maturation and perichondral bone formation. Mutation in *fam20b*, encoding a xylose kinase that phosphorylates the initiator xylose residue within the tetrasaccharide linkage region in proteoglycans, leads to lower levels of chondroitin sulfate proteoglycans and initiation of perichondral bone formation earlier. Also, levels of *ihha* and *ihhb* transcripts were up-regulated in chondrocytes of the ceratohyal in *fam20b* mutants. IHH and bone morphogenetic protein (BMP) mutually promote the expression of each other to regulate chondrocyte maturation and osteogenic induction. Here, we aim to link decreased cartilage proteoglycans to an increase in osteogenic growth factor signalling, hypothesizing that cartilage PGs normally inhibit BMP signalling. In order to evaluate changes in the BMP signalling pathway, cryosection immunocytochemical staining was performed for p-Smad and p-p38, two BMP signaling pathway markers. Mutant *fam20b* zebrafish chondrocytes had up-regulated BMP signalling. Transgenic BMP loss-of-function (hsp70I:dnBmpr1a-GFP) zebrafish embryos had downregulated BMP signalling and less perichondral bone, as determined by Alcian blue/Alizarin red staining, compared to wild-type fish. Also, early perichondral bone formation in *fam20b* mutants was phenotypically rescued by the BMP loss-of-function transgene, establishing a relationship between BMP and proteoglycans. Through these in vivo analyses, we propose a novel role of ECM proteoglycans as inhibitors of BMP signalling molecules that control bone formation. Funding sources: CIHR project grant.

**Program Abstract #313**

**Intracisternal administration of tanshinone IIA-loaded nanoparticles leads to reduced tissue injury and functional deficits in a porcine model of ischemic stroke.**

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Tanshinone-IIA (Tan IIA) has shown success in preclinical rodent studies of ischemic stroke but led to inconsistent efficacy in human patients. The physical properties of Tan IIA, including short half-life and low solubility, suggest that polylactic-co-glycolic acid (PLGA) nanoparticle (NP) delivery may lead to improved and more consistent therapeutic effects. The objective of this study was to evaluate the acute therapeutic effects of Tan IIA-NPs on cerebral pathology and consequent motor function deficits in a translational pig ischemic stroke model. Tan IIA-NP treatment lead to reduced brain swelling, midline shift, lesioning and hemorrhage and improved white matter integrity and diffusivity in stroke tissue. These tissue level improvements corresponded with an acute reduction in motor function deficits. These promising results in a translational pig model suggest acute Tan IIA-NP administration may decrease stroke pathology and improve functional outcomes and the quality of life of future
stroke patients. This work was supported by the National Institutes of Health, National Institute of Neurological Disorders and Stroke grant R01NS093314.

**Program Abstract #314**

**Co-option of a developmental transcriptional circuit driving cell migration by neural crest-derived cancer**

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During malignant transformation, cells harboring oncogenic mutations de-differentiate into a progenitor state to promote tumorigenesis. In our lab, we use the neural crest as a model for studying how this reactivation of developmental programs play a role in cancer progression. The neural crest is a migratory stem cell population in the vertebrate embryo, that gives rise to several adult derivatives such as glia, neurons, and melanocytes. Strikingly, neural crest-derived cancers like melanoma and neuroblastoma are prone to metastasis and are highly aggressive. Further, the process of neural crest migration is physiologically very similar to cancer metastasis, which hints at shared mechanisms of regulation. By analyzing the neural crest transcriptome, we found that at the onset of migration, these cells undergo a characteristic shift to aerobic glycolysis. This results in increased glucose consumption and lactate production, that are hallmarks of the Warburg effect (WE), a distinctive feature of cancer cells. The metabolic reprogramming promotes cell migration via activation of the YAP-TEAD transcriptional program, which directly controls the key regulators of epithelial-to-mesenchymal transition. Intriguingly, transcriptome and genomic profiling of neural crest-derived cancers further revealed that this regulatory circuit re-emerges in metastatic melanoma cells. Together, our findings suggest that downstream of the Warburg effect, the Yap/Tead signalling program promotes the re-emergence of developmental transcriptional circuits to control cancer metastasis. This work was supported by NIH grants R00DE024232 and R01DE028576 to M.S.-C., a Basil O’Connor Starter Scholar Award from March of Dimes.

**Program Abstract #315**

**Unique and Overlapping Effects of Triiodothyronine (T3) and Thyroxine (T4) on Sensory Innervation of the Chick Cornea**

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Multiple aspects of cornea development, including the innervation of the cornea by trigeminal axons, are sensitive to embryonic levels of thyroid hormone (TH). Although previous work showed that increased TH levels could enhance the rate of axonal extension within the cornea in a thyroxine (T4)-dependent manner, details underlying the stimulatory effect of TH are unclear. By examining the effects throughout all stages of cornea innervation of the two main THs, triiodothyronine (T3) and T4, we provide a complete characterization of the stimulatory effects of TH on corneal nerves and begin to unravel the underlying molecular mechanisms. During development, trigeminal axons are initially repelled at the corneal periphery and encircle the cornea in a pericorneal nerve ring prior to advancing into the corneal stroma radially from all along the nerve ring. Overall, exogenous T3 led to pleiotropic effects throughout all stages of cornea innervation, whereas the effects of exogenous T4 was confined to timepoints following completion of the nerve ring. Specifically, exogenous T3 accelerated the formation of the pericorneal nerve ring. By utilizing \textit{in vitro} neuronal explants studies we demonstrated that T3 acts as a trophic factor to directly stimulate trigeminal nerve growth. Further, exogenous T3 caused disorganized and precocious innervation of the cornea, accompanied by the downregulation of inhibitory \textit{Robo-Slit} signaling within developing eyefronts. Following nerve ring completion, the growth rate and branching behavior of nerves as they advanced into and through the cornea were found to be stimulated equally by T3 or T4. These stimulatory influences of T3/T4 over nerves likely arose as secondary consequences brought on by TH-mediated modulations to the corneal extracellular matrix, including keratan- and chondroitin-sulfate containing proteoglycans. These findings shed insight into the mechanistic basis of how T3 and T4 leads to accelerated cornea innervation.
Program Abstract #316

An Axon-Pathfinding Mechanism Preserves Epithelial Tissue Integrity

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Dividing cells often move apically within epithelial tissue layers, likely to escape the spatial confinement of their neighbors. Because of this movement, daughter cells may be born displaced from the tissue layer. Reintegration of these displaced cells helps support tissue growth and maintain tissue architecture. In the Drosophila follicular epithelium, reintegration relies on the immunoglobulin-superfamily cell-adhesion molecules (IgCAMs) Neuroglian and Fasciclin 2. These molecules have been described in epithelia, but are well-studied for their roles in neural development. We show here that reintegration works in the same way as IgCAM-mediated axon growth and pathfinding; it relies not only on extracellular adhesion but also mechanical coupling between IgCAMs and the lateral Spectrin-Based Membrane Skeleton. Our work indicates that reintegration is mediated by a novel epithelial cell-cell junction that is compositionally and functionally equivalent to junctions made between axons. This work was funded by NIGMS R01GM125839.

Program Abstract #317

Rap1 regulates actomyosin dynamics to drive rapid wound repair

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Collective cell movements contribute to tissue development and repair, as well as the spread of disease. We investigate collective cell migration during wound healing in the Drosophila embryonic epidermis, where cells coordinate their movements to rapidly seal lesions. Cells immediately adjacent to embryonic wounds internalize cell-cell adhesion molecules from the wound edge and polarize their actomyosin cytoskeleton to form a supracellular cable around the wound. Cable contraction and reinforced adhesion at former tricellular junctions coordinate cell movements to repair the wound. However, the signals that initiate the molecular rearrangements that drive wound closure are unclear. The small GTPase Rap1 is a mechanosensitive molecular switch that promotes cytoskeletal polarization, regulates cell-cell adhesion turnover, and interacts with actin regulators. Therefore, Rap1 may coordinate and integrate the adhesion and cytoskeletal rearrangements that drive wound repair. Reducing Rap1 activity slowed the rate of wound repair by 68%. The decrease in wound closure rate was accompanied by a 69% decrease in myosin accumulation to the wound edge. Conversely, increasing Rap1 activity resulted in wounds that repaired 14% faster and accumulated myosin 3-fold faster. Our results indicate that Rap1 contributes to rapid embryonic wound healing by controlling myosin dynamics. We tracked the dynamics of Rap1 and E-cadherin localization during wound closure and found that Rap1 and E-cadherin were concurrently depleted from the wound edge and localized to tricellular junctions. Our lab demonstrated that removal of E-cadherin from the wound margin is necessary for polarization of myosin. Thus, our data support a model in which Rap1 triggers the re-localization of E-cadherin, promoting the formation of a contractile actomyosin cable that drives rapid wound repair. This work is supported by a CIHR postdoctoral fellowship awarded to KR and funding from the CIHR awarded to RFG.

Program Abstract #318

Uncovering the Podocyte Foot Process Proteome

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Kidney podocytes form elaborate cellular protrusions termed foot processes. Multiple podocytes interdigitate their foot processes through unique cell-to-cell contacts thereby forming a molecular sieve. Compromise to foot process integrity is frequently observed in kidney disease and cannot be repaired. However, little is known about how these processes form during development and are maintained thus hindering preventive or regenerative therapies. Identifying the spatially localized foot process proteome would provide unprecedented insights into this specialized structure. To this end, we developed a novel proximity-dependent biotin identification (BioID)
mouse model. Podocin (Nphs2) is highly expressed and localized to the foot process. We knocked-in the BioID moiety with a flexible linker into the Nphs2 locus, generating a podocin-BioID mouse that would allow us to identify the in vivo foot process proteome. We validated the podocin-BioID fusion properly localizes in podocytes via immunofluorescence. We isolated biotinylated proteins and performed mass spectrometry analyses. We are identifying candidates for follow-up studies focused on defining their role in development and promoting/maintaining foot process integrity. Further characterization and functional significance of novel proteins will be tested utilizing Danio rerio (zebrafish). Zebrafish offer a well conserved podocyte and nephron structure and are highly amenable to rapid forward/reverse genetic approaches, allowing us to test candidate significance. In turn, we will confirm the functional significance of proteins characterized in zebrafish in our mammalian mouse model. These bioorthogonal assays position us to generate the unique podocyte foot process proteome and uncover novel proteins required for generating and supporting podocyte integrity necessary for proper blood filtration. Funding: GFG: F32 NIAMS, LO: R01-NIDDK, Vanderbilt O’Brien Kidney Center.

Program Abstract #319
Effect of Phenylalanine, Retinoic Acid, and 4-diethylaminobenzaldehyde on Proliferation of O9-1 Mouse Cranial Neural Crest Cells
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Maternal phenylketonuria [MPKU] is a syndrome of multiple congenital anomalies including cardiovascular malformations [CVMs], and brain and growth restriction when a mother with Phenylketonuria [PKU] does not control her dietary intake of Phenylalanine [Phe]. However, the mechanisms responsible for Phe-induced CVMs are poorly understood. Our lab has preliminary evidence that high levels of Phe could inhibit Retinoic Acid [RA] signaling, which typically promotes the expression of genes controlling proliferation, migration, and differentiation. Proliferation of the neural crest cells are important in formation of the outflow tract (OFT) and aortic arch arteries (AAA). We hypothesize that Phe inhibits proliferation, which may contribute to the defects seen in MPKU. We also looked at the effects of exposure to RA and 4-diethylaminobenzaldehyde [DEAB], a known RA inhibitor. We conducted in-vitro proliferation assays on mouse neural crest cells o9-1, to determine the effect of Phe, RA, and DEAB exposure on proliferation. Images were analyzed with ImageJ and GraphPad Prism. Present research suggests that Phe exposure causes a significant decrease in proliferation of cells. It has been shown that RA increases proliferation, and that DEAB decreases cell proliferation. In this way, Phe induced a similar response to DEAB, which suggests that it may act as an RA inhibitor. This could contribute to the CVMs observed in MPKU. This work is significant because it eludes to a potential mechanism that Phe could affect RA signaling, thus resulting in the types of defects observed in human MPKU. This project is supported by funding by CURE-STEM, College of Math and Science, UCO and Faculty on Campus Grant funding by The Office of Research and Sponsored Projects, UCO, and Oklahoma INBRE SMART Grant, Student Transformative Learning Record at UCO.

Program Abstract #320
Epithelial cell dynamics during small intestinal elongation
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In utero, the small intestine elongates rapidly to acquire a remarkable length which ensures a sufficient absorptive surface area for postnatal nutritional demands. However, the underlying mechanism of fetal small intestinal elongation remains unclear. Our previous study showed that the early fetal mouse intestinal epithelium is pseudostratified (E10.5–14.5). Nearly all epithelial cells are actively cycling with their nuclei traveling between basal and apical surfaces in concert with the cell cycle, a process known as interkinetic nuclear migration. By combining 3D high-resolution confocal imaging and 2D live imaging, we profiled behaviors of individual epithelial cells in detail and found that more than half post-mitotic daughter cells need to project a robust filopodial-like protrusion to make a basal path to return their nucleus, called "pathfinding" strategy. Interestingly, this filopodial "pathfinding" is guided by Wnt5a ligand, secreted by the underlying mesenchyme. Without Wnt5a, this "pathfinding" process was compromised, leading to loss of a subset of epithelial progenitors and slowing down SI elongation. However, it was unclear how "pathfinding" daughter cells recognize Wnt5a cue from the mesenchyme
and transduce that signals to instruct their filopodial projection. Two known Wnt5a receptors, Ror2 and Ryk, were investigated. Surprisingly, epithelial Ror2 is not required for intestinal elongation. Instead, Ryk, similar to Wnt5a, facilitates the proper projection of filopodial-like protrusion for pathfinding cells. Taken together, our study revealed that Wnt5a-Ryk navigated “pathfinding” is important for rapid fetal small intestinal elongation. This work is supported by the NIH (R01DK089933 and R01DK117981).

Program Abstract #321

Spatiotemporal regulation of thyroid hormone signaling specifies cone subtypes in human retinal organoids

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Trichromatic color vision in humans requires three cone photoreceptor subtypes that are sensitive to blue, green, or red light. We previously used human retinal organoids to show that cone subtype specification is controlled by thyroid hormone (TH) signaling, with low TH signaling early to specify blue cones and high TH signaling late to generate green/red cones. Here, we outline a project to determine how TH is regulated within the retina to control cone subtype specification. TH exists in two main forms, inactive T4 and active T3, which are regulated by deiodinase enzymes. DIO2 converts T4 into active T3, whereas DIO3 degrades T4 and T3. RNA-seq of retinal organoids showed that DIO3 expression peaks early then decreases whereas DIO2 expression increases late. We investigated the cell-type-specific expression of these regulators and found that DIO3 expression is confined to retinal precursor cells (RPCs) early and DIO2 is expressed in cones late. These data suggest that the large pool of RPCs early in development express DIO3 to limit TH. As developmental time progresses, RPCs differentiate and DIO3 expression decreases, reducing degradation of TH. A subset of RPCs differentiate into cone precursors that express DIO2, which increases local TH signaling and pushes the later-specified cones toward green/red fate. We are testing this model by visualizing expression of TH regulators in human retinas and organoids and using CRISPR to ablate function in organoids to determine their roles in cone subtype specification. These studies will inform the role of TH regulation in the generation of cone subtypes and will advance the use of retinal organoids to understand mechanisms of development. Funding Source: R01EY030872

Program Abstract #322

Molecular characterization of retinal ganglion cell subtypes in human retinal organoids.

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Retinal Ganglion Cells (RGCs) are the projection neurons that relay visual information from the retina to the brain. Death or damage to RGCs causes blinding conditions, including glaucoma. The study of RGC development in humans is challenging due to the lack of experimental access to viable human tissue. To obviate this obstacle, we study RGC development in human retinal organoids. Human retinal organoids are “mini-retinas” differentiated from human stem cells that recapitulate retinal development, including the generation of RGCs. Subtypes of RGCs can be defined by their morphologies, physiologies, and transcriptomes. ~18 RGC subtypes have been classified in macaque, yet the number and defining features of RGC subtypes in the human retina are unknown. The main goal of this project is to identify the subtypes of RGCs in the human retina and determine the developmental mechanisms that diversify their functions. To achieve this goal, we are characterizing RGC subtypes in human retinas and organoids with scRNA-seq and imaging-based expression analysis. Thus far, we have identified two subtypes of RGCs in human organoids based on their expression of the transcription factor Foxp1. Surprisingly, markers with subtype-specific expression in macaque and mice appear to be expressed in all RGCs in humans, suggesting an interesting evolutionary divergence. In the future, I will use CRISPR genome editing to mutate genes encoding subtype-specific transcription factors to identify regulators of RGC subtype specification. Our work will test mechanisms of RGC specification in developing human tissue, providing insights critical for understanding glaucoma progression and therapeutic applications.

Program Abstract #323

Novel regulatory role of miR-124 on mesodermal cell specification

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MicroRNAs (miRNAs) regulate gene expression by inhibiting translation in animal cells. MicroRNA-124 (miR-124) has been examined mostly in neurogenesis. This study discovers a novel role of miR-124 in regulating the specification of mesodermally-derived immune cells. Early in sea urchin development, mesodermally-derived immune cells give rise to blastocoelar cells (BCs) and pigment cells (PCs) that must make a binary fate decision to differentiate from one another. Results indicate that inhibition of miR-124 resulted in embryos with a significant expression expansion of BC-specific transcription factors (TFs) and a concurrent reduction of specified PCs. miR-124 directly suppresses *Nodal* that is active early in the oral ectoderm to promote BC fates and suppress PC fates. Because the presumptive BCs are located on the oral side of the embryo, BCs receive Nodal signaling temporally earlier than the PCs. Further, miR-124 also directly suppresses BC-specific TF, *Ese*. The overexpression of *Ese* is sufficient to reduce number of differentiated PCs, indicating its importance in PC specification. Overall, this study demonstrates miR-124 in regulating mesodermal cell specification, by directly suppressing *Nodal* and *Ese*. Funding: NSF CAREER for JLS, Grant-In-Aid of Research from Sigma Xi for KK.

Program Abstract #324
The amino acid transporter *Slc38a2/SNAT2* provides proline to fulfill energetic and biosynthetic demands during osteoblast differentiation.

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Osteoblasts are the chief bone forming cell. Osteoblast differentiation is associated with enhanced biosynthetic and energetic demands due to increased protein and collagen matrix production and secretion. To understand how osteoblasts fulfill these changing metabolic needs, we established a bioinformatic model based on transcriptional changes and protein amino acid composition to predict how amino acid demand changes during differentiation. Our model indicates proline demand increases during osteoblast differentiation. Consistent with this inference, osteoblasts increase proline consumption during differentiation. Thus, proline may be a critical regulator of osteoblast differentiation and function. However, how osteoblasts obtain or utilize proline is unknown. To resolve this question, we profiled the transcriptome of osteoblasts and identified *Slc38a2* (encoding Sodium-coupled Neutral Amino acid Transporter 2 - SNAT2) as the most highly expressed proline transporter in osteoblasts. We used a Crispr/CAS9 targeting approach to knockout *Slc38a2*. Osteoblasts lacking *Slc38a2* had significantly reduced proline uptake and reduced osteoblast differentiation *in vitro*. Conditional deletion of *Slc38a2* in either mesenchymal progenitors using *Prx1Cre* or preosteoblasts using *Sp7Cre* resulted in defects in both intramembranous and endochondral ossification due to delayed osteoblast differentiation and bone matrix production. Mechanistically, proline is primarily incorporated directly into protein as we were unable to trace proline carbon into any other metabolites. Additionally, proline is involved in energy production as inhibiting proline uptake or oxidation resulted in decreased oxygen consumption and increased AMPK phosphorylation hallmarks of energetic stress. Collectively, these data demonstrate osteoblasts require proline to fulfill both energetic and synthetic demands associated with osteoblast differentiation and bone matrix production. Supported by NIH R01AR071967.

Program Abstract #325
A Previously Uncharacterized Connexin Mediates Early Muscle Differentiation

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Animal behavior constitutes a series of movements that are achieved through the communication and coordination of the nervous system with the musculature. One method of fast intercellular communication used by both neurons and muscles is the specialized intercellular channels called Gap Junctions (GJs), which allow cells to share small metabolites and ions directly. In vertebrates, GJ channels are composed of a large family of highly conserved transmembrane proteins, called Connexins (Cx). While GJ communication within neurons and between muscles exists, little is known about the precise molecular makeup of the Cxs that form the GJ channels, nor how they contribute to the coordination of behavior. To address this gap, we are utilizing the embryonic zebrafish, and the very first behavior of the fish, spontaneous coiling, which requires GJ channels in both the
neurons and muscles. To identify the zebrafish Cxs required for coiling, we analyzed whole embryo single cell RNA-sequencing (scRNAseq), and looked for highly enriched Cx expression in neurons and muscles known to drive coiling. From this analysis, we identified a previously uncharacterized Cx, Cx46.8, expressed specifically in slow muscle cells. Using CRISPR/Cas9 we disrupted cx46.8 function and found that mutants display defects in the initiation, strength, and symmetry of coiling behavior. Preliminary data suggests that cx46.8 mutant have fewer slow muscle precursors and disorganization of myosin, suggesting that Cx46.8 is facilitating the development of slow muscle fibers. Our current model is that Cx46.8 mediates calcium signaling between developing slow muscle cells, facilitating differentiation. We are working to test this at a variety of molecular and functional levels. Further work will use similar approaches to identify the Cxs relevant to the neural circuits that drive coiling. Funding provided from the Genetics Training Grant T32 GM007413 to R.M.L, R01 NS105758 to A.C.M, and R24 OD026591 to A.C.M.

Program Abstract #326

gata5/6 broadly regulate the early specification of mesoderm lineages
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GATA4/5/6 play evolutionarily conserved roles in heart development, with loss of Gata5/6 resulting in a heartless phenotype in zebrafish. However, little is known about how gata5/6 mediate the transition from mesoderm progenitors to a committed cardiac fate. Here, we interrogated this process by performing single-cell mRNA-seq of gata5: GFP+ cells from wild-type and Gata5/6 morphant embryos spanning early gastrulation (6hpf) to early somite (13hpf) stages. As development proceeds, heterogeneity increased with 20 cell types evident by 13hpf. These lineages were either absent (cardiac, myeloid), reduced (pronephric), or expanded (hematopoietic, pharyngeal/cranial) upon Gata5/6 loss. We confirmed a cell-autonomous role for gata5/6 in regulating the specification of these lineages using gata5/6 mutants. Notably, pseudotime analysis of gene expression dynamics along the developmental trajectory of each lineage revealed that gata5 expression was highly dynamic and lineage specific. In particular, gata5 expression was maintained in the cardiac lineage, yet gradually turned off in the pharyngeal mesoderm, suggesting that the modulation of gata5 expression may be crucial for cardiopharyngeal cell fate divergence. We confirmed that cardiac progenitors are biased to a pharyngeal fate by CRISPR-mediated loss of GATA4/5/6 in the chordate model Ciona. Through single-cell open chromatin profiling at corresponding stages, we identified cardiac-specific gata5 regulatory elements, which could be responsible for maintaining its cardiac-specific expression. Our most recent data show a global downregulation of BMP signaling in Gata5/6 deficient embryos with a loss of accessible chromatin signatures near BMP target genes. Currently, we hypothesize gata5/6 facilitate the divergence of mesodermal lineages by regulating BMP signaling. Overall, our study reveals an evolutionarily conserved role for GATA4/5/6 in the diversification of multiple mesoderm lineages. Funding: CIHR, HSF and OTS.

Program Abstract #327

Gene dosage and environmental factors modulate phenotype variability in congenital anomalies
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Clinically, it is well established that individual congenital disorders may encompass a broad range of phenotypes with varying degrees of severity, but that no phenotypes are identical in all affected individuals. Such variability in genotype-phenotype correlation is particularly evident in rare craniofacial disorders, with Treacher Collins syndrome (TCS) as a prime example. Individual phenotypes range from being mild or unaffected, to severe micrognathia with cleft palate that compromise breathing and feeding. TCS is primarily associated with mutations in TCOF1, which encodes a nucleolar phosphoprotein called Treacle that is necessary for normal craniofacial development. Treacle specifically functions in rDNA transcription and ribosome biogenesis, which are essential for normal growth and proliferation. Treacle also plays a key role in DNA damage repair, protecting progenitor neural
crest cells from oxidative stress induced DNA damage and cell death. Currently more than 200 mutations spanning the entire length of TCOF1 have been identified, and collectively they are associated with considerable inter- and intra-familial phenotypic variability. In this study we explore the role of gene dosage and environmental factors on the pathogenesis of TCS in animal models which mimic the phenotypic variance observed in humans. Using six different strains of mice, we discovered that endogenous levels of Treacle protein and reactive oxygen species (ROS) vary according to genetic background. A mild phenotype correlates with high levels of Treacle and low levels of ROS, whereas a severe phenotype manifests with low levels of Treacle coupled with high levels of ROS. Phenotypic variability can be further modulated by exogenous stress or dietary antioxidant supplementation. Thus, genetic and environmental factors such as ROS can each modulate the outcome of a genotype resulting in phenotypic variance. This work is funded by Stowers Institute for Medical Research.

Program Abstract #328
Suture involvement in midface hypoplasia and Bmp7’s role
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Postnatal craniofacial growth occurs at fibrous sutures separating skull bones where stem cells differentiate into osteoblasts and deposit new bone. Much of the current knowledge regarding sutures stems from craniosynostosis, leaving sutures of the midface understudied. Little is known about spatial/temporal contribution of midface sutures to facial growth. We used a mouse model of midface hypoplasia based on Bone Morphogenetic Protein 7 (Bmp7) deletion from neural crest cells (Bmp7<sup>ncx</sup>) focusing on midface sutures. Histology revealed increased osteoclast activity within the mesenchyme and osteoclasts lining the inner surfaces of nasal bones and contralateral loss of osteoblasts. Midface tissue gene expression analysis showed an increase in Sca1, a marker observed in early intramembranous bone formation. A concomitant decrease in genes expressed in further differentiated cells like Fgfr2, Sp7, and Col1a1 was observed. Calvarial osteoblasts were isolated from E18.5 Bmp7 knockout and control embryos to identify intrinsic molecular changes. Osteoblasts lacking Bmp7 show complete loss of Wnt antagonist Dkk1. In combination with an increase in Wnt11 and dysregulation of genes like Bmp2 and 5, this change may lead to premature ossification. Rapid mineralization could cause osteoblast depletion, which the system must cope with through bone remodeling and cellular changes. These consequences are reflected in Bmp7<sup>ncx</sup> increased osteoclast presence, abnormal skull architecture, and expanded progenitor pool. Our data indicate that suture abnormalities directly contribute to midface hypoplasia. We also find that Bmp7 controls early bone development. Thus, changes to suture behavior are a likely and so far insufficiently considered contributor to midface hypoplasia. This insight into the functionality of sutures in midface hypoplasia is crucial in a field like pediatrics, where minimally invasive options are mandated. Funding: Women and Children’s Health Research Institute

Program Abstract #329
Fgf8 dosage levels contribute to asymmetries in jaw and pharyngeal pouch development
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Pharyngeal pouches provide structure and signals for morphogenesis of the craniofacial skeleton. If pouches fail to form, or are malformed, skeletal derivatives of the associated arch will be altered. Despite these roles in head development, pouch development is poorly understood in mammals. We first investigated the role Fgf8 has on pouch formation in mice at different Fgf8 levels, including a mild and severe mutant. In both mutants the first pharyngeal pouch (pp1) out-pockets laterally to contact the ectoderm but fails to extend proximo-distally. In mild mutants pp1 is reduced in size and extends proximo-distally. In severe mutants pp1 does not extend and remains circular. Moreover, the ectodermal cleft is hypoplastic and disorganized, contributing to the failure of separation between the first two arches. These data suggest Fgf8 has more roles in pouch formation beyond directing lateral out-pocketing, which may include regulating extension of the pharyngeal endo- and ectoderm. Although reductions in Fgf8 cause cell death in migrating neural crest, apoptosis is not sufficient to explain jaw defects in Fgf8 mutant mice. Instead we find evidence for disruptions to patterning as exemplified by alterations to the position of the hinge or joint of the jaw. Our data suggests that pouch shape does not vary bilaterally, but that at
E10.5 the right pouch is deeper than the left. Interestingly, Fgf8 mutant mice have directionally asymmetric jaws with the left side being more affected than the right. These data indicate an asymmetric contribution to facial development whereby higher levels of Fgf8 may promote accelerated development of the right side of the face in normal development that may be linked to asymmetry in heart development. Further, higher levels of Fgf8 expression on the right may partially buffer the right side of the face from disease processes. Funding source: NIDCR R15 DE026611

Program Abstract #330
Wnt Signaling Control of Secondary Palatogenesis Illuminated by Single-cell Transcriptomes
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Wnt signaling is integral to many different processes during embryonic development and helps coordinate the activity of several important pathways during craniofacial morphogenesis. Wnt5a is required for formation of the secondary palate, and is expressed in both major tissue lineages in the palatal primordia, epithelium and mesenchyme. Wnt5a has been demonstrated to contribute to a number of downstream functions, depending on context. Its loss of function results in cleft palate with defective mesenchymal proliferation, though the mechanism by which Wnt5a signaling contributes to these activities during secondary palatogenesis is unclear. In order to better understand the roles Wnt5a plays in the developing palatal shelves, we have used loxP-Cre targeting to conditionally ablate its activity in Pax3-expressing mesenchyme. Using 10X genomics 3’ chromium method, we have performed single cell RNA sequencing of palatal shelf tissue in E14.5 mouse embryos lacking mesenchymal Wnt5a and their wild type littermates. Based on the gene expression profiles of cells in the converging palatal shelves, we have defined subpopulations of differentiating tissue based on conserved markers across experimental groups to understand the transcriptomic landscape of the developing palate. We then compared expression within these subpopulations to assess how disrupted Wnt5a signaling affects gene expression within palatal mesenchyme, which groups of cells specifically are targeted, and which intermediate factors or pathways may link Wnt signaling with mesenchymal proliferation. This research is funded by the NIH National Institute for Dental and Craniofacial Research.

Program Abstract #331
A zebrafish model of ALX-linked frontonasal dysplasia
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The vertebrate midface comprises neural, ocular, and craniofacial lineages that are closely apposed during embryogenesis, develop in concert, and are influenced by poorly understood gene-environment interactions. In humans, mutations in the ALX family of homeobox transcription factors are linked with frontonasal dysplasia and ocular malformations that range from mild (coloboma) to severe (microphthalmia and anophthalmia). In vertebrate embryos, transcription of alx1, alx3, and alx4 is restricted to anterior cranial neural crest (aCNC), which originates in the dorsal neural tube, then migrates rostrally and gives rise to facial cartilages. This suggests Alx genes function cell-autonomously in the aCNC and non-cell-autonomously in the developing eye. The mechanism of Alx genes in either context is unknown. We generated zebrafish mutants at the alx1 locus and found that homozygous alx1 zebrafish develop with low-penetrance craniofacial, ocular, and vascular defects. Exposure to an environmental toxin (alcohol) during early embryogenesis increases penetrance of these malformations, suggesting that alx1 confers robustness against environmental insults in the aCNC lineage. Notably, transcription of alx3 is increased in alx1 homozygotes, likely due to transcriptional adaptation. To further probe Alx functions, we generated alx1;alx3 double mutant zebrafish. We show that alx1;alx3 mutants develop with highly penetrant cranial and ocular defects, characterized by significant loss of the median anterior neurocranium and aberrant retinal ganglion cell differentiation. This work establishes the first zebrafish Alx mutants as novel and powerful models for dissecting conserved gene-environment interactions during facial morphogenesis. Our research was supported by an AHA predoctoral fellowship to BY, Shriners Hospital research grants to JK and ECL, and grants from the NEI and UW VCRGE to YG.
Program Abstract #332
Modeling Cerebro-costo-mandibular syndrome (CCMS) in mouse by mutating Snrpb to understand its role in craniofacial development
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SNRPB, which codes for a core component of the spliceosomal small nuclear ribonucleoproteins is mutated in patients with a rare autosomal dominant disorder known as Cerebro-costo-mandibular Syndrome (CCMS). CCMS patients have mutations in the highly conserved alternative exon 2 (AE2) that has a pre-termination codon in \textit{SNRPB}. Mutations cause increased inclusion of AE2 resulting in nonsense-mediated decay of the transcript and is thought to lead to a general decrease of SNRPB. As SNRPB is a core protein of the splicing machinery in every cell, it was thought that the requirement of SNRPB would be equivalent in all the cells and tissues. However, CCMS patients have abnormalities mostly in craniofacial bones and ribs. The molecular etiology of how SNRPB mutation causes tissue-specific abnormalities seen in CCMS is unknown. We hypothesize that transcripts that are sensitive to the level of SNRPB during development are neural crest cell (NCC) specific, as these cells significantly contribute to craniofacial development. By using a mouse model, we aim to uncover the transcripts that are affected in CCMS due to the reduced SNRPB level. We have generated a \textit{Snrpb} conditional mutant mouse line with Loxp sequences and have mated the line with \textit{Wnt1}-cre mice to ask if the deletion of \textit{Snrpb} in NCCs causes craniofacial abnormalities. NCC-specific \textit{Snrpb} heterozygous mutant embryos show severe craniofacial malformations at embryonic day 12.5 and onward. \textit{Snrpb} haploinsufficiency in NCC is lethal as no animal was recovered at the time of weaning. Our preliminary cartilage and skeletal analysis of the mutant embryos show severe defects in the craniofacial structures. We will perform RNA sequencing of \textit{Snrpb} mutant NCCs to identify the transcripts presumably mis-spliced due to reduced SNRPB level. Our research findings will advance the knowledge of genes or pathways required for normal craniofacial development, which is interrupted in CCMS due to \textit{SNRPB} mutation.

Funding source: CIHR

Program Abstract #333
E-cadherin-mediated Cell Contacts Underlie Epithelial Symmetry Breaking \textit{in vivo}
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Epithelia provide resiliency to organs and act as a barrier to the environment, which depend on establishing polarized exteriorly-facing apical surfaces and basolateral surfaces oriented toward the body. Achieving this polarity requires symmetry breaking, a process that specifies the apico-basolateral axis based on prior asymmetric cues. Symmetry breaking occurs at the cellular level, such that each cell establishes an apico-basolateral axis, but also at the tissue level, as all cells in an epithelium orient this axis identically to generate a polarized tissue. Despite its requirement for establishing polarity, epithelial symmetry breaking remains poorly understood. To characterize the mechanisms underlying this process, we are studying the \textit{C. elegans} embryonic intestine, a simple epithelial tube composed of 20 ‘E’ cells that orient their apical surfaces toward a common midline surrounded by basolateral surfaces facing each other and non-E cells. This tissue is inherently asymmetric, as apical and basal surfaces form at homotypic (E/E) and heterotypic (E/non-E) contacts, respectively. We find this asymmetric contact is likely instructive for apico-basolateral symmetry breaking. In mutants with extra E cells, apical proteins localize to ectopically formed homotypic contacts, suggesting that E/E contacts inform apical polarization. We further tested a role for cell contacts by depleting the adhesion protein HMR-1/E-cadherin, which causes strongly delayed and uncoordinated apical polarization at the tissue level. Although individual E cells still aggregate apical proteins upon HMR-1 depletion, these domains exist as irregularly positioned patches that eventually coalesce near a common midline following an extreme developmental delay. Taken together, this work demonstrates that epithelia can break symmetry through the recognition of cell/cell contacts, a phenomenon largely unexplored \textit{in vivo}. Funding: CMB Training Grant (NIH 5 T32 GM007276), Stanford Graduate Fellowship
Program Abstract #334
Post-transcriptional tuning of signaling gradients mediates ectodermal patterning
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The neural crest is a stem cell population that gives rise to the craniofacial skeleton, the peripheral nervous system, and the pigmentation of the skin. Neural crest cells are induced adjacent to the forming neural plate by the combined action of signaling systems. Opposing morphogen gradients of Wnts and FGFs (Fibroblast Growth Factors) instruct ectodermal cells to decide between these two fates. Recent studies highlight the requirement of a precise combination of signals for proper ectodermal patterning, but we still have a superficial understanding of how levels of FGF and Wnt are fine-tuned to drive neural crest induction. Due to their diverse roles in development, we hypothesized that microRNA (miRNA) mediated gene silencing may play a role in modulating the activity of signaling pathways during early embryogenesis. To test this, we combined functional manipulation of the miRNA pathway with genomic and biochemical analyses. Our results show that knockdown of a key miRNA processing enzyme Dicer, causes ectodermal defects in avian embryos, with an expansion of the neural plate territory and loss of neural crest cells. Consistent with this phenotype, small RNA-sequencing of neural crest progenitors revealed that these cells express a subset of miRNAs that target essential components of the FGF signaling pathway, as confirmed by luciferase reporter assays. Inhibition or overexpression of these miRNAs caused a reduction or expansion in the number of neural crest cells, respectively, and also affected proper ectodermal patterning due to changes in FGF activity. Accordingly, we propose that neural crest induction relies on post-transcriptional attenuation of the FGF signals. Our results identify a post-transcriptional mechanism that reshapes morphogen gradients to accurately divide the ectoderm into distinct fields of progenitor cells. This work was supported by a Cornell CVG Scholars Award to J.C.

Program Abstract #335
Molecular basis of synovial joint site specification in a developing chick limb
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The initially contiguous embryonic cartilage template is iteratively segmented to give rise to most of the appendicular skeletal elements of vertebrates. Interestingly, in every species, the respective ratio of each skeletal element lengths stays constant suggesting tight molecular regulation towards determination of the segmentation site. The process of segmentation is initiated by the formation of a specialized structure called the Interzone where the cells are morphologically and molecularly distinct from the adjoining cartilage cells. However, the molecular mechanism that dictates the location of Interzone formation is still unknown. We attempted to investigate the molecular mechanism dictating the formation of the Interzone site using an analogous developmental process called somitogenesis, the process by which discrete somites are formed by iterative segmentation of a contiguous structure called paraxial mesoderm. Reciprocal gradients of RA and FGF signaling along the anterior-posterior axis of the growing embryo dictate somitogenesis. According to Olivier Pourquie’s model, at a particular threshold level of RA and FGF signaling, somite segmentation happens (Dubrulle and Pourquie, 2004). Interestingly, the same two signaling molecule gradients exist in limb context as well. Thus, by analogy, we hypothesized that the site of Interzone is also specified by RA and FGF gradients. To investigate the hypothesis, we chose chick as the model organism and used beads soaked in RA/FGF8/inhibitors of RA and FGF signaling pathways to perturb the gradients. We here demonstrated that these bead manipulations alter the femur-tibia length ratio and shift the expression domain of the Interzone marker gene, Barx1 (relative to body axis). We, hereby, establish that RA and FGF gradients dictate the site of joint formation. Funding: Department of Biotechnology (DBT)

Program Abstract #336
Alternative polyadenylation of the Hes7 3’UTR is required for proper segmentation
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The embryonic segmentation of vertebrate embryos into repeated structures called somites is regulated by a
genetic clock comprised of oscillatory genes which cycle with a period matching the species-specific rate of somite formation, for example, 2 hours in mice. Tight regulation of the genetic clock is crucial for axial skeleton development. In mice, Hes7 is a critical clock gene, and previous work has shown that sequences in the 3’UTR of the Hes7 transcripts are required for oscillatory expression. Here we show that the genomic region downstream of the Hes7 gene contains multiple alternative polyadenylation (APA) signal sequences, and their utilization in mouse embryos produces three distinct transcript isoforms, varying only in the length and sequence of the 3’UTR. All three isoforms are expressed in an oscillatory manner, however the longest isoform is depleted in the anterior PSM, suggesting tissue level changes to the regulation or production of the isoforms. Additionally, the longer isoforms have reduced translational efficiency, influencing the rate of oscillations through the amount of protein produced. Finally, we used CRISPR to mutate the endogenous Hes7 locus, blocking the production of longer isoforms of Hes7. These mice show an increase in the number of vertebrae produced and some minor abnormalities in some axial skeleton structures. The increased vertebral number would be consistent with a slight increase in clock period, suggesting that APA of Hes7 aids in setting the pace of the segmentation clock. Ongoing work is aimed at dissecting the mechanism behind polyA site selection by measuring the individual transcript isoform half-lives and looking at the relationship between the production of a tRNA that lies within the Hes7 3’UTR and polyA site selection. Funding: NIH R03-HD087909 to S. Cole, OSU Pelotonia Fellowship to K. Braunreiter

Program Abstract #337
In vitro characterization of the human segmentation clock
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The vertebral column is characterized by the periodic arrangement of vertebrae along the anterior-posterior axis. This segmental or metameric organization is established early in embryogenesis when pairs of embryonic segments called somites are rhythmically produced by the presomitic mesoderm (PSM). The tempo of somite formation is controlled by a molecular oscillator known as the segmentation clock. While this oscillator has been well characterized in model organisms, the properties of the human clock remain elusive. As it is not feasible to observe the human segmentation clock in vivo, we have established an in vitro system based on the differentiation of pluripotent stem cells towards PSM fate by dual Wnt activation and BMP inhibition (Chal et al. 2015, 2016). We first verified that in vitro derived cells faithfully recapitulate the segmentation clock by differentiating mouse embryonic stem cells harboring a Hes7 fluorescent reporter and confirming that the resulting PSM-like cells oscillate with normal period (i.e. 2.5 hours). Then, we deployed the same strategy for human induced pluripotent stem cells and observed oscillations of the HES7 cyclic gene with a 5-hour period. This simple and controllable in vitro system enabled us to probe the segmentation clock with unparalleled resolution at the single cell level. We found that the mouse and human segmentation clocks are similarly regulated by FGF, Wnt, Notch and Yap signaling. Furthermore, we demonstrate that FGF signaling controls the phase and period of the oscillator. This contrasts with classical segmentation models such as the “Clock and Wavefront” where FGF merely implements a signaling threshold specifying where oscillations stop. Overall, our work identifying the human segmentation clock represents an important breakthrough for human developmental biology. This work was funded by grants from the National Institute of Health (5R01HD085121, 1F31HD100033) and the NSF GRFP.

Program Abstract #338
Ex utero development of Mouse Embryos From Pre-Gastrulation to Advanced Organogenesis
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Establishment of the mammalian body plan occurs shortly after the embryo implants into the maternal uterus, and proper development of the embryo is dependent on its interaction with the uterine environment. While methods for in vitro culture of pre- and peri-implantation mouse embryos are routinely utilized, approaches for stable and robust culture of post-implantation embryos from egg cylinder stages until advanced organogenesis...
remain to be established. We develop and characterize herein highly efficient \textit{ex utero} post-implantation mouse embryo culture platforms, that enable appropriate development of embryos before gastrulation (E5.5) until the hind limb formation stage (E11). Thorough molecular and histological validation tests establish that the \textit{ex utero} developed embryos obtained by this approach are nearly indistinguishable to embryos developing \textit{in utero}. The amenability of the \textit{ex utero} culture system to a series of embryo micro-manipulations or electroporations, followed by long-term culture, was shown and used to assay primed pluripotent stem cell competence for yielding post-implantation chimeras. Establishment of a system to robustly grow normal mouse embryos \textit{ex-utero} from pre-gastrulation to advanced organogenesis represents an unprecedented dynamic and flexible tool to investigate mammalian embryogenesis. This work was funded by Pascal and Ilana Mantoux; Nella and Leon Benoziyo Center for Neurological Diseases; David and Fela Shapell Family Center for Genetic Disorders Research; Kekst Family Institute for Medical Genetics; Helen and Martin Kimmel Institute for Stem Cell Research; Flight Attendant Medical Research Council (FAMRI); Helen and Martin Kimmel Award for Innovative Investigation; Dr. Beth Rom-Rymer Stem Cell Research Fund; Edmond de Rothschild Foundations; Zanter Charitable Foundation; Estate of Zvia Zeroni; European Research Council (ERC-CoG); Israel Science Foundation (ISF); Minerva; Israel Cancer Research Fund (ICRF) and BSF.

**Program Abstract \#339**

**Investigating the relationship between epithelial cell reintegration and the cell cycle**

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Epithelial tissues are the most common tissue type in humans, and they serve various functions in the body, such as protection and secretion. Epithelial cells change shape (round up) and often move apically within the tissue layer to divide, likely to escape mechanical confinement. Thus, daughter cells may be born protruding from tissue layers. The process whereby mitotic daughter cells reincorporate into the layer is called cell reintegration. Thus, reintegration is a protective mechanism that guards tissue integrity. Alternatively, reintegration could also be an invasive behavior if the integrating cell has a foreign origin. Therefore, we first asked whether reintegrating cells require a pre-existing attachment to the layer. To address this question, we added mechanically-dissociated GFP-labelled cells onto a polarized MDCK cell layer and determined whether they incorporate into the layer. GFP cells were found both inside and apically attached to the layer, with various arrangements. We concluded that integrated cells do not need a pre-existing attachment to the layer, and that our \textit{in vitro} model is a valid tool to study epithelial reintegration. Despite the diverse GFP cell arrangements, we have often seen GFP cells in pairs inside the layer, suggesting that integrated cells are division products. This leads to our investigation of the relationship between reintegration and the cell cycle. Together with the definition of reintegration as a post-division behavior, our preliminary data shows that reintegration is a regulated behavior and is likely linked to the cell cycle. Future experiments with cell cycle manipulation will be conducted to continue testing this hypothesis. Our research is supported by NIGMS R01GM125839.

**Program Abstract \#340**

**Cis-clustering of Cdh3 is required for convergent extension.**

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Convergent extension is a conserved developmental process that drives axis extension in animals. During convergent extension, collective cell movement along the mediolateral axis leads to extension of the anterior-posterior (head-to-tail) axis. Cadherins, a family of cell adhesion molecules, play a vital role in convergent extension. In cell culture, the lateral (cis-) clustering of cadherin has been shown to be important for adhesion strength and cadherin function, but it is unclear if clustering is required \textit{in vivo}. To test if cadherin clustering is required for convergent extension, we expressed a mutant c-cadherin (Cdh3) construct that is unable to cluster. Our data show that embryos expressing mutant c-cadherin exhibit a swayed back and stunted growth which are phenotypes typical of convergent extension defects. Strikingly, however, these embryos do not display cell
cohesion defects typical of c-cadherin loss. Thus, our data indicate that cis-clustering of cadherin plays a specific and important role in collective cell movement, but is dispensable for homeostatic tissue integrity. This work was supported by grants from the NICHD (R21HD084072) and the NIGMS (R01GM104853) to J.B.W.

Program Abstract #341
Cell shape remodeling and phenotypic plasticity during extravasation in the larval zebrafish
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Cell shape has been used as an important descriptor of the organization and physiology of cells. Morphological features can denote different biological processes controlled by the interactions between cytoskeletal components, membrane proteins, and the cell microenvironment. Cancer cells are characterized by having different cell morphologies associated with tumor progression and metastasis. During the metastatic cascade, specifically, in extravasation, cancer cells display an arrangement of different morphologies associated with the type of migration, aggressiveness potential, and the interaction with endothelial cells. Remarkably, zebrafish is an excellent model for studying extravasation, as the optical clarity of the larvae allow for the visualization of xenografted cancer cells in the fish vasculature. This study quantitatively analyzed the morphological features and geometrical changes of cancer cells during extravasation and demonstrated that extravasated and non-extravasated cells exhibited distinct morphologies associated with their cell dynamics. Also, an important in vivo migration behavior is locomotion through confined spaces and the zebrafish vasculature is presented as a suitable three-dimensional microenvironment that allowed the characterization of this type of cancer cell migration. Finally, we developed a model that defines the relevancy of cell shape descriptors as good predictors of extravasation and as a tool that provides important insights into cell mechanics and phenotypic plasticity of metastatic cancer cells. Funding Sources: Damon Runyon Cancer Research Foundation; Pershing Square Sohn Cancer Research Alliance

Program Abstract #342
A mutation in the histone modification reader, Yeats2, results in defective collective cell migration in the zebrafish embryo
Jon Bell, Hillary McGraw
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Embryonic development requires the precise coordination of cell behaviors, such as proliferation, migration, and differentiation. During collective migration, cells move together as a coordinated cohort, while also maintaining proliferation and differentiation. When any of these cellular behaviors are disrupted, the results can lead to morphological defects in the embryo, and when these behaviors are inappropriately activated in the adult, result in tumor growth and cancer cell invasion. In a novel zebrafish mutant line, yeats2, collective cell migration is disrupted during development of the posterior lateral line (pLL) mechanosensory system. The pLL forms through the collective migration of the pLL primordium (pLLP), a cohort of ~100 cells that migration along the trunk of the embryo between 20-48 hours post fertilization. As the cells of the pLLP migrate, they continue to proliferate and differentiate to form the mechanosensory organs of the pLL. Zebrafish embryos with a mutation in the histone modification reader, yeats2, show defects in collective migration. Yeats2 is a member of the Ada-two A-containing (ATAC) complex and plays a role in the epigenetic regulation of gene transcription. Previous work has shown that Yeats2 expression is strongly upregulated in several cancers and is associated with increased tumor size, but it is not known if Yeats2 also has a role in cancer cell invasion. Further, the developmental role for Yeats2 mediated gene transcription has not been described. The yeats2 mutation in the zebrafish will allow us to uncover the function of Yeats2 protein during development and in disease. All work on this project is funded by Dr. McGraw’s start-up funds from the University of Missouri-Kansas City, School of Biological and Chemical Sciences.

Program Abstract #343
Endothelial sema3fb regulates angiogenic sprouting
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**Objective:** Sprouting angiogenesis is a dynamic process whereby endothelial cells (ECs) collectively migrate and connect to remodel into a hierarchically branched vascular bed. During vessel formation, migrating ECs in the nascent sprout adopt two distinct cellular states, the leading ‘tip’ and proliferating ‘stalk’ cell, with distinct gene expression patterns that help regulate and guide vessel patterning. We found that semaphorin3fb (sema3fb) is expressed within developing endothelial cells of the zebrafish embryo and we sought to identify its role in early vascular patterning.

**Approach and Results:** We generated CRISPR/Cas9 mutagenized sema3fbca305 zebrafish and assessed their early developing vasculature. We find that loss of sema3fb results in significantly shorter sprouts that fail to form proper network connections. Interestingly, we find that although cell numbers are unchanged in the shorter sprouts, individual endothelial nuclei are significantly larger and ‘clump’ together more frequently. Filopodia projections also appear aberrant and fail to resolve once connections are established. Gene expression analysis of isolated ECs from sema3fbca305 embryos reveals a shift to a more ‘tip-like’ expression profile which is partially rescued by targeted receptor inhibition.

**Conclusions and Significance:** Using a zebrafish model we determined that that sem3fb acts to guide the stereotypic patterning of blood vessels by regulating endothelial cell dynamics. Our data suggest that sema3fb acts via auto-secretory feedback to modulate gene expression and limit tip cell formation. Overall these data point to a novel mechanism for sema3fb whereby the vessel supplies the guidance molecule necessary for its formation. Funding Sources: SJC received a Natural Science and Engineering Research Council Discovery Grant RGPIN/06360-2014 (www.nserc.ca). CW received an Eyes High Studentship from the University of Calgary

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**Program Abstract #344**

**Zebrafish melanoma cells form focal adhesion-like structures during single cell migration in vivo**

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Cell migration is critical during many biological processes, including embryonic development, immune surveillance, and cancer. Focal adhesion complexes are recognized as an important structure that physically links the cytoskeleton of a cell to the extracellular matrix (ECM), thus facilitating cell migration. Cell culture studies have provided a wealth of information regarding focal adhesion biology, however, cell-matrix interactions through focal adhesion complexes during single cell migration in living organisms has yet to be clearly established. To address this challenge, we have taken advantage of optically transparent, genetically tractable zebrafish larvae to manipulate both the environment and our cells of interest, the highly migratory zebrafish melanoma (ZMEL) cells. We found that transplanted ZMEL cells disseminate to the skin. Strikingly, ZMEL cells form Paxillin or Ena/VASP positive punctate structures at the ventral surface of the cells that directly contact the native ECM of the zebrafish skin. We hypothesize that these structures are focal adhesion-like structures, and that these structures are critical to regulate cell migration in vivo. By performing FRAP experiments, we found that Paxillin has higher molecular mobility in cells in vivo compared to cell culture models, while the mobile fraction remains similar. Using a collagen reporter, our preliminary data suggest rearrangement of collagen fibers during ZMEL cell migration, suggesting that migrating ZMEL cells transduce force to the environmental ECM. We are currently characterizing focal adhesion-like structures in vivo at the ultra-structural level, and assessing their regulation and function. These studies will finally provide significant insight into focal adhesion-based cell migration in vivo and help us gain a better understanding on melanoma dissemination. This work is funded by R00CA190836.

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**Program Abstract #345**

**Ventral tissue fate in Drosophila leg is controlled in part by three distinct actions of the selector gene midline**

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The patterning of Drosophila limbs is controlled in part by the regional expression of selector genes, which are transcription factors that mediate binary patterning choices. The T-box transcription factor gene midline (mid) (fly Tbx20) acts as a selector gene, causing cells to adopt a ventral pattern instead as opposed to dorsal. The ventral signal Wg (fly Wnt) specifies ventral fate by activating mid in all ventral cells. Dorsal fate is controlled by Dpp (fly...
BMP). Our work shows that mid specifies ventral fate via at least three pathways. In the first pathway, mid inhibits dorsalization by blocking Dpp signaling. Ectopic dorsal fate induced by tkv\textsuperscript{D0}, an activated Dpp receptor, is inhibited by simultaneous expression of mid. As well, mid mutant cells have increased levels of phosphorylated Mothers-Against-Decapentaplegic (pMad), a readout of the level of Dpp signaling. In the second pathway, Mid acts as a direct transcriptional repressor for Dpp-target genes such as Dad. A minimal Dad-enhancer fragment both binds Mid in ChIP assays and is responsive to mid loss- and gain-of function. Lastly, mid directly promotes ventral fate. Genetic mosaics that lack Mid and are blocked for Dpp signaling are not rescued to ventral fate in all but one ventral structure. Thus, Mid also specifies ventral fate independent of Dpp signaling. Specification of ventral fate by mid requires a known repressing domain (eh1). Mid is a direct transcriptional repressor of several genes expressed in the ventral domain and a mid mutant in the eh1 domain is compromised in ventral fate specification in gain-of-function assays and rescue experiments. We propose that Mid specifies ventral fate though (1) inhibition of Dpp signaling, (2) acting as a transcriptional repressor and (3) coordinating the regulation of genes in the ventral leg. L.A.P. was funded by an ACHRI Graduate Scholarship. This work was supported by the CIHR.

Program Abstract #346
OCT4 labels two distinct stem cell types in somatic cell reprogramming
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Somatic cell reprogramming using Oct4, Sox2, Klf4 and c-Myc (OSKM) has long-been recognized to produce induced pluripotent stem cells (iPSCs). However, an additional, distinct stem cell type routinely arises during OSKM reprogramming, induced extraembryonic endoderm stem (iXEN) cells, but it is not yet understood why this second cell type arises. Both iPSCs and iXEN cells have promising therapeutic potential and provide novel in vitro developmental models. In order to understand how two separate cell types are forming, it is important to draw from our knowledge of iXEN cells and iPSCs embryonic counterparts: extraembryonic endoderm (XEN) and epiblast (EPI). In the embryo, Oct4 is expressed in, and required for, both EPI and XEN cell development. This leads to our hypothesis that Oct4 is expressed in both iXEN cells and iPSCs during reprogramming. We tested the hypothesis by evaluating the expression of OCT4 with XEN marker (GATA6 and SOX17) using flow cytometry analysis and immunofluorescent imaging throughout reprogramming. In addition, OCT4-eGFP reprogramming cells were single cell sorted to form clones and explore the cell fate of OCT4-positive cells. Our preliminary data show that OCT4 is indeed expressed in iXEN cells during reprogramming. OCT4 is co-expressed with XEN markers and OCT4-eGFP clones yield iXEN and iPSC colonies. This indicates that OCT4 has dual roles in reprogramming, as it does in the embryo. Further exploration of the dual role of OCT4 in reprogramming is essential as this will help us better understanding iXEN and iPSC formation and cultivate comparisons between development and reprogramming. Funding for this work was provided by NIH R35GM131759 awarded to A.R.

Program Abstract #347
The ETS Transcription Factor ERF is a Gatekeeper of Naïve Pluripotency
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Exit from the naïve pluripotent state during early embryonic development in mammals is essential for proper embryo implantation. Activation of the MAPK cascade by FGF4 is the key signal within the inner cell mass (ICM) of the embryo to promote this exit. Consequently, FGF4\textsuperscript{-/-} embryos are unable to progress to a later post-implantation primed pluripotent state and remain trapped into a naïve pre-implantation state. Despite the relevance of this transition, the molecular mechanism by which MAPK instructs this exit remains unknown. Here, we propose the transcriptional repressor ERF as the MAPK-dependent switch that allows the exit from naïve pluripotency. We used inducible ESCs to genetically eliminate all RAS proteins and observed that in Ras\textsuperscript{-/-} cells, ERF translocates to the nucleus and binds to enhancers of pluripotency and developmental factors. Importantly, ERF is evicted and kept inactive in the cytoplasm by MAPK-dependent phosphorylation. We show that ERF deletion...
rescues the ability to exit naïve pluripotency and the progression into a post-implantation state in Ras−/− cells. Indeed, RNAseq data revealed that, while Ras−/− cells failed in the downregulation of naïve genes upon differentiation, deletion of ERF restored the overall gene expression profile to a wild-type level. Interestingly, differentiated Ras−/− cells are reminiscent of the recently described rosette-stage pluripotency characterized by a transcriptional profile intermediate between naïve and primed states. Consequently, the absence of ERF overcomes the developmental blockage of Ras−/− cells from this intermediate state. Mechanistically, we show that ERF regulates negatively the expression of the de novo methylase DNMT3, essential for the extinction of the naïve transcriptional program. Our data revealed an essential role for ERF in the exit from naïve pluripotency as a coordinator of the progression to primed pluripotency. This work is funded by the Intramural Research Program at the NIH.

Program Abstract #348
The Effects of the Deletion of BRCA-Associated Protein 1 (BAP1) From the Skin Epidermis
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BAP1 is a nuclear protein that acts primarily as a histone deubiquitinase and tumor suppressor. In humans, its dysfunction has a role in many uncommon cancers, most notably, mesothelioma, in which it is implicated in up to 70% of cases. In addition to this, BAP1 is heavily involved in the epigenetic regulation of stem cells. Our lab studies the biology of the skin epidermis and pursuant to this goal has begun to study the effects of the loss of BAP1 on the maintenance and behavior of the skin epidermis in mouse. To do this, a line of B6 mice with BAP1 deleted in all tissues derived from keratin 14+ progenitors as well as a line of keratinocytes isolated from them have been studied to examine the possible changes this causes in behavior and appearance. Thus far, knockout mice have shown a striking phenotype, this being ventral side alopecia. Bearing this observation in mind, the examination of the origins and the full extent of this phenotype as it relates to hair follicle bulge capacity, skin carcinogenesis, and the development of skin appendages are now under investigation. All research was funded by the Developmental Biology Training grant; NIH grant # 2T32HD055164-11.

Program Abstract #349
Brain Games: The Interplay Between Neural Stem Cells and Vascular Cells During Mouse Cortical Development
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Neural stem cells (NSC) are derived from the ectodermal layer of a developing embryo and are critical to brain formation, including the multi-layered cortex. NSC must balance proliferation and differentiation to give rise to a variety of distinct cell types, among which are neurons and glia. As the cortex expands, blood vessels invest the developing neuroepithelium. Recent evidence suggests that vascular cells may play a role in regulating NSC proliferation as well as their “fate” choice. Our project is designed to investigate the interplay between NSC and vascular cells during cortical development using a Transwell-based in vitro model of heterotypic cell-cell interactions. We used RNA-Seq analysis to identify differentially expressed genes in NSC grown in solo culture or in coculture with brain-derived vascular cells. In the presence of vascular cells (either contacting or non-contacting coculture), NSC displayed increased expression of several genes associated with gliogenesis, including Glial fibrillary acidic protein (Gfap) and receptors for Leukemia inhibitory factor (Lif). Perivascular cells, not NSC or endothelial cells, express Lif transcript (qPCR) and produce Lif protein. We have treated NSC with Lif directly and evaluated changes to the neurospheres. Lif-treated NSC form higher numbers of small diameter neurospheres when compared to control cultures. To determine the population composition, we used a single-cell approach to parse out clusters of cells undergoing differentiation. Lif-treated NSC express Gfap and lose expression of Pax6. We hypothesize that Lif, produced by perivascular cells of the growing blood vessels, promotes gliogenesis in NSC. Our current efforts are directed toward further characterizing subpopulations in the single-cell system, as well as targeting the Lif signaling pathway to test our hypothesis. Funding: This research was supported by an NIH/COBRE grant 2P20GM104360-06A1 (PI R. Vaughan, Project Leader D. Darland).

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Program Abstract #350
Deciphering the role of CNKSR2 in the developing chick forebrain.
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Metabolism Related Genes (MRGs) are a special class of genes that encode metabolic enzymes and transporter proteins. One such MRG, Connector Enhancer of Kinase Suppressor of Ras 2 (CNKSR2), was found to be expressed in the roof plate of the chick forebrain. The expression of CNKSR2 coincided with the time-window wherein the roof plate starts to invaginate, leading to the separation of the two cerebral hemispheres. In a previous study, we showed that retinoic acid signaling (RA) regulates forebrain roof plate invagination partially through the regulation of cell proliferation. Moreover, CNKSR2 is known to interact with the Ras/Raf/MEK pathway, a regulator of cell proliferation. We observed that the forebrain roof plate midline exhibits low cell proliferation and low levels of pMAPK, a readout of active Ras/Raf/MEK signaling. Thus, we hypothesized that CNKSR2 possibly functions downstream of RA signaling to regulate forebrain roof plate invagination. To test our hypothesis, we carried out the loss and gain of function of RA signaling and found that this affected the expression of CNKSR2. We also carried out loss-of-function of CNKSR2 in the chick forebrain roof plate and observed invagination defects with an increase in cell proliferation and pMAPK in the midline. To identify potential downstream cell-cycle regulators of MAPK signaling, we performed an expression screen for cell cycle regulators. We found that CyclinD1 and CyclinB2 were differentially expressed in the forebrain roof plate. We propose that CNKSR2 inhibits cell proliferation in the forebrain roof plate midline by inhibiting MAPK and the downstream effector such as Cyclin D1. This project is currently being funded by the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India.

Program Abstract #351
Global process, tissue-specific defects: The role of RNA Polymerase I during cranial neural crest development
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Ribosomopathies are tissue-selective birth defects that result from disruptions in ribosome biogenesis, a global process essential for cell growth, proliferation and survival. Two examples are Acrofacial Dysostosis-Cincinnati Type (AFDCIN) and Treacher Collins syndrome (TCS), which are primarily defined by anomalies of the head and face. AFDCIN arises from mutations in POLR1A, the largest and catalytic subunit of RNA polymerase I (Pol I), which transcribes ribosomal RNA. TCS is caused by mutations in POLR1C and POLR1D, subunits of Pol I and III, and in TCOF1, a Pol I-associated factor. Zebrafish and mouse models of AFDCIN and TCS reveal that polr1a, polr1c, polr1d, and Tcof1 loss-of-function leads to reduced rRNA transcription by Pol I, which is a rate-limiting step of ribosome biogenesis. This results in p53-dependent neuroepithelial cell death and reduced proliferation of neural crest cells (NCC), which leads to hypoplastic craniofacial bone and cartilage. To understand the cell autonomous role of rRNA transcription and ribosome biogenesis in NCC, we conditionally deleted Polr1a, Polr1c, and Tcof1 from NCC in mice. Our data shows that Polr1a, Polr1c, and Tcof1 are essential for embryonic survival and that these genes play a specific role in rRNA transcription during NCC-derived cranioskeletal development. Additionally, we show that rRNA transcription is tissue-specifically regulated and that the neuroepithelium and NCC are highly susceptible to defects in rRNA transcription. Overall, our work provides novel insights into the dynamic roles of ribosome biogenesis in NCC and cranioskeletal development. Furthermore, this work will aid in identifying mechanisms underlying the pathogenesis and possible prevention of AFDCIN, TCS and other ribosomopathies and neurocristopathies. This work is supported by the Stowers Institute for Medical Research and the National Institute for Dental and Craniofacial Research.

Program Abstract #352
Neural Crest Specific Deletion of Mouse Sf3b4 Leads to Abnormal Craniofacial Phenotype
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Neural Crest Specific Deletion of Mouse Sf3b4 Leads to Abnormal Craniofacial Phenotype
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Nager and Rodriguez syndromes are two rare disorders that have been attributed to the haploinsufficiency of SF3B4, a core component of the U2 complex of the splicing machinery. Patients of both syndromes have craniofacial as well as limb abnormalities with variable expressivity including small jaw bones, small cheek bones, downward slanted eye lids, radial-ulnar defects, and thumb abnormalities. Using in situ hybridization, we showed that in mouse embryos, Sf3b4 was expressed ubiquitously from embryonic day (E)9.5 – E11.5. From E11.5 – E12.5, enriched expression of this gene was found in the maxillomandibular region, limb, and tail bud. To study the craniofacial abnormalities caused by haploinsufficiency of Sf3b4, we generated a conditional mutant mouse line using CRISPR/Cas9 and mated it to Wnt1-Cre2 transgenic mice to delete Sf3b4 specifically in cranial neural crest cells. Although heterozygous mutant embryos from these matings were normal, homozygous mutants have hypoplasia of the midbrain and abnormalities in the frontonasal prominence with variable severity. Thus, it appears that Wnt1-Cre2:Sf3b4-/- mutants mimic craniofacial phenotypes observed in Nager/Rodriguez syndrome patients. In the future, we will use this mutant mouse line to uncover the etiology of these two syndromes.

Program Abstract #353

Knockdown of hspg2 in zebrafish is associated with mandibular jaw joint fusion and neural crest cell dysregulation

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HSPG2 encodes for perlecan, a large proteoglycan that plays an important role in cartilage formation and basement membrane stability. Mutations in HSPG2 have been associated with Schwartz-Jampel syndrome and Dyssegmental Dysplasia Silverman-Handmaker Type, two disorders characterized by skeletal abnormalities. These data indicate a function for the gene in cartilage development and maintenance. However, the mechanisms in which HSPG2 regulates cartilage development are not completely understood. We explored the relationship between this gene and craniofacial development through morpholino-mediated knockdown of hspg2 in zebrafish. Knockdown of hspg2 resulted in a fusion of the mandibular jaw joint at 5 days post fertilization (dpf). Early NCC development was normal in morphant animals as measured by dlx2a and sox10 expression at 18 somites and 1 dpf. However, subsequent analysis at later stages of development (4 dpf) revealed a decrease in the number of Sox10+ and Col2a1a+ cells within the mandibular jaw joint region of morphants relative to random control injected embryos. Concurrently, morphants showed a decreased expression of nkx3.2, a jaw joint molecular marker, at 4 dpf. Collectively, these data suggest a complex role for hspg2 in jaw joint formation and late stage NCC differentiation. Financial support for this project was provided by the National Institute of Neurological Disorders and Stroke 1K01NS099153-01A1 given to Dr. Anita M. Quintana.

Program Abstract #354

The development of vascular malformations in a RASA1 Capillary Malformations-Arteriovenous Malformations (CM-AVM) zebrafish model.

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Mutations in the RASA1 (p120RasGAP) gene result in the development of capillary malformations and arteriovenous malformations (CM-AVM) but how RASA1 regulates blood vessel development remains unknown. I use the zebrafish model to image in real-time RASA1 mutant embryo vasculature in vivo to characterize aberrant cellular behaviours contributing to the formation of vascular lesions that offer hints at the underlying molecular mechanisms of CM-AVM. I found that mutant RASA1 embryos have AVMs at a high frequency and a massively enlarged vessel at the posterior of the caudal venous plexus (CVP) of the tail. A subtle enlargement of the veins is seen at 30hpf and by 2dpf there is significantly altered blood flow and pooling of blood in the malformation. Over time, the vascular lesion expands to involve the entire tail vasculature by 5dpf, with embryos developing paracardial edema. Mutants do not survive past 10dpf. Interestingly, the caudal veins seem to be primarily affected; the dorsal aorta, which runs parallel to the CVP, remains unaffected at early timepoints. Formation of a second plexus, the subintestinal venous plexus, is unaffected, suggesting specificity to the posterior vasculature. RASA1 can signal through multiple pathways. I found that MEK inhibition reduced vessel enlargement in mutants,
while alterations in VEGF signalling did not alleviate vascular malformations. Cell size is unaffected, hinting that mTORC signalling may not be aberrantly activated. A better understanding of the mechanisms behind the initiation and development of AVMs could result in the rapid repurposing of safe and effective drugs as novel treatments for CM-AVM, preventing the morbidity and mortality caused by this vascular disease. Funding Sources: the Heart and Stroke Foundation of Canada, the Canadian Institutes of Health Research, the Cumming School of Medicine.

Program Abstract #355
Determine the Effect of an Early-Onset Atrial Fibrillation-Associated TTN Missense Mutation on Cardiac Development and Function
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Mutations in cardiac ion channels, transcription factors, and myocardial structural proteins have been identified in patients with early-onset atrial fibrillation (EOAF). However, the underlying mechanisms by which structural proteins give rise to AF remain unclear. One such protein is titin (TTN), which is essential for sarcomere assembly during heart development and for the restoration of normal sarcomere length after cardiomyocyte contraction. Previous work generating a truncated TTN variant suggested that disruption of sarcomeric organization may lead to EOAF. However, it remains unknown what role a specific TTN domain might play in abnormal sarcomere development and/or maturation. We identified a missense TTN mutation (T23691I) in a patient with EOAF and showed co-segregation in the patient’s three-generation family. To elucidate the underlying mechanisms by which the T23691I mutation may cause AF, we are expressing and characterizing this variant in zebrafish. Thus far, F0 mutant zebrafish embryos generated with CRISPR-Cas9-mediated homology-directed repair demonstrate atrial enlargement, pericardial edema, and atrial regurgitant inflow. Ongoing studies of F1 progeny will test our hypothesis that the EOAF-causing TTN mutation affects sarcomere assembly and/or function during cardiac development. This research is funded by the National Institutes of Health.

Program Abstract #356
The role of collagen 11a2 in zebrafish vertebral development
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Cartilage tissue patterning depends on several proteins, one of which is collagen 11a2. In humans, mutations in this gene cause defects in bone and cartilage development. Work in the zebrafish shows robust expression of col11a2 in both the craniofacial cartilage and notochord, which is the precursor to the spine. Heterozygous zebrafish and homozygous mouse col11a2 mutants exhibit craniofacial defects. However, morpholino knockdown of col11a2 in zebrafish disrupts notochord structure, suggesting that loss of this gene product may affect spine development. To investigate the role of col11a2 in zebrafish vertebral development, I used CRISPR-Cas9 mutagenesis to create F0 mosaic mutants, which exhibit severe vertebral fusions throughout the spine. To examine the phenotype of stable mutants, I generated several indel mutant lines, bearing premature termination codons in col11a2, and characterized mild vertebral fusions in these animals. To circumvent the effects of genetic compensation in indel mutants, I created a transcriptless allele of col11a2 by deleting the entire locus. These homozygotes exhibit severe vertebral fusions. To determine when developing vertebrae fuse, I used in vivo bone staining to visualize the formation of these defects over time. I quantified the positions of fusions along the spine, and found that caudal vertebrae are consistently fused in col11a2 deletion homozygotes, in contrast to F0 mosaic mutants, which develop fusions along the entire spine. This suggests that genetic mosaicism in col11a2 produces a more severe defect than a stable mutation. To investigate this hypothesis, I will be creating col11a2 chimeras. To elucidate the role of the intervertebral disc in fusion formation, I will be using fluorescent reporters in the col11a2 deletion background. In this project, made possible by CIHR funding, I have created a new model of vertebral fusions and showed that col11a2 is necessary for proper vertebral development.
Program Abstract #357
Patterning the embryonic pulmonary mesenchyme
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Smooth muscle and smooth muscle-like tissues guide morphogenesis of several branched epithelia, including those in the lung, mammary gland, and prostate. Despite its importance for airway morphogenesis, it remains unclear how spatiotemporally patterned smooth muscle differentiation is achieved during lung development and whether smooth muscle is recruited from a distinct population of mesenchymal progenitors. Here, we carried out single-cell RNA-sequencing analysis of embryonic mouse lungs. Computationally clustering mesenchymal and smooth muscle cells did not uncover a distinct progenitor population but instead revealed that the embryonic pulmonary mesenchyme contains a continuum of cell states. Mesenchymal clusters correspond to spatially distinct sub-epithelial and sub-mesothelial compartments and express different sets of genes associated with Wnt signaling. Consistently, manipulating Wnt signaling affects the size of these compartments. Time-lapse imaging analysis revealed that we found that sub-epithelial mesenchyme directly gives rise to airway smooth muscle. Analysis of reconstructed differentiation trajectories from sub-epithelial mesenchyme to smooth muscle revealed that pathways related to cell cytoskeleton and adhesion and Wnt signaling are activated early on in differentiation, and that proliferative metabolism is systematically downregulated. Finally, we provide evidence that Wnt-dependent increases in mesenchymal cells that express high levels of F-actin at the earliest stages of differentiation can influence epithelial morphogenesis. Our work provides the first single-cell view of embryonic airway smooth muscle differentiation and sheds light on how signaling pathways are integrated to pattern the pulmonary mesenchyme during lung development. Funding: NSERC postgraduate scholarship; CFUW pre-doctoral fellowship; HHMI Faculty Scholar’s Award; NIH/NICHD R01 HD099030.

Program Abstract #358
Non-canonical Fzl1/2/7 and Nodal signaling cooperate to initiate the specification of dorsal territories in sea urchin embryos
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The establishment of the major body axes is a crucial event in early animal development. In many metazoans, including vertebrates, the Wnt and TGF-β signaling pathways play key roles in many developmental processes and are essential for the specification and patterning of the anterior-posterior (AP) and dorsal-ventral (DV) axes, respectively. In the deuterostome sea urchin embryos, early AP patterning depends on integrated information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways, which form an interconnected Wnt signaling network. In addition, members of the TGF-β superfamily (Nodal and BMP2/4) produce opposing morphogen gradients that pattern the three germ layers (endoderm, mesoderm, and ectoderm) along the DV axis. While AP and DV body axis specification and patterning mechanisms are closely related spatially and temporally, the current model is that early they act independently of one another. Here, we used morpholino perturbation approaches to analyze the function of the non-canonical Wnt16-Fzl1/2/7 signaling pathway during early AP and DV patterning. Our functional data indicate that the Fzl1/2/7 signaling is necessary for the specification of the dorsal ectodermal territory, but not the ventral territory. We also show that during early cleavage stages Fzl1/2/7 signaling controls the initial activation of the dorsal territory specifier, bmp2/4, independently of early Nodal signaling, but Fzl1/2/7 signaling is not necessary for its maintenance during later gastrula stages. Taken together, our results suggest that Fzl1/2/7 and Nodal signaling pathways work cooperatively to activate the BMP2/4 signaling pathway that is essential for the specification and patterning of dorsal territories, connecting AP and DV gene regulatory networks (GRNs). (Funding sources: R15 NICHD [grant number 1R15HD088272-01] and the Department of Biological Sciences at Auburn University).

Program Abstract #359
microRNA-31 regulates skeletogenesis by direct suppression of Eve and Wnt1
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microRNA-31 regulates skeletogenesis by direct suppression of Eve and Wnt1
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microRNAs (miRNAs) play a critical role in a variety of biological processes, including embryogenesis and the physiological functions of cells. Highly conserved microRNA-31 (miR-31) has been found to be involved in cancer, bone formation, and lymphatic development. In the sea urchin, we previously discovered miR-31 knockdown (KD) embryos have shortened skeletal spicules, mispatterned skeletogenic primary mesenchyme cells (PMCs), and shifted and expanded Vegf3 expression domain. Vegf3 itself does not contain miR-31 binding sites; however, we identified its upstream regulators Eve and Wnt1 to be directly suppressed by miR-31. Removal of miR-31’s suppression of Eve and Wnt1 resulted in skeletal and PMC patterning defects, similar to miR-31 KD defects. Additionally, removal of miR-31’s suppression of Eve and Wnt1 resulted in expansion and anterior shift of expression of Veg1 ectodermal genes, including Vegf3. This indicates that miR-31 indirectly regulates Vegf3 expression through direct suppression of Eve and Wnt1. Furthermore, removing miR-31 suppression of Eve is sufficient to cause skeletogenic defects, indicating a novel role of Eve in regulating skeletogenesis and PMC patterning. Overall, this study identifies the molecular mechanism of miR-31’s regulation of skeletogenesis and PMC patterning through its cross-regulation of Wnt signaling ligands and transcription factors of the endomesodermal gene regulatory network. Regulation of miR-31 in sea urchin Vegf signaling in skeletogenesis provides a deeper understanding of post-transcriptional regulation of evolutionarily conserved analogous developmental processes such as Vegf signaling in vertebrate angiogenesis. This work is supported by NSF CAREER Award IOS-1553338.

Program Abstract #360

**BMP signaling gradient interpreted through concentration thresholds in dorsal-ventral axial patterning**

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Bone Morphogenetic Protein (BMP) patterns the dorsoventral (DV) embryonic axis in all vertebrates, but it is unknown how cells along the DV axis interpret and translate the gradient of BMP signaling into differential gene activation to specify distinct cell fates. To determine the mechanism of BMP morphogen interpretation in the zebrafish gastrula, we identified 57 genes that are directly activated by BMP signaling. By using Seurat analysis of single-cell RNA-seq data, we found that these genes are expressed in at least three distinct DV domains of the embryo. We distinguished between three models of BMP signal interpretation in which cells activate distinct gene expression through interpretation of thresholds of: 1. the BMP signaling gradient slope, 2. BMP signal duration, or 3. the level of BMP signal activation. We tested these three models using quantitative measurements of phospho-Smad5 and by examining the spatial relationship between BMP signaling and activation of different target genes at single cell resolution across the embryo. We found that BMP signaling gradient slope or BMP exposure time did not account for the differential target gene expression domains. Instead we show that cells respond to three distinct levels of BMP signaling activity to activate and position target gene expression. Together, we demonstrate that distinct phospho-Smad5 threshold levels activate spatially-distinct target genes to pattern the DV axis. Funding: R35-GM131908, T32-HD083185, F31-GM123633.

Program Abstract #361

**Shining a light on ciliary signaling: The role of ciliary cAMP in Hedgehog-dependent fate specification**

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The diffusible second messenger cAMP communicates the activity of many different G-protein-coupled receptors (GPCRs) to the cell and modulates Hedgehog (HH) signaling. How cells discriminate cAMP produced by different signaling events is poorly understood. The primary cilium is a cellular projection that coordinates vertebrate Hedgehog signal transduction and is decorated with select GPCRs. We hypothesized that ciliary and extraciliary cAMP communicate different information to the cell. To test this hypothesis, we developed an optogenetic system to generate cAMP specifically inside or outside of cilia. We found that ciliary cAMP, but not cytoplasmic cAMP, inhibited zebrafish HH signaling, indicating that ciliary and cytoplasmic cAMP have distinct biological effects. As an orthogonal test, we developed designer ciliary and nonciliary GPCRs to modulate either ciliary or extraciliary cAMP levels. We found that similar to optogenetic cAMP generation, ciliary GPCRs, but not plasma
membrane GPCRs, attenuated HH signaling. Modeling suggested that local production of cAMP can explain
differential activation of an effector in the cilium and cell body. We discovered that there is a ciliary pool of the
cAMP effector Protein Kinase A (PKA). Blocking the activity of ciliary PKA, but not extraciliary PKA, activated HH
signal transduction, indicating that ciliary PKA interprets ciliary cAMP. Our results reveal that ciliary and
extraciliary cAMP have impart different information, demonstrating how cells distinguish signaling with a shared
second messenger. This work was funded by grants from the NIH (AR054396 and HD007470).

Program Abstract #362

In vivo and in toto imaging for the reconstruction of multilevel dynamics
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We aim to decipher and compare the cell behaviours and signalling activities that underlie tissue patterning in
zebrafish embryos and embryoids derived from early blastulas. Our strategy is based on long-term in toto imaging
of fluorescently labelled specimens and the reconstruction of the cell lineage tree. Zebrafish embryoids, also
known as pescoids, made by separating the blastoderm from the yolk cell at the 256-cell stage, were cultured and
imaged in Ringer’s 1x solution. We achieved an optimal trade off between spatial and temporal resolution, signal
to noise ratio and field of view while minimising phototoxicity by two-photon point laser scanning or single-
photon light sheet microscopy. The cell lineage of embryos and pescoids developing until 12 hpf were
reconstructed and compared. The cell proliferation rate, cell division orientation, cell trajectories and pattern
formation revealed by transgenic reporters were analyzed. Pescoids retain some of the individual and collective
cell behaviours characteristic of vertebrate gastrulation. This work has received funding from the European
Union’s Horizon 2020 Research and Innovation Programme ImageInLife under the Marie Sklodowska-Curie grant
agreement No. 721537.

Program Abstract #363

Mechanics of Cell Packing in the Notochord
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The notochord, the defining feature of chordates, is essentially a soft cylinder patterned in early development by
a small number of interior cells. Our analysis of packing patterns of vacuolated cells in zebrafish notochords
reveals that the characteristic “staircase” pattern, or the alternate patterns observed in mutants, are governed by
a simple and robust geometric measure. We have also noted that the notochord is weakly elliptical in cross
section. From these observations, and from similar observations in a model gel system, we have identified a
bidirectional interaction between cell packing pattern and the cross section of the surrounding tube. We model
the mechanics of the notochord tube three ways, identifying a second key nondimensional ratio governing the
pattern formation, and revealing previously unobserved packing patterns. This work has been partially funded by
the Simons Foundation, grant 524764.

Program Abstract #364

A novel microsphere-based cell tagging method for large-scale tissue flow mechanics in embryos and animals
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Animal epithelial tissues are typically rigid structures supporting organs & embryos. However, epithelial tissues
are also known to exhibit liquid-like cellular ‘flows’ during various physiological activities and during embryonic
development. Our current understanding of these tissue ‘flows’ is still very limited due a lack of tractable, generic
methods. The technical challenges include the need to obtain cellular resolution data at extremely large (~10 mm)
fields of view, and to carry out live long-duration imaging (hrs/days) of highly motile animals with minimal light
exposure. Here, we address these issues by developing a novel method to tag cells in epithelial tissues.
Fluorescent microspheres are first coated with a lectin that specifically binds to cell membranes. These ‘adhesive’
microspheres are then sprinkled on animals and embryos to tag a high density of cells in the tissues – thus
enabling quantitative live mapping of large-scale, long-duration tissue flow-fields. Next, computational mechanics
techniques such as particle tracking and particle imaging velocimetry (PIV) can be used to visualize flows such as vortices, to quantify local cellular speeds, and to characterize tissue properties such as stress/strain. We demonstrate the success of this method in two ‘flat’ animal systems – (i) the early divergent *Trichoplax adhaerens*, and (ii) the avian model *chicken*. In *Trichoplax*, this method enabled the discovery of physiological tissue fractures and healing, and helped demonstrate motility-induced elastic-ductile-brittle tissue transitions. In the early developing *chicken* embryo, we quantified large-scale bilateral counter-rotating cellular flows (‘polonaise movements’) during gastrulation. We believe that our technique will find potential applications in a wide range of model & non-model animal systems, both in the context of developmental as well as organismal biology. This work has been funded by NIH (T.M.), NSF (M.P.), CZI BioHub (M.P.), and HHMI (M.P.).

**Program Abstract #365**

*The SensorOverlord predicts the accuracy of measurements with ratiometric biosensors*

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Two-state ratiometric biosensors change conformation and spectral properties in response to specific biochemical inputs. Much effort over the past two decades has been devoted to engineering biosensors specific for ions, nucleotides, amino acids, and biochemical potentials. The utility of these biosensors is diminished by empirical errors in fluorescence-ratio signal measurement, which reduce the range of input values biosensors can measure accurately. Here, we present a formal framework and a web-based tool, the SensorOverlord, that predicts the input range of two-state ratiometric biosensors given the experimental error in measuring their signal. We demonstrate the utility of this tool by predicting the range of values that can be measured accurately by biosensors that detect pH, NAD+, NADH, NADPH, histidine, and glutathione redox potential. The SensorOverlord enables users to compare the predicted accuracy of biochemical measurements made with different biosensors, and subsequently select biosensors that are best suited for their experimental needs. Some strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). The research was supported by a Northeastern TIER1 award and a National Science Foundation CAREER grant (1750065) to J.A.

**Program Abstract #366**

*Mechanochemical symmetry breaking during morphogenesis of lateral-line sensory organs*

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The development of mechanosensory epithelia, such as those of the auditory and vestibular systems, results in the precise orientation of mechanosensory hair cells and consequently directional sensitivity. After division of a precursor cell in the zebrafish lateral line, the daughter hair cells differentiate with opposite mechanical sensitivity. This process produces neuromasts containing equal numbers of hair cells of two opposite polarities, half of them sensitive to caudal water movement and half to rostral flow. Through a combination of theoretical and experimental approaches, we show that Notch1a-mediated lateral inhibition produces a bistable switch that reliably gives rise to hair cell pairs of opposite polarity (Jacobo, Dasgupta, *et al.*, 2019). Using our mathematical model of the process, we predict the outcome of several genetic and chemical alterations to the system, which we then confirm experimentally. Following the predictions of our model, we are able to alter the ratio of rostral to caudal cells in the neuromast by titrating the concentration of different regulators of the Notch pathway. Further, we show that spontaneous symmetry breaking drives polarity-dependent movements of hair cells that rearrange the developing cell pairs and establish a mirror-symmetric organization of the organ (Erzberger, Jacobo, *et al.*, 2020). We model these reconfigurations by describing the effective surface tensions of the hair cells and the changes in these quantities throughout the developmental process. These results provide a unified experimental and theoretical framework to describe the polarity selection and cellular organization of the neuromasts of the zebrafish lateral line. A.E. was supported by a Feodor Lynen Fellowship from the Alexander von Humboldt Foundation and A.J. by an F.M. Kirby Postdoctoral Fellowship from Rockefeller University. A.D. is a Postdoctoral Associate and A.J.H. an Investigator of Howard Hughes Medical Institute.
Program Abstract #367

Hox genes coordinate adult tissue segmentation and behavior to regulate asexual reproduction

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Hox genes are highly conserved transcription factors that regulate A/P segmental identity during embryonic development. While Hox gene expression perdures in adult animals, their roles in this context are still poorly understood. Our recent work uncovered a previously unsuspected segmentation process underlying asexual reproduction in the planaria Schmidtea mediterranea. These highly regenerative flatworms undergo transverse fission, sequentially tearing off posterior tissue fragments that regenerate into clonal progeny. Mechanically vulnerable planes progressively segment the animal along the A/P axis in coordination with growth. Fission planes arise at fixed distances along the A/P axis, generating uniformly sized progeny. Establishment of these planes is independent of other A/P regulators such as the Wnt and TGF-Beta signaling pathways, and their molecular basis is still unknown. Hox genes have conserved roles in A/P segmental identity across metazoans and, despite considerable effort, their function in planaria remains largely a mystery. We tested the extent to which Hox genes regulate fission-associated segmentations and fission behavior. Silencing of each of the 13 planarian Hox family members revealed 5 Hox genes required for fission. Among these, silencing hox3b results in supernumerary segments, while silencing post2b eliminates segmentation altogether. The opposing roles of hox3b and post2b are similarly conserved in the regulation of fission behavior. Silencing of hox3b increases the frequency of fission behavior, but animals fail to complete the fission process. In contrast, silencing of post2b eliminates fission behavior entirely. In summation, our study establishes a molecular basis for the emergence of fission planes, identifies roles for planarian Hox genes as mediators of adult tissue segmentation and behavior, and provides the first lines of evidence that Hox genes function to regulate asexual reproduction in an adult organism.

Program Abstract #368

How hierarchical protein pattern formation couples cell shape information to biochemical dynamics

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Many developmental processes rely on the precise positioning of proteins to ensure proper morphogenesis. Such protein patterns are susceptible to cell and tissue shape changes, raising the question of how these patterns can be robust in a mechanically dynamic environment. Here, we elucidate a mechanism that pattern protein localization robustly despite cell shape deformations. By combining quantitative experiments in starfish oocytes with mathematical modelling, we find that cell shape information encoded in a cytosolic gradient can be decoded by a bistable front of a RhoA regulator. In turn, this bistable front precisely positions RhoA by locally triggering excitable dynamics. We posit that this hierarchical coupling between a biochemical gradient and protein self-organization provides mechanochemical feedback for cell shape sensing and control in early oocyte and embryo development. This research was supported by the National Science Foundation (NSF) CAREER Award #1848247 and the Deutsche Forschungsgemeinschaft (DFG) SFB 1032.

Program Abstract #369

Thyroid hormone mediates proximo-distal patterning in zebrafish fin skeleton.

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The mechanisms that regulate organ growth and positional identity are of great importance in development, and these processes can theoretically be decoupled to produce major changes in form and function. The morphology of fins is enormously diverse across teleost fishes, including variation in the size and proximo-distal characteristics of the fin skeleton. Zebrafish fins are composed of segmented rays that taper and shorten distally. In the full-grown caudal fin, each ray bifurcates multiple times at stereotyped locations along the proximo-distal axis. Bioelectric signaling regulates fin length, and dysfunction in ion channels and gap junctions changes growth and ultimate length of the rays, while proximo-distal features are generally maintained in proportion in these mutants. In this study, we propose a novel role for thyroid hormone in establishing proximo-distal morphologies
of the zebrafish fin skeleton during both development and regenerative growth. We found that thyroid hormone is necessary for morphogenesis of distal features, including fin ray bifurcations and the shortening and tapering of ray segments. Fins of hypothyroid fish are severely proximalized in both ray morphology and gene expression profiles along the fin axis, and such proximalized phenotypes can be rescued during regeneration by exogenous thyroid hormone. Nonetheless, thyroid hormone titer has little effect on the overall size and length of the fins, suggesting that thyroid hormone mediates skeletal patterning in the fin via pathways distinct from bioelectric signaling. We demonstrate that thyroid hormone is upstream to shh-mediated processes underlying ray bifurcation, and that the hormone might regulate proximo-distality through changes in global methylation patterns. In all, our results provide insights into mechanisms underlying different aspects of proximo-distal identity and patterning, and how these mechanisms might be modulated to produce novel phenotypes. NSF CAREER1845513.

Program Abstract #370
Insights into BMP-mediated patterning with in vivo optogenetics
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Signaling molecules activate distinct patterns of gene expression to coordinate embryogenesis, but how spatiotemporal expression diversity is generated is an open question. In zebrafish, a BMP signaling gradient patterns the dorsal-ventral axis. We systematically identified BMP target genes with RNA-sequencing. Using lightsheet microscopy and NanoString molecular barcoding, we found that BMP target genes have diverse spatiotemporal expression patterns. Transcriptional responses to optogenetically generated high- and low-amplitude BMP signaling pulses suggest that spatiotemporal expression is not defined by different signaling activation thresholds. In addition, we observed negligible correlations between spatiotemporal expression and transcription kinetics in response to BMP signaling pulses. In contrast, spatial differences between BMP target genes largely collapsed when FGF and Nodal signaling were inhibited. Our results challenge the basic morphogen model and indicate that combinatorial signaling by BMP, FGF, and Nodal is a major driver of spatial diversity in BMP-dependent gene expression.

Program Abstract #371
A multicellular crosslink of genetic networks underlying robust tissue patterning of cells in vertebrate embryos
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Cells of a gastrulating vertebrate embryo rely on chemical and mechanical cues to commit into patterning decisions in a timely manner at correct locations. Consecutive groups of cells riding out presomitic mesoderm (PSM) make repetitive decisions to mark sizes of forming somites —vertebrae, muscle, and skin precursors—. Somites form with regular periodicity and scale in sizes. PSM cells facilitate their “clock”’s (Notch signaling oscillators) and “ruler”’s (signaling gradients) as “sailors on an open sea would do” (Lewis, 2008) to achieve this robust patterning. Here we developed a tissue culture method, enabling us to untangle mechanistic input from axial elongation and chemical input from signaling gradients, for live imaging of zebrafish tail explants. This method combined with time-controlled and space-restricted perturbations let us to answer a long-debate on “which signaling pathway provides positional information” (Simsek and Ozbudak,2018). We showed fold-change detection of FGF signaling among neighbor cells is the “ruler” used for segmental commitment. We also showed Wnt signaling acts permissively upstream of FGF signaling for both segmental determination and further genetic commitment (Keskin,Simsek,et al.,2019). However, the bottleneck mechanism that integrate temporal and spatial information from the clock and FGF gradient is still missing. Various congenital disorders (spondylocostral dysostosis, infantile myofibromatosis) are associated with misregulation of both Notch and RTK signaling, pointing to potential defects in crosstalk between two pathways. In that venue, we observed correlations between clock, FGF, and Wnt signaling components, analyzed time-course data in response to genetic and chemical perturbations and performed mechanistic modeling. This revealed a post-translational coupling between “clock”’s and “ruler”’s of PSM cells for robust patterning, as an unprecedentedly conserved phenomenon from fish to mammals. The work is funded by NIH GM122956.
Program Abstract #372
Investigating the role of the Planar Cell Polarity Pathway during kidney development in zebrafish
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The Planar Cell Polarity pathway (PCP) establishes polarity within a sheet of cells, such that cells align along a single plane. Defects in PCP result in developmental phenotypes in tissues, including cysts in the forming kidney. Kidney cysts are the main symptom of cystic kidney diseases like autosomal dominant polycystic kidney disease (adPKD), which are likely due to disrupted kidney cilia organization by PCP defects. To date, we lack comprehensive knowledge of how PCP functions to orchestrate cilia development. The Intraflagellar Transport (IFT) Proteins, part of a PCP sub-pathway, regulate cilia organization and movement; defects in both PCP and IFT genes result in cyst formation. To investigate the synergy of PCP and IFT pathways in kidney development, we use zebrafish embryos, for their external, transparent development and conserved kidney development with mammals. PCP genes prickle1b, ift57 and kif3a have been implicated in adPKD and cilia formation; however, how they function to affect cilia formation is not well known. We sought to characterize their spatiotemporal expression patterns and function during kidney development. To this end, we have cloned the prickle1b, ift57 and kif3a genes. Using whole-mount in situ hybridization at 24, 48, and 72 hours post fertilization (hpf), we have discovered prickle1b is expressed in the developing kidney. We also aim to examine the expression patterns of ift57 and kif3a. To detect differentiated cilium on the kidney, we use immunohistochemistry to visualize acetylated α-Tubulin protein localization, a component of cilia, and high resolution confocal microscopy. Better understanding of the role PCP plays in development will improve our understanding of how and why kidney cysts occur, which is critical to further understanding cystic kidney disease pathology. Thank you to the Uribe Lab in the Biosciences Department at Rice University, Society for Developmental Biology, and Choose Development! Fellowship Program.

Program Abstract #373
Decreased levels of miR-28 and miR377 in hyperglycemic conditions demonstrate low mineralization in mESCs
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The American Diabetes Association reported in 2018 that approximately 34.2 million Americans are living with diabetes which is about 10.5% of the population. Furthermore, approximately 1.4 million children and adults are living with type 1 diabetes. Diabetes is a metabolic disease that is characterized by the presence of elevated glucose levels, and sadly the rates of diagnoses will continue to increase worldwide. Diabetes has been shown to cause numerous health problems, one being improper bone development, which could lead to other bone diseases such as osteoporosis or even bone fractures. Our lab previously shown that two miRNAs, miR28 and miR377, are among many miRNAs that were significantly regulated during osteogenesis of mouse embryonic stem cells (mESCs). These two miRNAs directly target AMP kinase (AMPK), which is a critical kinase activated during a hyperglycemic stress response and is encoded by the Prka gene. To uncover how miR28, miR377 and AMPK control lineage fate early on during specification, mRNA expression of genes associated with neural crest cells, a potential precursor to bone, was examined in both glucose concentrations of 5 mM (low) or 25 mM (high/diabetic). Furthermore, miR28 was found to target FoxO1, a transcription factor downstream of AMPK, which may transcriptionally mediate the metabolic response. In addition to being a target of miR28, the expression and localization of FoxO1 was impaired in response of hyperglycemic conditions and was rescued to the homeostatic state when miR28/377 were overexpressed. Ultimately, these results indicate that osteogenesis is reduced in the presence of high glucose and that manipulation of candidate miRNAs may represent a feasible treatment option to prevent developmental consequences of hyperglycemia. Funding provided for this research is from the National Institute of Dental and Craniofacial Research (NIH).
Program Abstract #374
Female-specific upregulation of insulin pathway activity mediates the sex difference in Drosophila body size plasticity
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Nutrient-dependent body size plasticity differs between the sexes in most species, including mammals. Previous work in Drosophila showed that body size plasticity was higher in females, yet the mechanisms underlying the sex difference in body size plasticity remain unclear. Here, we discover that a protein-rich diet augments body size in females and not males because of a female-specific increase in activity of the conserved insulin/insulin-like growth factor signaling pathway (IIS). This increased IIS activity was triggered by a diet-induced increase in stunted, and required Drosophila insulin-like peptide 2, illuminating new sex-specific roles for these genes. Importantly, we show that sex determination gene transformer regulates the diet-induced increase in stunted and IIS activity, and mediates the sex difference in body size plasticity. This identifies one sex-specific mechanism underlying the nutrient-dependent regulation of IIS activity and body size plasticity, providing vital insight into conserved mechanisms that mediate sex differences in phenotypic plasticity. Funding for this study was provided by grants to EJR from the Canadian Institutes for Health Research (PJ1-153072), Natural Sciences and Engineering Research Council of Canada (NSERC, RGPIN-2016-04249), Michael Smith Foundation for Health Research (16876), and the Canadian Foundation for Innovation (JELF-34879), and to IMA from the European Research Council (ERCAdG787470). JWM was supported by a 4-year CELL Fellowship from UBC, LWW was supported by a British Columbia Graduate Scholarship Award, ZS was supported by an NSERC Undergraduate Student Research Award, and BH was supported by an European Molecular Biology Organization Fellowship (aALTF782-2015).

Program Abstract #375
Adipose mitochondrial metabolism couples nutrients to systemic insulin signaling and growth
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The larval fat body (equivalent to the vertebrate adipose tissue and liver) is central regulator of whole-body physiology and growth in Drosophila. In nutrient-rich food, activation of TOR pathway in the fat body promotes endocrine signaling to the brain leading to the release of insulin-like peptides (ILPs) causing increased systemic insulin and growth. In contrast, in low nutrients, this insulin signaling is reduced and growth is decreased. An important question is how nutrients control fat body to mediate these effects on endocrine signaling and growth. Here we describe a role for mitochondria in this process. We found that in rich nutrients, fat body mitochondria were large with sparse cristae, and levels of both TFAM, a transcription factor which controls expression of the mitochondrial genome, and OxPhos activity were low. However, when larvae were switched to low nutrient food, which delayed growth and development, TFAM levels and OxPhos activity were increased and fat body mitochondria became smaller with dense cristae. To explore the significance of this finding we used RNAi to knockdown TFAM, which lead to reduced OxPhos activity. We saw that fat body specific TFAM RNAi caused an acceleration of growth and development in rich nutrients, and was sufficient to reverse the delay in development seen in low nutrient food. These effects were accompanied by reduced fat body expression of Eiger/TNF-alpha, a negative regulator of brain ILP expression, and Imp-L2, an inhibitor of insulin signaling, and reduced whole-body expression of FOXO target genes. These effects are consistent with an increase in systemic insulin signaling. Also, we found that fat body TFAM RNAi increased expression of key glycolytic genes. Based on these findings we propose that a mitochondria-mediated switch in adipose OxPhos vs glycolytic metabolism can couple nutrient availability to fat-body mediated changes in systemic insulin signaling and growth. Research funding sources: CIHR and CRS.
Program Abstract #376
Sensory Sensitivity in a Developmental Genetic Disorder: Association of Tactile and Auditory Sensitivity with Autism Traits in Cornelia de Lange Syndrome.
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Background: Cornelia de Lange Syndrome (CdLS) is a rare genetic disorder, caused by cohesin-related genes, resulting in multisystem developmental deficits and low adaptive sensory processing (ASP). ASP depends on developmentally-determined habituation to sensory stimuli, and is abnormal in individuals with autism. Given that the developmental wnt pathway is involved in sensory receptor development and function it may have a role in ASP. The relationship between ASP, autistic traits and putative protein networks is explored using CdLS as a model disorder. Methods: Forty-one youth with CdLS were administered the Childhood Autism Rating Scales (CARS) to assess autistic traits and the Dunn Sensory Profile (DSP) to assess sensory sensitivity. CdLS diagnoses were ascertained clinically and CARS behavioral domains were extracted (repetitive behaviors, language, sensory). String-db.org, a database for protein-protein interactions, was used to explore connections between CdLS causal genes, and Wnt pathway components.

Results: The total CARS score was strongly associated with tactile sensitivity (p < 0.001), while other specific associations with tactile sensitivity were: social cognition (p=0.002), verbal (p=0.006), obsessive-compulsion/repetitive (p=0.006), and global sensory (p=0.006). In string-db.org protein networks, wnt proteins are functionally related to the NIPBL CdLS causal gene, via a sonic hedgehog (SHH) developmental pathway.

Conclusion: CdLS may serve as a model disease to study the links between ASP and specific developmental pathways (cohesin-WNT-SHH). Of sensory domains, tactile sensitivity is the most associated with autism traits in this model.

Program Abstract #377
TAEL 2.0: An improved optogenetic gene expression system for zebrafish
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Inducible gene expression systems are valuable tools for studying biological processes. We previously developed an optogenetic gene expression system called TAEL that is optimized for use in zebrafish. When illuminated with blue light, TAEL transcription factors dimerize and activate gene expression downstream of the TAEL-responsive C120 promoter. By using light as the inducing agent, the TAEL/C120 system overcomes limitations of traditional inducible expression systems by enabling fine spatial and temporal regulation of gene expression. Here, we describe ongoing efforts to improve the TAEL/C120 system. First, we modified the TAEL transcriptional activator by adding a C-terminal nuclear localization signal (TAEL-N) and observed significantly improved light-induced expression of a reporter gene (176-fold compared to 82-fold for unmodified TAEL). We then made modifications to the C120 regulatory element by replacing the minimal TATA box of the original construct (C120T) with the basal promoter of the cFos gene (C120F) and observed additional improvement with 44-fold induction for C120F compared to 3-fold for C120T. We found that TAEL-N and C120F in combination, referred to as TAEL 2.0, consistently induced not only higher levels of reporter gene expression but at a faster rate and with comparable background and toxicity compared to our previously published TAEL system. With these improvements, we were able to create functional stable transgenic lines to express the TAEL 2.0 transcription factor either ubiquitously or in the developing endoderm. We demonstrate that the ubiquitous line in particular can be used to induce expression at late embryonic and larval stages, addressing a major deficiency of the original TAEL system. We believe this improved optogenetic expression system will be a useful resource for the zebrafish community. This work was supported a CRCC grant from the University of California Office of the President.

Program Abstract #378
Transcription Factor 21 Controls Nephron Progenitor Cells and Podocyte Development through regulation of Wnt/β-catenin signaling
Gal Finer¹,², Tomoko Hayashida¹,², Tomokazo Suma³, Xiangmin Zhao¹,², Tuncer Onay¹, Yoshiro Maezawa⁴, Susan
Congenital anomalies of the kidney and urinary tract (CAKUT) is the leading cause of renal failure in the first three decades of life. We have previously shown that Transcription Factor 21 (Tcf21) regulates GDNF in the metanephric mesenchyme and is critical for branching morphogenesis. We now aim to decipher a role of Tcf21 in nephron progenitor cells (NPC) dynamics and podocyte development. During normal kidney development, canonical Wnt/β-catenin signaling initiates nephrogenesis by inducing NPC to undergo mesenchymal to epithelial transition (MET). However, following initial stabilization, β-catenin levels must decrease for MET to progress normally. Hence, discrete levels of β-catenin appear to promote two disparate fates of NPC: self-renewal vs. differentiation.

It remains unclear however, what drives this change and causes NPC to exit/maintain their self-renewal phase. Tcf21 knockout in NPC (Six2CreTcf21f/f) led to arrested nephron epithelialization at the pretubular aggregate stage. Subsequently, the mature kidney of Six2CreTcf21f/f mice showed paucity of podocytes, cells that arise from NPC and that are critical for an intact filtration barrier. Additionally, Six2CreTcf21f/f kidneys demonstrated low expression of Cited1, a hallmark of uncommitted NPC and a β-catenin target, but high Wnt4, another β-catenin target that marks early differentiation. The Cited1lowWnt4high state in the mutant kidneys suggested persistent stabilization of β-catenin in the differentiating progenitors. In uninduced mesenchymal cells, over-expression of wild-type Tcf21 led to enhancement of markers of self-renewal: Cited1, Tafa5, and Pla2g7. Over-expression of mutated-Tcf21 abrogated that enhancement. These data suggest that Tcf21 modulates Wnt/β-catenin signaling and regulates NPC toward self-renewal and podocyte differentiation. Search for direct targets is underway.


Program Abstract #379
Microenvironment- and cell type-specific functions of core gene regulatory network component, FOXD3, in human germ layer development and neuroblastoma

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Musculoskeletal conditions affect approximately 25% of the worldwide population, and neurocristopathies encompass at least 66 known disorders afflicting roughly 1 in 70 people globally. Production of relevant cell types via stem cell differentiation or direct cellular programming, both for therapies and modeling of human diseases, has progressed significantly. However, while it is well established that a common gene regulatory network (GRN) is deployed in multiple spatiotemporal instances during development to generate mesoderm-derived musculoskeletal and ectoderm-derived neural crest progenitors, it remains unclear how this core GRN produces both outcomes. Using high-content analysis, we interrogated contextual determinants of effects generated by overexpression of a core GRN element, FOXD3, in human embryonic stem cells and a neuroblastoma cell line. This approach exposed interactions that separately regulate cell fate specification, apoptosis, and epithelial-mesenchymal character. Further, we identified a single developmental condition that promoted population-limiting FOXD3 activity similar to that observed in neuroblastoma and other cancer cell lines. RNA-seq comparison revealed that whereas 175 loci were found to be differentially regulated by FOXD3 overexpression in both conditions, only 3 loci were regulated in a way that correlated with phenotypic outcomes across multiple microenvironments and cell lines. Taken together, these results break down key GRN-microenvironment interactions in formation of progenitor populations of interest in musculoskeletal, craniofacial and peripheral nervous system disorders. Further, this study provides new insights that may lead to improved targeting or differentiation strategies for clinical interventions into the cell types of these systems, which are superficially similar but developmentally distinct. Funding for this project was provided by the New York Stem Cell Initiative (NYSTEM C30161GG).

Program Abstract #380
The Development of Neural Crest Cells and Chromatophores in Agalychnis callidryas
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Neural crest cells (NCCs) are a group of pluripotent cells whose fate is dependent on their migratory pathways during embryonic development. Once pre-migratory neural crest cells delaminate from the neural tube, the cells are influenced by surrounding structures and cues to reach their target. Trunk neural crest cells (tNCCs) have been seen to follow a dorsolateral migration pattern that will give rise to pigmented cells. In some organisms, pigmented cells can be divided into two, melanophores, dark cells, and chromatophores, colored cells. In *Agalychnis callidryas*, the red-eyed tree frog, it has a distinct coloration: red eyes, green head and trunk, a variation of blue, orange, or red legs, blue and yellow striped flank, and a cream-colored torso. Its variety helps us look at the origin of melanophores and chromatophores prior to metamorphosis. These cells were distinguished by rtPCR and immunofluorescence with specific markers such as HNK1, FoxD3, MelanA, CSF1R, Sox10, MitF, and AcetTub to distinguish NCCs and pigmented cells. Using these markers, ectodermal expression along with vital labeling of premigratory NCCs confirms the presence of NCCs in the ectoderm. Both chromatophore and melanophore precursor cells were distinguished along with clear visualization of the melanocytes due to their cell shape and color. This work was supported by NIH MBRS-RISE (Grant No. GM063787).

Program Abstract #381
**Srcap-mediated H2A.Z localization is required for zebrafish neural crest cell differentiation**
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Precise regulation of stem cell specification and their progressive fate restriction is crucial for embryogenesis. One such population is the multipotent neural crest cells (NCCs) that arise during gastrulation and later differentiate into multiple cell types including neurons, cartilage and bones in the face, and smooth muscle cells in the heart. Whereas cell signaling pathways and transcription factors involved in NCC development have been well characterized, the underlying molecular roles of epigenetic regulators during neural crest fate transitions remain largely unknown. The chromatin remodeler **SRCAP** is often disrupted in Floating-Harbor Syndrome, a rare human disease where several NCC-associated tissues and organs are affected. Recent studies of frogs (Greenberg et al. *Cell* 2019) indicate that these defects might arise through disruption of the histone variant H2A.Z. Here we show that the zebrafish histone remodeler Srcap, which deposits H2A.Z throughout the genome, is critical for proper NCC development. Zebrafish lacking Srcap exhibit heart pericardial edema and jaw defects. We found that NC-derived cartilage is disrupted in the **srcap** null fish, and our preliminary studies suggest that these defects arise through failed NCC differentiation. Taken together, our study supports a model that precise localization of the histone variant H2A.Z play crucial roles in establishing proper differentiation trajectories during neural crest fate transition. Funded through the University of Rochester School of Medicine, and the Wilmot Cancer Institute.

Program Abstract #382
**Profiling genes regulated by the neural crest-essential methyltransferase NSD3**
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Development of the neural crest, a stem-cell like population unique to vertebrate embryos, is controlled by a gene-regulatory network. While network factors are well-defined, their expression and activity are also regulated by post-translational modifications like methylation. Our previous work showed that NSD3 is a neural crest-essential methyltransferase. To define the genome-wide transcriptional impact of NSD3 knockdown in premigratory neural crest cells, we compared gene expression in NSD3 morpholino (NSD3 MO) or mismatch control (mmMO) electroporated chick cranial neural folds by RNAseq. 769 genes had statistically significant changes in expression levels after NSD3 knockdown. Gene ontology analysis revealed significantly overrepresented categories among affected genes, including sympathetic nervous system development and neurogenesis (downregulated), and ribosomal small subunit biogenesis and RNA processing (upregulated). Additionally, several known regulators of neural crest development (Sox9, Pax7, TFAP2B, TFAP2E, Zic1) were significantly downregulated after NSD3 knockdown, consistent with our previous findings that NSD3 is essential for neural crest specification. To validate our RNA-seq data, we selected three novel, putative regulators of neural crest development: **Astrotactin-1** (*Astn1*), **Dispatched-3** (*Disp3*), and **Tropomyosin-1** (*Tpm1*). These genes, which are expressed by premigratory cranial neural crest cells, exhibit NSD3-dependent changes in gene expression in
embryos when assayed by whole mount in situ hybridization. Specifically, *Astn1* and *Disp3* expression were significantly decreased upon NSD3 knockdown, while *Tpm1* expression was significantly increased, mirroring RNA-seq results. Together, this study provides insight into the transcriptional impacts of NSD3 and offers a resource of putative novel regulators of neural crest development. Funding: MOD 5-FY-0-39; NSF IOS-1052102, 1354809.

**Program Abstract #383**

**Deregulation of fibroblast growth factor, insulin and folate signaling mediates hypomineralization associated with tobacco exposure**

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According to a report by the U.S. Department of Health and Human Services, cigarette smoking is the cause of upwards of 480,000 deaths per year. Additionally, exposure to second-hand smoke results in more than 41,000 deaths annually. All the while global demographic data suggests that smoking is on the decline, 10-12% of women in the U.S. still consume tobacco products during pregnancy. While numerous studies demonstrate the adverse effects of tobacco-related products towards health and developing embryos, the ramifications towards embryonic skeletal development remain unclear. Specifically, the role tobacco exposure plays in altering signaling pathways that activate transcriptional programs necessary for embryonic cell fate specification is not well understood. Here, we show that exposure to Camel Snus tobacco extract (STE) caused metabolic bone disease characterized by hypomineralized bones in both zebrafish and mice *in vivo* and inhibition of human embryonic stem cell (hESC) osteoblast differentiation *in vitro*. In STE treated differentiating hESCs transcriptome analysis revealed variations in fibroblast and insulin growth factor, folate, WNT and BMP signaling pathways. GO analysis suggested these differentially expressed genes to be associated with adversely affecting skeletal development. Exogenous treatment of FGF17 and folate mimicked the negative effects of tobacco exposure on osteogenic differentiation, while insulin coadministration rescued osteogenesis, causally corroborating the respective up- and down-regulation of these pathways observed with RNA-seq. Together, these data implicated that STE tobacco dysregulates osteogenic signaling pathways that impact bone lineage specification resulting in skeletal developmental defect. Funded by Tobacco Related Disease Research Program grants 19KT-0017H, 20DT-0038 and 24DT-0002.

**Program Abstract #384**

**Investigating the Effects of Histone H3.3 Point Mutations in Neural Crest Cells and Craniofacial Development**

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The vertebrate head is composed of a conglomerate of cells, with a majority deriving from a population of multipotent precursor cells called neural crest cells (NCC). NCCs are governed by a complex of regulatory genes whose coordinated expression equip them with their multipotency and specialization pathways for differentiation. Histone proteins, which are fundamental to both the structural integrity and organization of DNA, play an important role in gene expression. Furthermore, the modification of histone tails is now known to be an essential mechanism for the proper expression of a large number of genes. A point mutation in the gene h3f3a encoding the histone variant H3.3, was shown to disrupt neural crest cell (NCC) induction and generation of cranial NCC derived head bones in zebrafish. The authors proposed that incorporation of histone H3.3 and remodelling of the H3.3 tail may be required for expression of genes important for NCC specification and differentiation, respectively. Nevertheless, it remains to be confirmed if disrupted modification of histone tails is important in NCCs. We propose that modification of the H3.3 tail is required for coordinated expression of genes important for NCC migration and/or differentiation. To test this hypothesis, we used CRISPR/Cas9 to generate a mouse line with a conditional missense mutation in H3f3a. The mutation generated was previously shown to significantly decrease K36 methylation of the histone H3.3 tail, a mark thought to be crucial in preventing premature expression of genes crucial to NCC development. The Wnt-1 Cre2 mouse line will be used to drive expression of this mutation in NCCs. Alcian Blue Staining will be used to analyze cartilage in E14.5 embryos and alizarin red will be used to stain bones formed in heads of E17.5 embryos. This study will elucidate the importance
of K36 methylation and more generally epigenetics, in NCC formation, migration and/or differentiation. CIHR, NSERC.

**Program Abstract #385**

**To transcribe or not to transcribe: How HoxB coding and non-coding RNA transcripts are regulated by enhancer elements**

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Correct expression of *Hox* genes is critical to ensure proper development of an embryo. *Hox* genes are transcription factors which are expressed in specific spatio-temporal domains to define the anterior-posterior (A-P) axis and confer segmental identity to the embryo. In mammals, there are 4 *Hox* gene clusters (A-D) which encode for both coding and non-coding transcripts. The domains of expression of *Hox* genes must be tightly maintained and are regulated by growth factors such as Retinoic Acid (RA). The signaling of RA is integrated into *Hox* gene expression through local and long-range enhancer elements that are interspersed within and around the *Hox* gene clusters. In addition to enhancer elements, long non-coding RNA (lncRNA) transcripts that are transcribed from within the clusters potentially add complexity to this regulatory landscape. The focus of my research is to understand how shared enhancer elements, that work on multiple genes over a long range, together with lncRNAs participate in regulating *Hox* genes. I am using the HoxB cluster as the model to study this regulatory interplay. I have optimized the use of single molecule Fluorescent In Situ Hybridization (smFISH) technique in mouse embryos, and we are using a deep neural network approach to detect all nascent transcripts. This enables us to quantify the expression profiles and co-localizations of coding and non-coding transcripts in an unbiased and high-throughput manner in cells over the whole embryo. My results show that cis-enhancer elements within the cluster have different inputs towards transcription, and at specific segments in the embryo can act antagonistically to regulate transcription of HoxB genes. I also observe that the enhancer elements may regulate transcription of the Hobbit lncRNA. We are currently employing CRISPR approaches to better understand the role of Hobbit towards the regulatory dynamic of the HoxB cluster. This research is supported by the Stowers Institute for Medical Research.

**Program Abstract #386**

**A roadmap of ribosome heterogeneity and its impact on cellular differentiation**

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The ribosome is a complex macromolecular machine that has recently been suggested to have specialized functions in organismal development. To determine the magnitude of ribosome composition changes and its functional contribution to cell fate specification, we developed a sophisticated model system to measure ribosome heterogeneity by quantitative mass spectrometry on a day-by-day basis as human embryonic stem cells differentiate in a step-wise fashion down endoderm and mesoderm lineages. In this first-ever roadmap of ribosome composition dynamics during cellular differentiation, we identified numerous core ribosomal proteins (RPs) as changing significantly in abundance in actively translating ribosomes during cell fate specification. At the organismal level, we further generated a unique loss-of-function mouse model where a heterogeneous RP, which is progressively incorporated into the ribosome during mesoderm differentiation, remains associated with the ribosome but is no longer functionally active. This allowed for previously unattainable genetic analysis of RP function in cell fate specification and embryonic development, which is uncoupled from more housekeeping functions of RPs. We observed a remarkable series of phenotypes that include striking defects in mesodermal cell populations including posterior body truncations. Ribosome profiling in these mutant mice revealed altered translation of known regulators of mesoderm formation, including members of the Wnt signaling pathway, suggesting that specialized ribosome components may regulate embryonic development through selective translation of core developmental signaling networks. These findings set the stage for studies of ribosome
heterogeneity and specialization, creating a tool box for reprogramming and studying the functions of ribosomal heterogeneity from cells to organisms. This work was supported by the NSF, NIH, and NYSCF.

Program Abstract #387
Using an RNA-guided DNA binding platform to study transcriptional corepressor specificity across the Drosophila melanogaster genome
Ana-Maria Raicu
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DNA-binding transcriptional repressors regulate gene expression through interactions with corepressor proteins that act as scaffolds to create large repression complexes across the eukaryotic genome. The developmental importance of transcriptional corepressors such as Rb and CtBP have been extensively studied, especially in Drosophila melanogaster. Rb and CtBP are found on thousands of gene promoters, which they potentially directly regulate. One challenge of uncovering their mechanisms of repression and context-specificity during development is the general reliance on global knockdown or overexpression studies to determine specific functions. An in vivo approach to directly assess and compare the activities of different corepressors in the same context would provide the necessary setting to understand repressor selectivity and mechanism. To address this problem, we engineered flies expressing a nuclease dead Cas9 enzyme (dCas9) fused to the Drosophila retinoblastoma tumor suppressor proteins (Rbf1 and Rbf2) and deployed them to various genomic targets through association with gene specific guide RNAs. We found that Rb proteins can function as repressors in gene-specific ways when tethered to dCas9. When targeting the E2F2 promoter, wild type Rb proteins can mediate potent gene repression, while specific mutant versions have a more limited ability. Notably, a form of Rbf1 that lacks an “instability element” previously shown to be critical for activity of the endogenous protein is functional when tethered, pointing to a recruitment role for this domain. By comparing Rb to other corepressors like the Drosophila CtBP using dCas9, we will study different repression pathways in eukaryotes and show how genomic, temporal, and tissue-specific contexts impact corepressor activities. This work is supported by the National Institutes of Health Grant R01GM124137 and by the MSU College of Natural Sciences Dissertation Continuation Fellowship to AMR.

Program Abstract #388
Regulatory roles of microRNAs in early development
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The microRNAs (miRNAs) are small non-coding RNAs that repress the translation and reduce the stability of target mRNAs in animal cells. In early development, cell specification and pattern formation are controlled by signaling pathways, transcription factors and post-transcriptional regulators. However, even the most comprehensive models of developmental gene regulatory networks exclude post-transcriptional regulators. Moreover, how post-transcriptional regulators coordinate with other regulatory mechanisms of gene expression remains mostly unknown. Our research goal is to understand how miRNAs, as key post-transcriptional regulators, coordinate with signaling pathways and transcription factors to drive development. To achieve this goal, we use the sea urchin as a model, as it has an exceptionally well-described gene regulatory network and most of its miRNA families consist of a single species, which makes it amenable to unique, single miRNA perturbation analyses, as well as for examining their global targets. One of the miRNAs we focus on is miR-31; its knockdown results in extra spicule rudiment, decreased spicule length, and defective skeletal patterning. In addition, miR-31 suppresses multiple transcription factors and effector genes within the skeletogenic gene regulatory network. To further identify the global function of miR-31, we conducted experiments with biotinylated miR-31 pull down assays and proteomic analyses of control and miR-31 knockdown embryos. Results identified additional function of miR-31 to potentially suppress genes involved in regulating actin dynamics. Since miRNAs, signaling pathways, and transcriptional networks are highly conserved in animals, the results from this study serve as a paradigm for understanding coordinated regulatory networks used to build an embryo. This work is funded by the NSF Career Award #IOS-1553338.
Program Abstract #389

Unique homeobox codes delineate all neuron classes of the nematode Caenorhabditis elegans
Molly Reilly, Oliver Hobert
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It is presently not known whether neuronal cell type diversity, defined by cell type-specific anatomical, biophysical, functional and molecular signatures, can be reduced to relatively simple molecular descriptors of neuronal identity. We examined the expression of 101 of the 102 homeodomain proteins encoded by the Caenorhabditis elegans genome, using reporters that contain the full intergenic genomic context of the respective homeobox genes. This analysis revealed that the complete set of 118 C. elegans neuron classes can be described individually by unique combinations of this single family of transcription factor proteins, thereby providing the simplest currently known descriptor of neuronal diversity. Furthermore, computational and genetic loss of function analysis corroborates that homeodomain proteins not only provide unique descriptors of neuron type, but also play a critical role in specifying neuronal identity. 85 neuronally expressed C. elegans homeodomain proteins have previously been shown to play a role in neuronal identity specification. We extended this functional analysis by examining 4 homeobox genes that were not previously implicated in neuronal identity specification and found they indeed specify the fate of neurons where they are expressed. We speculate that the pervasive employment of homeobox genes in defining unique neuronal identities reflects the evolutionary history of neuronal cell-type specification. This work was funded by a predoctoral fellowship to Molly Reilly (F31 NS105398), by NIH R21 NS106843, and by the Howard Hughes Medical Institute.

Program Abstract #390

Observing wound healing in Clytia hemisphaerica muscle-like cells.
Elizabeth Lee
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Epithelial wound healing is a necessary process allowing organisms to repair integral barriers. The emerging model cnidarian system Clytia hemisphaerica is ideal for investigating wound healing due to its transparency and the easy visualization of its epithelial tissues. We want to understand how the muscle-like epithelial tissue of the Clytia jellyfish heals wounds. This tissue consists of epitheliomuscular cells, a specialized form of epithelial cell with contractile capabilities. To understand wounding in epitheliomuscular cells, we wounded this tissue in live medusa by gently scratching the lower surface (subumbrella) and observed wound closure using DIC imaging. Unlike the squamous epithelial tissue of the upper surface (exumbrella), the epitheliomuscular tissue generates two overlapping layers of lamellipodia during wound closure. To understand this novel result, we are defining the morphology of the epitheliomuscular cells in Clytia using dye injections, confocal imaging, and reconstruction. Together, these observations characterize a complicated wound healing process in a specialized epithelial cell type with a putative unusual structure. This work is supported by BSD internal funds from the University of Chicago.

Program Abstract #391

Description of serotonin-LIR and SCPb-LIR in the juvenile nervous system of Berghia stephanieae
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Adult nudibranchs are neurobiology models due to their stereotyped ganglia and large, identifiable neurons. Inaccessible embryos, however, have hampered studies of neural development at juvenile stages in current nudibranch models. Berghia stephanieae retains the benefits of current systems and is easily reared in lab and produces accessible embryos, enabling studies at all stages of its development. To compare different stages of the nudibranch nervous system a set of distinct neural features must be established. For example, immunohistochemical labeling of small cardio peptide b (SCPb) and serotonin neurotransmitters mark distinct neurons of the adult brain. The distribution of SCPb and serotonin in juvenile B. stephanieae has not been described before and can provide insight into when landmark neurons arise in development. The juvenile and adult central nervous system of B. stephanieae consists of four laterally paired ganglia: rhinophore ganglia, cerebropleural ganglia (CPL), pedal ganglia (PG) and buccal ganglia (BG). SCPb-like reactivity was found in the CPL,
PG, and BG. While multiple immunoreactive cellular regions were found in the CPL, a pair of anterior neurons was consistently found between animals. Consistent pairs of neurons were identified in the PG and BG as well. We also found paired projections from both the left and right CPL connecting to the peripheral nervous system. In comparison, serotonin-like cell reactivity was limited to the CPL and the PG, each with three consistent patterns. This reproducible reactivity in the juvenile stage provides interesting similarities to neuroanatomical features in adult nudibranchs, despite the juvenile’s smaller nervous system. Other features were variable however, and highlight elements that may be changing during *B. stephanieae* brain development. Funding provided by SDB Emerging Research Organisms Grant (2019) and NIH BRAIN Award 1U01NS108637-01.

Program Abstract #392
**Developing Genetic Tools to Establish *Biomphalaria glabrata* as a Model Organism**
Davoneshia Lollis, Heather Tsong, Maura Boerio, Wesley Chou, Daniel Wagner
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Model organisms provide a means of understanding and comparing the physiological processes, genetic expression, and anatomical composition of many organisms. While the mollusca phylum consists of a large and diverse group of organisms, a mollusc model combining all the features of modern molecular genetics does not currently exist. However, we have previously identified the freshwater snail, *Biomphalaria glabrata*, as a possible candidate because of their sequenced genome, reproductive qualities, and convenient maintenance requirements. Moreover, deeper understanding of these snails may result in discoveries with implications to alleviate the devastating effects of the parasitic disease, schistosomiasis. Establishing *B. glabrata* as a model organism requires tools that allow for detecting and manipulating gene expression. Thus, my work has focused on the optimization of a whole-mount *in situ* hybridization (WISH) protocol for *B. glabrata* embryos. This optimized protocol will allow for detecting expression of mRNAs transcribed from specific genes during embryonic development. By studying and modifying existing WISH protocols for other snail species, I have successfully created a protocol that treats embryos with the conditions typical for WISH but still conserves their anatomical structure. I have also successfully transcribed *in situ* probes that should show strong localized genetic expression in the embryos. Future work will focus on further alterations of the protocol to ensure hybridization of these probes results in robust detection of mRNAs. Once this protocol is optimized, we will examine the expression of the *B. glabrata* orthologs of diverse developmental regulatory genes to define signals that pattern the early snail embryo. *Thank you to the Wagner Lab, the Rice Sustaining Excellence in Research Program, and the Society for Developmental Biology Choose Development! Fellowship Program for support of this project.*

Program Abstract #393
**Development of pouch young of the sugar glider (*Petaurus breviceps*), a novel marsupial model organism**
Bruce Ostrow
*Grand Valley State University, USA*

Marsupials are ideal organisms to study mammalian development because much of their development occurs in the marsupium, which allows easy access to the pouch young. The sugar glider *Petaurus breviceps* is a small gliding marsupial native to Australasia and available in the USA as pets. To investigate sugar glider development, we established a breeding colony of 14 animals. We discovered it is feasible to repeatedly expose pouch young from the marsupium for observation and then tuck them back into the pouch for further development without harming the young or the mother. From our observations of >100 offspring, we determined the progressive development of various morphological features. Sugar glider young are born blind, pink, and hairless, with well-developed forelimbs and nares. They crawl to the marsupium and attach to a teat for two months, obtaining nutrition through lactation. The eyes present initially as pigmented retinae under the skin, the orbits bulge externally by 17 days postpartum (dpp), the eyeslits appear at day 36 (d36), and the eyes open 3-7 days after the young emerge out of the pouch. Ear pinnae appear as bulges by 4 dpp, become free at the ventral tip by d14, become fully free at d18, and point dorsally starting at d22. Pigmentation appears on the ear pinnae at 56 dpp and on the dorsum and tail at d60. Fur develops initially as a fine pelage at 60 dpp and continues to lengthen and spread over the integument until the young are fully furred by d71. The patagium forms initially in the axillary region starting at 6 dpp and grows posteriorly and laterally until it fills in the interlimb space by d25. Both sexes
form a phallus by 12 dpp and phallus differentiation between sexes is not apparent until d76. Our study demonstrates that sugar gliders are a useful model for researching animal development. Information gained from studying *P. breviceps* may be applicable to other gliding species and to bats. Supported by GVSU’s Professional Development Fund.

Program Abstract #394
Size and shape control in an early diverging animal
Pranav Vyas
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Animals have traditionally been characterized as clonal cell populations that undergo specific developmental programs that guide the morphogenesis of organs with complex tissue architectures and in turn whole animals. Though surprisingly, very early diverging metazoans display simple tissue architectures with a lesser division of labour that allows high plasticity and scalability. High plasticity in such tissues allows dynamic shape changes due to cell-cell contact shifts at time scales of the order of several minutes. Whereas, a scalable tissue architecture allows size variation of over several orders of magnitude with identical replication of design. For such tissues, size and shape are deeply interdependent due to scale-dependent physical constraints. In this study, we explore the nature of tissues in early diverging metazoans through the organism *Trichoplax adhaerens* from the phylum Placozoa. Using high throughput long term scanning microscopy, we collect datasets on shape and size variation in these animals in lab culture conditions and quantify their temporal trajectories. Using perturbations to these 'natural' trajectories, we decouple size and shape parameters to decipher the principles that the system uses to exercise control over growth. Utilizing large datasets and growth-based models, our study aims to provide abstract principles that would have governed size and shape control in very primitive ‘animal-like’ multicellular clusters. Funding - Fellowships: Stanford Graduate Fellowship, Stanford Interdisciplinary Graduate Fellowship - BioX, Lab: HHMI, CZI-Biohub

Program Abstract #395
Genetic Knockout of pigmentation in the squid *Doryteuthis pealeii*
Karen Crawford1,2, Caroline Albertin2, Juan Diaz Quiroz2, Namrata Ahuja2, Kristen Koenig3, Joshua Rosenthal2
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Research using squid as a model led to seminal breakthroughs in diverse areas of biology and biophysics. Despite their biological novelties, such as possessing by far the largest brains among invertebrates, highly plastic systems for camouflage, and an advanced system for recoding genetic information as it passes through mRNA, squid and other cephalopods are not widely used in research today. This is mostly because at present they are genetically intractable. In this work we demonstrate the first gene knockout in a cephalopod. The pigments in squid chromatophores and retinas are ommochromes. In many organisms, the first step in ommochrome biosynthesis is catalyzed by Tryptophan 2,3 Dioxygenase (TDO). We show that in the squid *Doryteuthis pealeii* TDO message is highly expressed in chromatophores and eyes, and that pigmentation can be blocked in developing embryos using TDO-specific antagonists. Likewise, TDO knockouts in developing embryos using the CRISPR-Cas9 system efficiently eliminated pigmentation. By fertilizing oocytes *in vitro* and precisely timing CRISPR-Cas9 delivery, the degree of pigmentation could be finely controlled. Genotyping of hatchlings through deep sequencing revealed highly efficient gene knockouts (routinely greater than 90%) in the G0 generation. These results open a pathway for exploring gene function in cephalopods and the associated biological innovations. This work was supported by NSF IOS 1827509, NSF IOS 1664767, NSF DBI 1723141, The Grass Foundation for the Doryteuthis Genome Project, The MBL Whitman Fellowship Program for K. Crawford, The Hibbitt Family for their support of C. Albertin, the office of the NIH Director 1DP5OD023111-01 and the John Harvard Distinguished Science Fellowship for their support of K. Koenig, and Charles and Patricia Robertson and The Owens Family Foundation for their support of J. Rosenthal.
Program Abstract #396
Embryonic formation of the cardiac jelly extracellular matrix assists in providing mechanical cues for proper heart development
Paige Ostwald, Neha Ahuja, Brandon Hylton, Deborah Garrity
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Every year, congenital heart defects affect 1% of births in the United States. The prevalence of some of these defects has been increasing, inciting a more thorough understanding of the developmental pathologies behind these defects. Previous studies have shown that the embryonic heart requires specific cues for valve specification and development. Primarily, mechanical cues coming from blood flow and myocyte contractions and molecular cues from intercellular signaling are necessary for proper development. As the atrioventricular (AV) valve begins forming, an extracellular matrix called the cardiac jelly (CJ) begins to accumulate between the inner endocardial and the outer myocardial cell layers. The CJ provides structure and aids in transferring mechanical and cellular cues. Previous studies found that CJ is necessary for proper valve development, but how it responds to mechanical stimuli, how it influences morphogenesis and how it may assist in heart function is unclear. Our work uses morpholinos to alter three targets in the CJ: hyaluronan synthase 2, nephronectin, and VE-cadherin 5, causing a decrease, increase and compromised cardiac jelly, respectively. We predicted that an appropriately structured CJ requires mechanical and genetic cues to provide an adequate environment for cardiomyocyte and chamber formation in order to support heart function. We found that the absence of early mechanical forces (i.e. heart contractions) expands the CJ associated with the AV junction. With wildtype mechanical cues but reduced gene expression, the CJ is compromised, leading to altered cardiac chamber shape, altered cardiomyocyte area and a decrease in heart function. In conclusion, the presence and amount of cardiac jelly is dependent upon mechanical function, whereas altering its structure results in abnormal morphogenesis, which in turn further decreases the mechanical function of the heart. Funding provided by the American Heart Association grant number 17GRNT33460256.

Program Abstract #397
The developing epicardium sustains cardiac chamber morphogenesis
Giulia Boezio1, Josephine Gollin2, Nana Fukuda1, Felix Gunawan1, Didier Stainier1
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The epicardium constitutes the outermost layer of the heart and has emerged as a crucial player during cardiac regeneration. However, the role of epicardial cues during cardiac morphogenesis is surprisingly understudied. To investigate this question, we generated three zebrafish models of impaired epicardial development by mutating epicardial-enriched transcription factor genes, tcf21 and wt1a, and ablating tcf21+ cells. All these genetic manipulations resulted in a reduction or complete absence of epicardial covering of the developing ventricle. Remarkably, in all three models, the larval heart exhibits abluminal cardiomyocyte extrusion, as well as a severe reduction in ventricle size and impaired cardiac function. Furthermore, allowing the epicardium to regenerate after ablation partially rescued the cardiac defects. These observations suggest that epicardial cells function not only as a scaffold that physically supports cardiac morphogenesis, but also as a paracrine signaling center essential for myocardial wall integrity. Employing deep phenotyping and transcriptomic analyses, we aim to elucidate how the epicardium affects the morphogenesis of the cardiac chambers and to identify the molecular pathways involved in this crosstalk, which is also potentially at play during cardiac regeneration. Funding Sources: Max Planck Society, Leducq foundation, and the European Research Council.

Program Abstract #398
Identification of a role for RA in hepatic patterning and induction in the mouse.
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Retinoic acid (RA), a metabolite of vitamin A, is a morphogen that is essential for normal embryonic development. Although RA signals are required for liver development in a variety of vertebrates, a role for RA during murine hepatic specification has yet to be defined. To examine the requirement for RA in murine liver induction we performed whole embryo culture with the small molecule RA inhibitor, BMS493, to attenuate RA signals
immediately prior to hepatic induction and through liver bud formation. The BMS493-treated embryos demonstrated a significant loss of hepatic specification that was confined to the anterior liver bud. Examination of RA-attenuated embryos demonstrates that while the liver bud displays normal expression of various foregut endoderm markers as well as the hepatopancreatobiliary domain marker, PROX1, the dorsal/anterior liver bud excludes the critical hepatic marker, HNF4a, indicating that RA signals are required for dorsal/anterior hepatic specification. Notably this patterning defect correlates with the known dorsal-ventral gradient of the RA synthesis enzyme, \textit{Aldh1a2}. In further support of a role for normal levels of RA in proper hepatic patterning, we examined embryos with a hypomorphic mutation in the RA synthesis enzyme, RDH10, and find they exhibit a similar reduction of the anterior liver bud. Finally, the addition of exogenous RA prior to hepatic induction leads to a uniformly reduced liver bud. Taken together our \textit{ex vivo} and \textit{in vivo} evidence demonstrate the requirement of appropriate RA gradients for normal hepatic patterning and specification. Funding: NIH grant R56Dk12363

Program Abstract #399

The Kinesin-9 Family: Non-Canonical Motile Ciliary Kinesins
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Motile cilia are microtubule-based, whip-like projections that produce laminar flows across the surface of epithelia in a variety of different tissues across the development of vertebrate organisms. Cilia rely heavily on kinesins for their formation, maintenance, length regulation, and motility. Several kinesins have been suggested to hold a ciliary function but remain relatively understudied. Here, using several different vertebrate organisms, we report the previously under-studied Kinesin-9 family members, Kif6 and Kif9, have separate functions in motile cilia formation and function. First, we report that mutations in \textit{Kif6} lead to hydrocephaly in mice and hydrocephaly and scoliosis in zebrafish. We demonstrate that these phenotypes are associated with loss of ependymal cell cilia in both mice and zebrafish, while cilia formation in other multiciliated cell lineages are not affected. Additionally, we report that human mutations in \textit{KIF6} are linked to macrocephaly and intellectual disabilities. By contrast, we demonstrate that mutations in \textit{kif9} cause defects in pronephric function in larval stage zebrafish, displaying phenotypes associated with loss of motile cilia function. Using \textit{Xenopus laevis} epidermal multiciliated cells, we show that Kif9-GFP has dynamic localization within axonemes, at basal bodies, and at a liquid-like, phase separated organelle, the Dynien Arm Assembly Particle (DynAP). Within DynAPs, Kif9-GFP shows dynamic turnover rates and co-localizes within a subdomain specific to inner dynein arm subunits. We hypothesize that Kif9 is required for trafficking a subset of cargo assembled within the DynAP into the cilium. Future work will aim to understand how mutations in \textit{kif9} affect motile cilia in the zebrafish pronephros and how Kif9 is contributing to motile cilia function through identifying interaction partners and potential cargoes. Studies supported by NIGMS R01HL117164 and Provost Graduate Excellence Fellowship through UT Austin.

Program Abstract #400

The role of Ankrd11, a KBG syndrome risk gene, in cardiovascular development

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KBG syndrome is a rare neurodevelopmental disorder characterized by global developmental delay, intellectual disability, and heart and brain defects. The disorder is caused by the haploinsufficiency of the chromatin regulator ANKRD11 (Ankyrin Repeat Domain 11), which controls global gene expression by regulating histone acetylation levels. While KBG syndrome patients display multiple heart defects, which often require surgery, the role of ANKRD11 in heart development is not known. To address this gap, I am using a mouse model with conditional knockout of Ankrd11 in the neural crest, a tissue that contributes to heart structures that are abnormal in KBG patients. My results demonstrate that embryonic neural crest conditional knockouts of Ankrd11 (Ankrd11\textsuperscript{lox/lox}; Wnt1Cre2 or Ankrd11\textsuperscript{cko}) display persistent truncus arteriosus, where the aorta and pulmonary trunk fail to separate. Moreover, Ankrd11\textsuperscript{cko} embryos show severely enlarged hearts and pale bodies, indicating that the heart defect causes deficient blood circulation. Since \textit{in utero} blood flow impairment can lead to brain abnormalities, I am also analyzing the brain vasculature in these embryos for potential compensatory increase in
blood vessel density. I am using fluorescent labeling of intact vasculature coupled with the tissue clearing technique CLARITY to image the brain 3D blood vessel network. My initial results suggest Ankrd11\textsuperscript{cko} embryos may have aberrant brain vasculature development. In summary, I show that Ankrd11 contributes to development of cardiac neural crest-derived tissues, which may have implications for brain vasculature and function. This advances our knowledge of the origins of KBG syndrome and the role of chromatin regulators in cardiovascular development. This work was funded by Alberta Innovates and WCHRI Summer Research Studentships as well as University Hospital Foundation (Gilbert Winter K. Fund)

Program Abstract #401

Iroquois homeobox genes Irx1 and Irx2 play essential and overlapping roles in mammalian embryonic development

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Mammalian Iroquois homeobox (Irx) genes are organized in two paralog clusters of three genes each. Irx1 and Irx2 are two members of the IrxA cluster and exhibit overlapping expression in the developing mouse embryo, which is likely regulated by shared enhancers. However, the role of Irx1 and Irx2 in mammalian development is poorly understood. We have previously shown that genetic knockout (KO) of Irx2 results in fertile and viable mice with no marked abnormality, pointing to the possibility of genetic compensation between Irx1 and Irx2. We have generated a new KO mouse line of Irx1 by deleting exon 2, which harbours the homeobox. These Irx1\textsuperscript{KO} mice show perinatal lethality as well as reduced viability and body size. To investigate the overlapping functions of Irx1 and Irx2, we deleted Irx2 in the Irx1\textsuperscript{KO} background and found that all Irx1\textsuperscript{KO}Irx2\textsuperscript{Het} mice die shortly after birth. These mice exhibit defects in the intestinal villi and reduced alveolar space. These results suggest that Irx2 compensates for Irx1 in the developing lung and intestine. Genetic removal of both copies of Irx2 in the Irx1\textsuperscript{KO} background results in embryonic lethality at around E11.5. Although Irx1\textsuperscript{KO}Irx2\textsuperscript{KO} embryos appear comparable to the WT littermates at E9.5 and E10.5, quantification of PECAM-stained yolk-sac vascular network demonstrate a significant disruption in the remodeling and maturation of yolk-sac vasculature. In addition, immunostaining results shows a reduction in vascular branching into the labyrinth of the placenta. Together, these observations illustrate the overlapping functions of Irx1 and Irx2 in mammalian development and uncover their essential functions in yolk-sac and placenta development. Further investigation is required to deduce the lineages and molecular mechanisms involved in the Irx1 and Irx2 regulation of extraembryonic angiogenesis. This work was supported by the Canadian Institutes of Health Research and Alexander Graham Bell Canada Graduate Scholarship.

Program Abstract #402

C. elegans establishes germline versus soma by balancing inherited histone methylation

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Embryos undergo extensive reprogramming at fertilization to prevent the inappropriate inheritance of histone methylation. In C. elegans, the H3K4me2 demethylase, SPR-5, and the H3K9 methyltransferase, MET-2, are maternally deposited into the oocyte where they cooperate to reestablish the epigenetic ground state of the newly formed zygote. Previous work from the Strome and Kelly Labs demonstrates that maternally deposited MES-4 maintains H3K36me2/3 at germline genes between generations to help re-establish the germline. To determine whether the MES-4 germline inheritance system antagonizes spr-5; met-2 maternal reprogramming, we examined the interaction between these two systems. Here, we show that the progeny of spr-5; met-2 mutants display a severe developmental delay that is associated with the ectopic expression of MES-4 germline genes in somatic tissues. By performing ChIP-seq on L1 progeny from spr-5; met-2 mutants, we find that MES-4 germline genes ectopically accumulate H3K36me3 in somatic tissues. Additionally, knocking down MES-4 suppresses the ectopic expression of MES-4 germline genes and rescues the developmental delay. We also show that the developmental delay is dependent upon the H3K4 methyltransferase, SET-2. Together, these data suggest a model where SPR-5; MET-2 maternal reprogramming antagonizes H3K36me2/3 to enable the proper
transgenerational control of germline versus somatic cell fates. Without SPR-5; MET-2reprogramming, somatic cells struggle to specify their proper cell fate amongst the noise of inappropriate germline gene transcription, leading to developmental delay. A similar mechanism may underlie the developmental delay of Soto and Kabuki Syndrome patients who have mutations in histone modifying enzymes. This work was funded by a grant to D.J.K. (NSF IOS1931697); B.S.C. was supported by the Fellowships in Research and Science Teaching IRACDA postdoctoral program (NIH K12GM00680-15); and B.S.C. was also supported by NIH F32 GM126734-01.

Program Abstract #403
Unraveling the molecular pathways that drive oligodendrocyte fate specification in the developing cerebral cortex using single-cell RNA sequencing
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Proper assembly of neural circuits in the developing brain requires coordinated production of many different neuronal and glial cell types. Progenitors in the dorsal forebrain produce excitatory neurons, astrocytes, oligodendrocytes and olfactory bulb interneurons. A major goal is to understand the molecular mechanisms that specify these different cell fates. Studies from our lab and others have shown that Sonic hedgehog (Shh) signaling is important for oligodendrocyte fates. Here, we use publicly available single-cell RNA sequencing data to propose a working model for how Shh signaling influences cell fate decisions in the developing dorsal forebrain. We first analyzed a dataset in which Shh was overexpressed in the mouse dorsal forebrain. Consistent with previous findings, we identified a progenitor state along the progression from radial glial cell (RGC) to either oligodendrocyte precursor cell (OPC) or olfactory bulb interneuron (OB-IN). This progenitor state is enriched for Shh pathway genes and transcription factors traditionally associated with the ventral forebrain. Further, we identified genes uniquely upregulated along the OPC lineage that define a pre-OPC state. Using wild type datasets, we identified pre-OPCs as early as E13.5 and found that some RGCs express pre-OPC markers at E11.5. Finally, we identified a pre-OPC state in data from human fetal cortex, and found that pre-OPCs shared some common gene expression profiles with certain human RGC subtypes. Together these data led to a working model whereby RGCs in the dorsal forebrain have the potential to generate either “dorsal-like” excitatory neurons or “ventral-like” OPCs and OB-INs. We hypothesize that the fate choices made from this bi-stable state depend on extrinsic signaling pathways that help to drive or maintain specific transcriptional networks. Funding: NIH R01-NS109239, R56-NS109239 (SJF); RNA Biosciences Initiative Informatics Fellows, Dev Bio & Regenerative Medicine Training Program (CCW).

Program Abstract #404
The complex Nuclear Receptor Element (cNRE) is necessary and sufficient to drive the atrial expression in mice and zebrafish
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Cardiac chamber specification is a complex process orchestrated by multiple signaling pathways and morphogenetic movements. It has been previously demonstrated that the SMyHC3 promoter expression is suppressed in ventricles via a VDRE/RARE element; however, the sequence driving SMyHC3’s atrial-specific expression remains elusive. Here, we propose a new dual cis-regulatory element (the complex Nuclear Receptor Element, cNRE) within the SMyHC3 promoter that is responsible for both ventricular repression and atrial activation. Calorimetry, binding assays, and mutation analysis demonstrated that the cNRE is composed of three tandemly arrayed hexads harboring binding sites for multiple nuclear receptors, being the chicken ovalbumin promoter-transcription factor II (COUPTF-II) one of its ligands. Proteomic studies evidenced that COUPTF-II binds to Androgen Receptor (AR), and transactivation assays in HEK 293T cells showed a synergistic interaction between these two repressors leading to activation of the SMyHC3 promoter in a cNRE-dependent manner. We then tested the cNRE relevance in transgenic mouse and zebrafish and found that it holds the information required to atrial activation and ventricular repression of the SMyHC3 promoter, as well as to change the expression pattern of a ventricular promoter towards an atrial promoter in zebrafish, even outside of its native context. In summary, we...
provided evidence that the cNRE holds the information to drive atrial-specific expression of cardiac genes via the synergic activity of COUPTF-II and AR, and this information crosses the species barrier. Taking together, these findings evidenced a whole new mechanism underlying the atrial-specific expression of the SMyHC3 promoter and unveil new roles for known transcription factors in the context of cardiac chamber specification. This work was funded by The Sao Paulo Foundation (FAPESP – grant numbers 2013/22695-0, 2015/12549-2 and 2018/09839-7).

Program Abstract #405
Identifying Gene Targets of Retinoic Acid During Retinal Neurogenesis
Raina Sacksteder, Margalit Leiser, Gwen Miller, Kara Cerveny
Reed College, United States
The generation of mature neurons during development or repair relies on the integration of intrinsic and extrinsic signals. Retinoic acid (RA) is a locally-generated extrinsic signal that can promote the formation of neurons by triggering transcription of target genes when it binds its intracellular retinoic acid receptors (RARs) that are bound to specific retinoic acid response elements (RAREs) in the DNA. Previous studies have shown that precise control of RA pathway activity ensures typical retinal neurogenesis. To determine how RA regulates neurogenesis in the developing eye, we performed a series of gene expression assays to identify immediate or secondary targets of retinoic acid in developing zebrafish eyes. Immediate targets are transcribed when RA binds a RAR bound to an upstream RARE, whereas secondary targets are activated only when RA first activates expression of a regulatory gene which then goes on to trigger expression of the secondary target gene. To understand how RA promotes neurogenesis, we first performed an RNA-Seq experiment on RNA from eyes of 28 hpf zebrafish (Danio rerio) embryos that were treated with the RAR agonist AM580 in the presence and absence of the ribosome-inhibitor cycloheximide. Validation with in situ hybridization and RT-qPCR analysis revealed that cyp26a1, dhrs3a, nr6a1b, and tshz1 are upregulated by AM580 in the presence and absence of cycloheximide, suggesting that these genes are immediate targets of RA. Furthermore, scanning 10 kb upstream of these genes identified RARE motifs that likely contribute to the direct regulation of tshz1, cyp26a1, and dhrs3a by RA. Our findings provide new insights into how RA may regulate retinal neurogenesis and lay the foundation for future investigations into a RA-regulated gene regulatory network in the developing vertebrate retina. Funding was provided by Reed College and the MJ Murdock Charitable Trust.

Program Abstract #406
Investigating the cis-regulatory logic that underlies horizontal cell fate specification in the developing retina
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During retinal development, uncommitted progenitors divide to form cells that will acquire specific fates. These include the six classes of neural cells that make up the retina and the more than 100 cell subtypes that they encompass. We are interested in uncovering the genetic mechanisms that underlie this process and that direct retinal cell development. Horizontal cells (HCs) are highly-specialized interneurons located in the inner nuclear layer of the retina that provide inhibitory feedback to photoreceptors. Varying numbers of horizontal cell subtypes are present in the vertebrate retina and are easily distinguished by morphology and molecular markers. Knowledge of the cis-regulatory modules, or enhancers, that regulate the expression of genes required for the generation of HCs can add a level of resolution to current models of development. These elements will therefore provide a foothold for the study of the gene regulatory networks that direct HC development, as well as serve as much-needed tools to target developing HCs. Here we present a screen for cis-regulatory elements near the HC-associated genes Ptf1a, Onecut1, Tfap2a, and Lim1, which resulted in the identification of several novel enhancers that display preferential activity in HCs. We detected binding sites within these elements for specific transcription factors that are required for activity of the enhancer and thereby regulate gene expression. Furthermore, we have uncovered a potential role for nuclear receptors as negative regulators of HC development. Support for this project was provided by National Science Foundation grant CAREER 1453044 (to MME).
**Program Abstract #407**

**Inhibitor-of-Differentiation 4 (Id4): a dominant-negative helix-loop-helix transcription factor with dose-dependent effects on inner ear hair cell differentiation**

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A complex network of basic Helix-Loop-Helix (bHLH) transcription factors regulates inner ear hair cell fate decisions during their embryonic production. The proneural bHLH Atonal-homologue 1 (Atoh1) promotes adoption of the hair cell fate. Atoh1 expression is repressed by bHLH members of the Hairy and Enhancer-of-Split (Hes) family, the major downstream effectors of Notch signalling during the lateral inhibition of hair cell formation. An additional class of dominant-negative HLH proteins, encoded by the Inhibitor of Differentiation (Id) genes 1-4, could also potentially interfere with Atoh1 and other bHLH expressed in the inner ear. Among these, we found that Id4 is expressed within the prosensory domains of the developing inner ear, but does it act as a negative regulator of hair cell formation? We show that the overexpression of Id4 in the embryonic chick inner ear strongly inhibits Atoh1 expression and hair cell differentiation. However, Id4 is normally expressed in differentiating hair cells, indicating that at endogenous gene dosage, it is unlikely to interfere with Atoh1 function. Furthermore, there is no excess or premature differentiation of hair cells in the inner ear of an Id4-deficient mouse model, even in conjunction to a pharmacological blockade of Notch activity in vitro. Whilst the precise role of Id4 remains unclear, these paradoxical findings highlight the critical importance of dosage in the bHLH proteins network at the core of hair cell fate decisions.  

**Funding sources:** AOHL, BBSRC

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**Program Abstract #408**

**The Vangl2 interactome during convergent extension reveals complex linkages to the actin and septin cytoskeletons**

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Tissue morphogenesis requires specific signaling pathways to govern precise behavior of individual cells and the collective tissue. This is exemplified by the requirement of Planar Cell Polarity (PCP) signaling to govern convergent extension (CE) and tissue elongation in model organisms and humans. During this collective cell movement, cells must rapidly rearrange the cytoskeleton to elongate and establish anteroposterior polarity before individual cells can intercalate. Despite their importance during CE, how PCP proteins function during this dynamic process remains unclear. To gain insight into how these proteins guide polarized cell behaviors, we utilized affinity purification and mass-spectrometry to probe the Vangl2 interactome during CE. We identified interactions between Vangl2 and key components of the cytoskeleton, including several actin regulators, microtubule associated proteins, and a specific septin heterotetramer. Utilizing subcellular time-lapse imaging of PCP proteins, we observed their dynamic, asymmetric localization, interaction with the remodeling actin cytoskeleton and co-localization with septin proteins. Through loss-of-function studies we have gained insight into a key role of septins in regulating the actin cytoskeleton and the localization of key PCP proteins during CE. By combining tissue-specific proteomics and advanced imaging approaches we have furthered our understanding of the molecular processes governing cellular movements in CE and provided new insights into the basic biology of vertebrate development.  

This work is supported by NIGMS.

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**Program Abstract #409**

**Glypican4 regulates planar cell polarity of endoderm cells by controlling N-cadherin localization**

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The endoderm, the deepest germ layer, contributes to the development of the gut and associated organs. During embryogenesis, the endoderm undergoes convergence and extension (C&E), transforming from a broad endodermal sheet into the long, narrow gut tube. Non-canonical Wnt/Planar Cell Polarity (Wnt/PCP) signaling has been implicated in endoderm morphogenesis. In mutants for *glypicanc 4* (Gpc4, a heparan-sulfate proteoglycan that promotes Wnt/PCP signaling) and *vanGogh-like 2* (Vangl2, a core Wnt/PCP protein), the endodermal sheet is significantly widened and the gut tube is enlarged. However, the underlying cellular and molecular mechanisms
are not clear. We found that during early- and mid- segmentation, endodermal cells progressively elongate and polarize along the mediolateral axis, and undergo intercalation during C&E. Such endoderm cell polarity is lost, and the intercalation is impaired in either gpc4 or vangl2 mutants. Notably, GFP-Vangl2 is enriched at the anterior edge of elongated endodermal cells, which is disrupted in gpc4 mutants. These findings indicate that during segmentation, endodermal cells acquire planar cell polarity and that this requires Wnt/PCP signaling. In gpc4 mutants, the N-cadherin expression on the plasma membrane of endodermal cells is increased and reducing N-cadherin expression partially suppresses their defects of endodermal cell polarity as well as gut-tube. Furthermore, N-cadherin endocytosis is impaired in gpc4 mutants, and endocytosed N-cadherin is colocalized with Rab5c-YFP. Overall, our findings indicate that Gpc4 controls endoderm C&E by regulating endodermal planar cell polarity through influencing N-cadherin localization. Currently, we are investigating the mechanisms by which Gpc4 regulates N-cadherin localization. Funding: NIH, NSF

Program Abstract #410
Perivascular Sonic hedgehog signaling regulates microvascular patterning and stability in cleft lip pathogenesis
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Disruption of the Sonic hedgehog (Shh) signaling pathway results in craniofacial malformations, including orofacial clefts of the lip and palate. In normal craniofacial morphogenesis, Shh signals to cranial neural crest cells (cNCCs), driving their proliferation and differentiation. Shh signaling was recently discovered to regulate the angiogenic transcriptome in cNCCs, including expression markers of pericytes, the mural cells that regulate angiogenesis and microvascular stability. Here, we examined microvascular morphogenesis in a mouse model of Shh pathway antagonist-induced cleft lip as well as the impact of cNCC-specific Shh pathway activation in a cNCC-endothelial cell coculture system. During cleft pathogenesis in vivo, attenuated tissue outgrowth was associated with altered microvascular morphogenesis, including reduced vessel length, junction number, and endpoints in the medial nasal processes that form the upper lip. We then established an in vitro coculture system in which human umbilical vein endothelial cells form cords that physically associated with cNCCs. Although Shh pathway perturbation did not affect cord formation in endothelial cells alone, cNCC-autonomous Shh pathway activity significantly delayed degeneration of endothelial cord networks in coculture. Taken together, these findings support the premise that Shh pathway activation in cNCCs promotes pericyte-like function and supports microvascular stability. In addition to suggesting a novel mechanism of action of Shh signaling in craniofacial development, these studies also identify perivascular differentiation and microvascular morphogenesis as new focuses for understanding the causes of craniofacial malformations. This work was supported by T32 ES007015 to HC, R25 ES020720 to VM, and the UW OVCRGE Fall Competition Award to RL.

Program Abstract #411
Mapping the relationship between proliferation and morphology in the mouse face
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There is a long-standing prediction that small changes in proliferation and apoptosis during the time frame of facial morphogenesis act to shape the face. Further, many studies show genetic alterations that cause structural birth defects affect local proliferation or apoptosis. Yet, it is unclear how much of local change in regional proliferation would be necessary to cause a defect. Here, we set out to understand the relationship between growth, morphology and proliferation and test that prediction that targeted proliferation shapes the developing face by quantifying proliferation and apoptosis in 3D and relating it to the growth of the face. We use whole mount staining for proliferation and apoptosis markers, whole tissue clearing methods, lightsheet microscopy and atlas and machine learning based quantification methods to identify individual proliferating or apoptotic nuclei within a 3D tissue structure at a set time point. We also employ geometric morphometric analysis of the same tissue structure to quantify overall morphology. By collecting data at various time points across facial development (E9.5-E11.5) and quantifying the age of each embryo, we are able to relate cell biological level growth to tissue level growth and morphological change and relate these two parameters in a way not performed
Program Abstract #412

**Sonic Hedgehog Regulation of Frem1 in the Cranial Neural Crest Mesenchyme and Midfacial Development**

Matthew McLaughlin, Miranda Sun, Robert Lipinski

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Face shape variation is a pillar of societal interaction, and facial malformations are among the most common human birth defects. However, our understanding of specific mechanisms that drive facial development remains limited. The Sonic Hedgehog (Shh) signaling pathway is a master regulator of facial development, and our laboratory recently uncovered a regulatory association between Shh signaling and Fras1-related extracellular matrix gene 1 (*Frem1*) in mouse cranial neural crest cells (cNCCs). Remarkably, a recent analysis also revealed a strong association between *FREM1* variants and human face shape variation. Here, we present *Frem1* as a novel target of Shh signaling in cNCCs. *In situ* hybridization analysis revealed that *Frem1* is expressed concomitantly with Shh target *Gli1* in vivo in the cNCC mesenchyme of the developing upper lip and palate, and preliminary data suggested that Shh signaling directly regulates *Frem1* transcription in cNCCs *in vitro*. This is the first report of *Frem1* expression in cNCCs and Shh as an upstream regulatory mechanism of *Frem1* expression during midfacial morphogenesis. Identifying the function of *Frem1* in guiding cNCC biology will shed light on its role in face shape variation and potential role in orofacial clefting. Funding Sources: Hilldale Undergraduate/Faculty Research Fellowship.

Program Abstract #413

**Differences between natural pregnancy and diapause: a cautionary tale**

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Diapause, or delayed implantation, is a mammalian phenomenon wherein both blastocyst and the uterine environment are paused in a pregnancy. Mice undergo lactational diapause, or ‘natural diapause’ (ND), where, if a mating event occurs upon birth of the first litter, implantation in the following pregnancy is delayed. This is achieved by high prolactin levels preventing the estrogen surge necessary for embryo attachment. Diapause can be artificially induced by removing the source of estrogen (ovary), termed ‘Artificial Diapause’ (AD). Using 3D imaging, we compare embryo location, embryo movement patterns, and progesterone (P4) levels during natural pregnancy (NP), ND, and AD. We previously showed that embryos in NP exit the oviduct at gestational day (GD) 3 of pregnancy, move *unidirectionally as clusters*, then *scatter and space out bidirectionally*, prior to attachment at GD4. Surprisingly in ND, embryos are present as clusters outside the oviduct on GD3, pause for ~24-hours, followed by only a *unidirectional scattering and spacing movement* on GD4, and a second ~24-hour pause prior to attachment. Thus, we discovered that in ND, there are two pauses both before and after embryo spacing, and embryo movement patterns differ from NP. Predictably, AD shows the embryo movement patterns of NP. We also discovered that ND showed lower levels (~10ng/ml) and AD showed high levels (>120ng/ml) of P4, compared to NP (~40ng/ml). Thus, pregnancy outcome is not impacted under delayed implantation because mammals adapt their uterine environment to ensure successful pregnancy. Understanding these adaptations, provides an opportunity to determine key players that are naturally modulated to allow implantation success. We can then harness this knowledge for guiding successful outcomes in the clinic where implantation rates are low, e.g., during infertility and for artificial reproductive technologies. This project was funded by startup funds provided by Michigan State University.

Program Abstract #414

**The ciliary protein Arl13b regulates axon guidance and cerebellar development in the mouse hindbrain**

Sarah Suciu, Alyssa Long, Tamara Caspary

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The ciliopathy Joubert Syndrome (JS) presents with physical anomalies, intellectual disability, and is diagnosed by the hindbrain “molar tooth sign” (MTS) malformation. The MTS results from a combination of hypoplasia of the...
cerebellar vermis and inappropriate targeting of the white matter tracts of the superior cerebellar peduncles (SCPs), which create a deepened interpeduncular fossa. ARL13B is a cilia-enriched, regulatory GTPase established to regulate cell fate, cell proliferation and axon guidance through vertebrate Hedgehog (Hh) signaling. In patients, point mutations in ARL13B cause JS. In order to understand the etiology of the MTS, we used mouse models to investigate the role of ARL13B in MTS formation during cerebellar development. We found ARL13B regulates both SCP targeting and cerebellar vermis size. However, despite finding that SCPs can use Hh as a guidance cue, our data argue that the JS-causing R79Q mutation in ARL13B does not disrupt Hh signaling nor does it impact SCP targeting. We observed the entire cerebellum was small upon complete Arl13b deletion consistent with the known role of Hh in regulating proliferation throughout the cerebellum. In contrast, only the cerebellar vermis was small in the Arl13b R79Q/R79Q mice. Similarly, mice expressing a cilia-excluded variant of ARL13B that transduce Hh normally, specifically displayed a small cerebellar vermis. Taken together, our data indicate that disruption of at least one specific ARL13B effector within cilia gives rise to the MTS in a Hh-independent manner. Thus, we conclude that MTS formation is due to disruption of a subset of ARL13B function. This work was funded by T32GM008490 and F31NS101806 to S.S. as well as R01NS090029 to T.C.
exposed guts revealed severe abnormalities in endoderm cell shape, adhesion and epithelial architecture, indicative of failure to execute the cell rearrangement events that drive intestine elongation. As an herbicide, ATR inhibits the photosynthetic electron transport chain (ETC) in chloroplasts, leading to the overproduction of reactive oxygen species in plants. However, ATR has also been shown to elicit oxidative stress in mammalian cell culture and vertebrate animal models, suggesting that ATR-induced intestinal defects may be a result of mitochondrial ETC dysfunction and/or activation of the oxidative stress response. Indeed, ATR-exposed embryos exhibit other phenotypes indicative of oxidative stress, including hemorrhaging and cardiac hypertrophy. Moreover, exposing *Xenopus* to diuron, another herbicide that inhibits the ETC, closely phenocopies exposure to ATR, as does exposure to high glucose or ethanol levels, both of which are known to induce oxidative stress in the developing embryo. Importantly, the intestinal shortening and malrotation phenotypes exhibited by ATR and diuron can be rescued after pretreatment with ascorbic acid, an antioxidant. Our results reveal a potentially novel mechanism of action of ATR on intestinal morphogenesis and suggest that oxidative stress may underlie the etiology of a common birth defect. This work was funded by the National Institute of Environmental Health Sciences (T32ES007046).

**Program Abstract #421**

**A non-proteolytic requirement for a single proteasomal lid subunit, RPN-12, in *C. elegans* germline sex determination and oogenesis**

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The 26S proteasome is a highly conserved complex known foremost for its essential proteolytic function. However recent evidence demonstrates that specific proteasomal subunits perform non-proteolytic roles in cellular processes such as transcription, mRNA export, and chromatin structure. We are investigating roles of individual proteasomal subunits during germline development and reproduction in *Caenorhabditis elegans*. We will demonstrate that *C. elegans* RPN-12, a 19S regulatory particle subunit of the proteasome, is not essential for the proteolytic activity of the proteasome but crucial for reproduction. A null RPN-12 mutant, *rpn-12*(av93), is homozygous viable, however >64% of the hermaphrodites lack sperm. When sperm are introduced via mating, *rpn-12*(av93) hermaphrodite oocytes are fertilized but the animals fail to reach wild-type reproductive capacity indicating oogenesis defects. Loss of RPN-12 also causes nuclear accumulation of WEE-1.3, a meiotic kinase crucial for oocyte quality and partially suppresses the *wee-1.3(RNAi)* infertility phenotype. This is not due to alterations in proteasomal activity because chemical inhibition of proteolysis using bortezomib does not produce similar phenotypes, suggesting a potential non-canonical role for RPN-12 in WEE-1.3 regulation. Our expression data indicates RPN-12 is ubiquitously expressed in the germline and soma at all developmental stages. We propose that RPN-12 plays a role in both hermaphrodite germline sex determination and in oocyte quality. Our study identifies important, non-proteolytic roles for a specific proteasomal subunit in reproduction and supports the continued investigation into non-canonical roles of well-studied proteins. This study will contribute to dissect the intricate and complex molecular pathways that govern gamete production crucial for healthy reproduction in humans worldwide. This work is supported by the Department of Defense Grant W911NF-18-1-0465

**Program Abstract #422**

**The role of RACK-1 in regulating stem cell proliferation in the *C. elegans* germ line.**

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Stem cells are central to the development of multi-cellular organisms, including *C. elegans* and humans. Key to their function is their ability to differentiate into specialized cells or proliferate to maintain their population for future use. We are focusing on identifying the molecular mechanisms that regulate the proliferation/differentiation decision of stem cells using the *C. elegans* germ line as a model. *C. elegans*’ germline stem cells (GSCs) proliferate to maintain the stem cell pool and differentiate to produce gametes. The conserved STAR family, RNA binding protein, GLD-1/Quaking, promotes differentiation and is required to maintain a proper balance between proliferation and differentiation. When *gld-1* activity is lost, along with *gld-2* activity, which functions redundantly with *gld-1* to promote differentiation, a germline tumour of proliferating stem cells is
formed. Previous research has shown that the pattern of GLD-1 germline accumulation is key to controlling its activity. We have found that GLD-1 subcellular localization is likely also involved in regulating GLD-1’s activity. We identified *rack-1* as a regulator of GLD-1 subcellular localization. *rack-1* mutants have lower overall levels of GLD-1 and a severe disruption in GLD-1’s subcellular localization; while wild-type GLD-1 is cytoplasmic, in *rack-1* mutants GLD-1 localizes to germ granules. Moreover, in other genetic backgrounds, a loss of *rack-1* phenocopies a reduction or loss of *gld-1* activity, suggesting that the mislocalized GLD-1 has reduced activity. Our research reveals a novel mechanism, where the activity of GLD-1 is regulated by its subcellular localization. This provides an additional layer of regulation in the proliferation/differentiation decision of *C. elegans GSCs*. Funding: NSERC (KV) (DH)

**Program Abstract #423**

**Germ plasm localized RNAs are conserved across Danionin fish embryogenesis**

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In many animal species, germ cell specification requires the inheritance of germ plasm, a biomolecular condensate containing maternally-derived RNAs and proteins. In the model vertebrate system *Danio rerio* (zebrafish), germ plasm is organized at two primary levels: i) as homotypic ribonucleoparticle (RNP) microstructures and ii) as massive supramolecular aggregates comprised of RNPs and associated components. However, the precise composition of germ plasm components and how they influence germ cell fate remains poorly understood. To date, 13 RNAs have been reported to localize to the germ plasm masses that form in the furrows of the earliest embryonic cell divisions in zebrafish; however, studies in other model organisms have shown that while a few of these germ plasm components, such as products of the gene *nanos*, are highly conserved across animal lineages, others appear to be species-specific. Here, we aim to determine if, within a restricted phylogenetic space, RNA components of germ plasm are conserved as a core set. In order to address this question, we use the *Danio* and *Devario* genera as a model vertebrate phylogenetic system by systematically testing for the presence of known zebrafish RNAs in three other species within this clade: the orange-finned danio *D. kyathit*, the pearl danio *D. albolineatus*, and the giant danio *Devario aequipinnatus*. We find that all tested zebrafish germ plasm RNAs are also localized to the germ plasm masses in each of these Danionin species, indicating a high level of conservation of germ plasm components at this phylogenetic scale. Our data also indicate that the patterns of germ plasm RNP localization and organization across early embryonic development are conserved within this clade. Funding was provided by the Laboratory of Genetics, College of Agricultural and Life Sciences, and the School of Medicine and Public Heath at UW-Madison and NIH grants GM007133 and GM065303.

**Program Abstract #424**

**Serotonylated proteins in mammalian spermatozoa: intracellular visualization and possible functions**

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The spermatogenesis and spermatozoa activity is under the regulation of numerous external and internal factors to ensure the optimal reproductive strategy of the species. Among chemical regulators, the serotonin (5-HT) plays an important role in mammalian gametogenesis. In addition to membrane receptors-mediated mechanisms for 5-HT action, the growing shreds of evidence suggest the non-canonical intracellular action of 5-HT via transglutaminase-mediated posttranslational protein modifications (serotonylation). We visualized serotonylated proteins in rodents’ testis and spermatozoa and suggested their role in spermatozoa activation and capacitation and mature spermatozoa motility. We combined a modified click-reaction method with propargylated serotonin (5-PT) and azide-biotin or azide-Alexa488 with immunostaining and 3D confocal imaging and performed detailed visualization during rat and mice spermatogenesis. Serotonylated proteins are located in a zone of late spermatids within the testis and in the acrosome and the proximal part of the flagellum of mature isolated spermatozoa. The positive reaction occurs in the same regions after 5-PT click-reaction, immunostaining with anti-5-HT, and anti-Nε(γ-glutamyl) lysine antibodies. Cystamine (transglutaminase inhibitor) decreases the staining signal intensities
confirming the transglutaminase-dependent specificity of all labeling. Several flagellar structural proteins
demonstrate a high degree of colocalization with 5-PT and anti-5-HT labeling suggesting them as candidate targets
for serotonylation. Our finding of serotonylated proteins in specific zones of the mammalian testis and within
mature spermatozoa could underlie decreased male fertility side effects of antidepressants most of which are
serotonin-specific reuptake inhibitors, thus decrease 5-HT intracellular content. The work was supported by
Russian Science Foundation # 17-14-01353.

Program Abstract #425

Prenatal FGFR2 signaling is required for postnatal PDGFRα+ myofibroblast differentiation and function during
secondary septation

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To treat neonatal lung diseases like Bronchopulmonary Dysplasia (BPD) where alveolar septation is halted, we
must first understand the molecular/cellular networks required for proper septation. We have previously
identified FGFR2 signaling as critical for late lung development, but the exact role it plays remains uncertain.
Prenatal inhibition of FGFR2 (E16.5-E18.5) causes alveolar simplification, and inhibition during recovery after
partial pneumonectomy inhibits re-septation via inhibition of PDGFRα+ myofibroblast differentiation (Hokuto
2003)(Perl 2009). Here, we strove to understand the relationship of FGFR2 signaling and PDGFRα+ progenitor cell
differentiation during late lung development. We expressed the soluble, extracellular dominant negative FGFR
(SPCrtTA/tetOFGFRfc, SPC/FC) E16.5-E18.5 in transgenic mice with a GFP knock-in to the PDGFRα locus (PDGFRα-
GFP). By E18.5, there are significantly more PDGFRα+ cells, more PDGFRα+/ADRP+ lipofibroblasts, and less
PDGFRα+/ACTA2+ myofibroblasts compared to wildtype. dnFGFR2 epithelium is more proliferative at E18.5
compared to WT. By PN7, there is significant alveolar simplification, failure of septal tip extension, and excessive
PDGFRα+/FN1+/WNT2+ matrix/lipofibroblast differentiation. Distal epithelium exposed to dnFGFR2 E16.5-E18.5
(“dnFGFR2 Epi”) retains expression of Sox9, suggesting failure to properly mature. In mouse organoids derived
from PN7 fibroblast (FBs) and epithelium, FBs from mice exposed to dnFGFR2 E16.5-E18.5 (“dnFGFR2 FBs”)
preferentially supports AT1 cell differentiation. However, the same FBs fail to contract collagen properly,
suggesting a shift away from contractile myofibroblasts and towards alveolar niche-supporting
matrix/lipofibroblasts. We hypothesize that prenatal FGFR2 signaling E16.5-E18.5 autonomously directs
differentiation of distal PDGFRα+ mesenchymal progenitors into myofibroblasts, which are required for proper
postnatal septation. Funded by CCHMC NIH T32 HL 007752-25.

Program Abstract #426

Identification of Signaling Pathway Genes Expressed During Axolotl Taste Bud Development

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Specification and determination of vertebrate taste buds occur in the oropharyngeal endoderm during embryonic
gastrulation and neurulation. Later in development, progenitor patterning, innervation and taste cell
differentiation occur within oral tissues. Evidence in mice and zebrafish suggest that several signaling pathways,
including Notch and FGF, play a role in taste cell differentiation in embryos. The Mexican salamander, Ambystoma
mexicanum (axolotl), provides an excellent model system to study cell signaling and gene regulation mechanisms
during taste cell differentiation since taste buds develop directly in the oral epithelium rather than in papillae. In
addition, taste buds develop normally in oropharyngeal endoderm explants (OPE) isolated from early embryos.
We have used several approaches to investigate the potential role of signaling pathways in the development of
taste buds in explants and whole embryos. We performed RNA-seq analysis using de novo assembly to
investigate the differential expression of genes in oropharyngeal explants at several stages of taste bud
development. We identified expression of genes associated with Notch and FGF signaling in the explants and
found that more than 1000 unique genes were differentially expressed during taste cell differentiation stages. We
used RTqPCR to validate the expression of several of these genes. In order to begin analyzing the possible function of Notch signaling during axolotl taste bud development, we analyzed the effect of a Notch pathway inhibitor, DAPT, on the number of taste buds in developing OPEs and in whole embryos. Our preliminary expression and functional results suggest that Notch may play a role during axolotl taste cell differentiation. This research was funded by a Connecticut College Research Matters award and an NSF RCNUBE Award to Juniata College: 1248096.

Program Abstract #427
Pou4-2 function is required for mechanosensory neuron differentiation in planarians
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BRN3/POU4 transcription factors are essential for differentiation and function of neuronal subtypes in diverse organisms. In C. elegans, unc-86 controls terminal differentiation of touch receptor neurons, and in mammals, pou4f3 is required for differentiation and maintenance of hair cells. Due to their ability to regenerate any lost or damaged tissue from a population of pluripotent stem cells, the planarian Schmidtea mediterranea is an excellent model in which to study factors that direct stem cells to particular fates in vivo. Our previous work found that SOXB1-2 transcription factor activity is required for differentiation and regeneration of sensory neurons in S. mediterranea. Upon soxB1-2 inhibition, planarians exhibit seize-like movements and have impaired sensing of water flow (rheosensation). Analysis of factors downstream of soxB1-2 revealed that RNAi knockdown of pou4-2, the BRN3/POU4 homolog, disrupted normal rheosensation, suggesting it is involved mechanosensory neuron differentiation. Whole-mount in situ hybridization (WISH) analyses showed pou4-2 is expressed in a stereotypical striking mechanosensory neuron pattern, lining the head periphery, body margin and dorsal ciliated stripe. In addition, WISH for genes marking two populations of ciliated neurons in the rheosensory organ, pkd1L-2 and hmcn-1-L, were also downregulated after pou4-2 RNAi, indicating that pou4-2 is involved in a regulatory cascade downstream of soxB1-2 that leads to terminal differentiation of mechanosensory neurons. To further examine the role of POU4-2 activity in sensory neuron regeneration and function, we will perform RNA-seq following pou4-2 RNAi to uncover candidate target genes, and assess their roles in regenerating planarians. These experiments are anticipated to expand our understanding of how POU4-2 specifies sensory neuron populations. [This work was funded by NSF IOS-1350302 and NIH R01GM135657 to R.M.Z.]

Program Abstract #428
Phosphatidic acid promotes ectopic Notch signaling by affecting Sanpodo and receptor trafficking during development of the sensory organ of D. melanogaster
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Binary cell-fate decisions mediated by Notch signaling are fundamental for tissue differentiation and homeostasis. Cell-fate decisions after asymmetric cell divisions of the sensory organ precursor cell are tightly regulated by Notch and Sanpodo trafficking. Sanpodo is a transmembrane protein that complexes with the Notch receptor, which works by promoting the signal activation, but it is inhibited in Numb positive cells. Phospholipase D derived phosphatidic acid is a well-known regulator of other signaling pathways, such as EGFR, by affecting receptor trafficking. However, it is still unknown whether phosphatidic acid regulates Notch-mediated binary cell-fate decisions during asymmetric cell divisions. Here we show that increased phosphatidic acid leads to defects in cell fate, which are consistent with ectopic activation of Notch in daughter cells where it is normally inactive. Removing a copy of Notch or Sanpodo suppresses dominantly this phenotype, while null mutants of Numb or the α-subunit of Adaptor Protein complex-2, two traffic regulatory proteins, enhance it. By using an ex vivo endocytosis assay we show that phosphatidic acid promotes internalization of Notch receptor. Moreover, we show that Sanpodo localization decreases at acidic compartments. Together these data suggest that high levels of Phospholipase D derived phosphatidic acid promote ectopic Notch signaling by inhibiting Sanpodo trafficking towards degradation in acidic compartments in daughter cells that inherit Numb. This work was supported by Biomedical Neuroscience Institute, Iniciativa Científica Milenio ICM P09-015F
Program Abstract #429
Coordinated loss of cilia during *Xenopus* development
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The skin of *Xenopus* embryos contains numerous multiciliated cells (MCCs) that work to create a directed fluid flow over the epithelial surface. To generate this flow MCCs become extremely specialized, containing approximately 150 evenly-spaced motile cilia each anchored into the cell via a centriole/basal body and an extensive interconnected cytoskeletal network. It has been proposed that MCCs have the potential to lose their cilia and undergo transdifferentiation into a mucus-secreting cell. The process of transdifferentiation is still poorly characterized for simple differentiated cells, let alone for highly specialized differentiated cells with many complex structures like MCCs. By performing a time-course analysis of cilia-induced flow during development, we found that flow begins to decrease at stage 38 (ST38) until it ultimately disappears by ST48. To determine if cilia are reabsorbed or shed, we performed biochemical analysis of the media and found ciliary proteins enriched in the media during this developmental window. Interestingly, when we chemically induce ectopic deciliation at ST38, MCCs are able to restart the MCC transcriptional program and regrow their cilia. Furthermore, ectopic deciliation extends the lifespan of MCCs suggesting there is a defined timeline from the onset of ciliogenesis to cilia loss. The temporal regulation of cilia loss in MCCs is further supported by skin transplants where we placed skin from “young” embryos onto “older” embryos and found that while the older host cells lost cilia by ST47 the younger transplanted tissue (ST46) maintained cilia. Collectively, this suggests that cilia loss in MCCs is a cell-autonomous, transcriptionally defined process. We plan to use lineage tracing studies combined with scRNA Seq to determine novel mechanisms that control cilia loss during development. This work is funded by the Northwestern University T32 Training Grant in Cutaneous Biology (T32AR060710) and GM089970 from the NIH/NIGMS (BJM).

Program Abstract #430
daf-16/FOXO acts via lin-41 to block adult cell fate in *C. elegans* dauer larvae
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Many adult stem cells must maintain multipotency during long periods of quiescence to properly function. The genetic mechanisms by which this occurs can be studied in *C. elegans* dauer larvae. Dauer is a quiescent and stress-resistant stage adopted midway through larval development in response to adverse environments. By contrast, favorable environments promote continuous development. If dauers encounter a favorable environment, they exit dauer and resume development normally. The conserved DAF-16/FOXO transcription factor facilitates dauer entry, and we are interested in its role in promoting multipotency during dauer. We are specifically interested in epidermal seam cells, which are multipotent in larvae and terminally differentiate at adulthood. We find that daf-16(0) dauers precociously express the adult cell fate marker col-19::gfp, suggesting that multipotency is compromised. During continuous development, downregulation of lin-41 allows derepression of lin-29 which in turn activates col-19::gfp transcription. If daf-16 works through lin-41 and lin-29 during dauer, reduction of lin-41 should also result in col-19::gfp expression during dauer. By contrast, misexpression of lin-41 or loss of lin-29 should block col-19::gfp expression in daf-16(-) dauers. We find that RNAi knockdown of lin-41 indeed causes col-19::gfp expression during dauer. Furthermore, when downregulation of lin-41 is prevented, col-19::gfp expression is greatly reduced in daf-16(-) dauer larvae. Surprisingly, lin-29 is completely dispensable for the col-19::gfp phenotype in daf-16(0) dauers. We used RNA-seq to look more broadly at changes in gene expression in daf-16(0) vs. control dauers, and find over 2000 genes that are differentially regulated, including over 200 transcription factors that could impact col-19::gfp expression. Our findings indicate that daf-16 blocks adult cell fate via a combination of characterized and novel factors. This work is supported by NIH R15GM117568.
Program Abstract #431

**Forebrain malformations resulting from Sox10cre-mediated cell autonomous Hedgehog pathway modulation: new roles for the neural crest in CNS morphogenesis**

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Sonic Hedgehog signaling is required for morphogenesis of the forebrain and face. Shh is expressed in the prechordal plate and adjacent neural ectoderm, where its domain expands to the level of the prosencephalon, then to a parallel field of expression in facial surface ectoderm. These ectodermal fields of Shh expression activate pathway activity in the cranial neural crest (cNC) mesenchyme that forms the connective tissue of the face and head. Fitting this classic paradigm, Shh inhibition targeting activity in the prechordal plate or neuroectoderm causes the forebrain malformation holoprosencephaly (HPE), while targeting activity in the cNC mesenchyme causes midfacial hypoplasia and/or orofacial clefts. Here we examined the impact of cNC cell-autonomous Shh pathway ablation or overactivation using Sox10Cre to target Smoothened (Smo), the obligate transducer of the Shh signaling pathway. cNC-targeted Smo deletion resulted in midfacial hypoplasia and, surprisingly, full blown HPE, including a single forebrain ventricle, with absent or deficient medial ganglionic eminences and olfactory bulbs. cNC-targeted expression of activated Smo resulted in partially reciprocal outcomes, including facial overgrowth and hyperplastic medial ganglionic eminences, bilateral ventricular enlargement, and expansion between the cerebral cortices. These findings challenge the dogmatic paradigm of sequential Shh signaling in forebrain and face morphogenesis and more broadly suggest that neural crest cells play an active role in forebrain morphogenesis. This work was supported by the UW OVCRGE Fall Competition Award to RJL.

Program Abstract #432

**alx3 Controls Mediolateral Patterning in Zebrafish Frontonasal Skeleton.**

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How skeletal cells reproducibly form cartilaginous and bony elements in the vertebrate head has been intensely studied. In contrast to the mechanisms patterning the lower jaw and upper lip, relatively little is known about the patterning of the frontonasal skeleton. In zebrafish larvae, frontonasal Neural Crest Cells (fNCC) contribute to the anterior neurocranium, including the frontonasal skeletal elements: the ethmoid plate cartilage and the parasphenoid bone. Here, we characterize the frontonasal population with single-cell transcriptomics. These analyses reveal that the fNCC have a distinct identity compared with other NCC populations. Deeper clustering analysis of just the fNCC demonstrates at least three subpopulations. These subpopulations are consistent with previous fate mapping studies indicating three morphological domains in the frontonasal skeleton. We find that combinatorial expression of the alx gene family defines the three domains. To test if these genes function in frontonasal skeletal development, we mutagenized the entire alx gene family. Surprisingly, only alx3 produced mutant craniofacial phenotypes in an otherwise wild-type background. Interestingly, zebrafish heterozygous for alx3 also show frontonasal skeletal defects with low penetrance, implying that our alx3 mutant alleles are partially dominant. Time-lapse recordings indicate that alx3 function is not required for fNCC migration. However, cell morphology, proliferation, differentiation, and tracking analyses suggest that medial cells are transformed to a more lateral cell identity in the ethmoid plate cartilage of alx3 mutants. Our data support a model where alx genes function to pattern mediolateral identity in the frontonasal skeleton. This function may be conserved across vertebrates because humans with mutations in ALX genes display frontonasal mediolateral dysmorphologies like hypertelorism. NIH grants R00 DE024190-04/04S1, and the RNA Bioscience Initiative at CU-AMC.

Program Abstract #433

**Spatial and temporal changes in EMT underlie evolution of cnidarian gastrulation**

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Animals demonstrate the extreme diversity of morphogenetic processes involved in gastrulation. Among them,
cell ingression based on the process of EMT is one of the most widespread morphogenetic movements. In the embryo of reptiles, birds and most mammals, cells ingress through the primitive streak and generate mesoderm and endoderm. Cell ingression provides mesoderm internalization in many insects, skeletogenic mesenchyme formation in sea urchins. The process of cell ingression may significantly vary between species. It is known that the process of EMT is highly dynamic and the cells undergoing EMT "move through a spectrum of intermediary phases" between full epithelial and full mesenchymal states (Nieto et al., 2016). The objects of our study are cnidarians belonging to the class Hydrozoa: Clytia hemisphaerica, Rathkea octopunctata, and Obelia longissima. In all these species, gastrulation is based on the unipolar cell ingression. The endoderm is formed through mass EMT at the future oral pole of an epithelial blastula. To understand the species-specific features of cell ingression, we have characterized in detail successive cell morphology changes during EMT by electron microscopy and confocal imaging. These changes accompany bottle cell formation followed by ingression in the oral domain and cohesive migration of ingressed cells towards the aboral pole inside blastocoel. We have found that spatial and temporal differences in the dynamic of EMT (e.g. number and location of involved cells, timing of intercellular contact reduction, and order of cell shaping events) are responsible for the species-specific embryonic morphology and might underlie evolutionary changes of cnidarian gastrulation. (Supported by Federal Project of IDB RAS 0108-2019-0003).

Program Abstract #434
Intracellular serotonin as a regulator of morphogenetic movements in a gastropod mollusk
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Gastrulation is one of the basal morphogenetic events during the development of all living creatures, which possesses at least two germ layers. While increasing data demonstrate the regulatory role of certain genes and their products in establishing the body pattern during gastrulation, the impact of small molecules, such as monoamines, is still underestimated. Serotonin (5-HT) appears as early as the zygote stage and located within cytoplasm and cell nuclei in cleaved blastomeres in both vertebrates and invertebrates (Ivashkin et al., 2019). Increased 5-HT levels during the cleavage stage of Lymnaea stagnalis resulted in irreversible malformation at the gastrula stage (Ivashkin et al., 2015). We analyzed in detail the cell movements during Lymnaea gastrulation in normal conditions and under a high serotonin level. Embryos were incubated in immediate serotonin precursor (5-HTP) to increase the intracellular 5-HT level. Blastula and early gastrula stages were immunostained for 5-HT and tubulin. Cells shape and position were determined using phalloidin and nuclei staining. Results were analyzed using LCSM with 3D. Incubation with 5-HTP increased the 5-HT level within all embryonic cells. Under such conditions, we observed disorder in cell differentiation, delay in proliferation rate, changes in the number of contacts, and the shape of vegetal pole cells. Also, there was no actin-dependent contraction at the apical pole of the cells, which is usually prerequisite blastopore formation. Such 5-HT-induced changes led to abnormal movements of the cell layers during gastrulation and the subsequent formation of exogastrula. Inhibition of transglutaminase rescues such malformation. We hypothesized that intracellular 5-HT might be involved in transglutaminase-mediated peptide modification - serotonylation. The particular targets for serotonylation affecting morphogenetic movements need further investigation. The work was supported by Russian Science Foundation #17-14-01353.

Program Abstract #435
Regulated Sonic hedgehog signaling is required for patterned apical constriction and cranial neural tube closure
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The development of functional tissues requires the precise interpretation of positional information, and disruption of this information can have severe developmental consequences that result in birth defects. Failures in neural tube closure are among the most common such defects, impacting 1:1,000 pregnancies worldwide, with exencephaly—a failure to close the presumptive brain—accounting for approximately one-third of cases. Over 200 mutant mouse strains exhibit exencephaly, but the cellular mechanisms that drive cranial closure remain
opaque. Using high-resolution microscopy and quantitative image analysis methods, we identified a novel pattern of spatially regulated epithelial remodeling in the cranial neural tube, in which closure is initiated by the sustained apical constriction of a broad population of lateral cells, while midline cells remain static. To identify the positional signals that generate these patterned cell behaviors, we examined mutant alleles of Ift122 and Ttc21b, two members of the conserved intraflagellar transport A (IFT-A) complex that regulates cilia function and Shh signaling. IFT-A mutants display an early failure in cranial neural fold elevation, which is required to convert the cranial neural plate from convex to concave, resulting in highly penetrant exencephaly. These defects result from a failure of apical constriction in lateral cells that is associated with defects in apical actomyosin organization. These defects are due to a dysregulated pattern of the Shh signaling response in IFT-A mutants, as genetic activation of the Shh signaling response throughout the midbrain neuroepithelium recapitulated the exencephaly defects of IFT-A mutants. These results reveal a novel pattern of cell remodeling required for cranial neural tube closure and uncover a key source of positional information governing this pattern. This work is supported by an NIH/NINDS F32 fellowship to ERB (NS098832) and by the Howard Hughes Medical Institute.

Program Abstract #436
Tissue-level imaging and analysis reveals region-specific role of Shroom3 in regulation of actin & N-cadherin-based junctional dynamics during Neural Tube Closure
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Neural tube defects (NTDs) are serious birth defects resulting from failures of neural tube closure (NTC), a morphogenetic process that forms the central nervous system of chordates. In humans and animal models, NTDs display variable penetrance in brain and spinal regions, indicating functional differences in cell behavior between regions. Shroom3 regulates apical constriction (AC), a process by which cells reduce their apical area to induce tissue bending during NTC. Mutation of Shroom3 results in strongly penetrant brain NTDs but weaker penetrance of spinal NTDs. We have a poor understanding of the cell behavioral differences that contribute to this region-specific phenotype in Shroom3 and essentially all NTD genes. To better understand these region-specific differences, we used time-lapse confocal microscopy to image actin and N-cadherin, then used Tissue Analyzer to track thousands of wild-type and Shroom3 mutant cells and cell junctions in brains and spines of Xenopus embryos. Using this wealth of data and mathematical modeling, we make several new insights into NTC. First, that medioapical actin accumulation drives AC in both the brain and spine and that this is defective in Shroom3. Second, that accumulation of actin and clearing of N-cadherin drive junction constriction and elongation respectively, and that these behaviors are polarized based on junction orientation in the spine but not in the brain. Finally, we show that Shroom3 cells are capable of junctional actin and N-cadherin enrichment and constriction but have excess elongating junctions that likely contribute to AC failure in the brain. Overall, these findings represent a new paradigm in both understanding region-specificity of NTD defects and tissue-level analysis of vertebrate morphogenetic processes. This work was funded by NIH NICHD Ruth L. Kirschstein F32 to AB and NICHD R01 to JW.

Program Abstract #437
Analysis of the talpid2 reveals impaired osteogenesis and bone-remodeling as mechanisms for ciliopathic micrognathia
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Ciliopathies are a class of developmental disorders caused by disruptions in the structure or function of primary cilia. Oral-Facial-Digital (OFD) syndrome is a human ciliopathy that presents with craniofacial anomalies, such as micrognathia, hypoglossia, cleft lip and cleft palate. This syndrome has a high public health impact, for its treatment is only with an invasive surgery in order to treat feeding and breathing difficulties. OFD is caused by mutations in several genes that affect the primary cilia assembly, including C2 Ca(2+)-dependent domain-containing 3 (C2CD3), which is highly expressed in the bone. Our previous work identified the ta2 as a bonafide avian model for OFD, thus we sought to understand the cellular and molecular basis of ciliopathic micrognathia. microCT analysis demonstrated decreased bone deposition in ta2 embryos relative to control embryos. Molecular
analysis confirmed that expression of transcription factors necessary for skeletal development of osteogenic
genes, revealed there was a two-fold reduction in \textit{HAND2} expression, whereas \textit{DLX5} and \textit{RUNX2} were
upregulated in the ta\textsuperscript{2} mandibles. Interestingly in addition to impaired differentiation, cell proliferation of skeletal
progenitors was increased. These results suggested that loss of cilia disrupted the balance between proliferation
and differentiation in cells fated to become craniofacial skeletal elements. Finally, molecular analysis of markers
for osteoblasts and osteoclasts revealed that there was decreased bone deposition accompanied by increased
bone resorption. Taken together, our results suggest that the ciliopathic micrognathia is a consequence of both
impaired skeletal differentiation, reduced bone deposition and increased bone remodeling caused by the reduced
C2CD3-dependent ciliogenesis. Funding: National Institutes of Health (R35 DE027557 - SAB) and Shriners Hospital
(S43938 - SAB)

Program Abstract #438
The highest levels of transcription factor Dorsal dampen, not promote, gene expression by regulating enhancer
action.
Jihyun Irizarry, James McGehee
\textit{California Institute of Technology, United States}\nMorphogen concentration instructs spatial expression of target genes, but as morphogens are dynamic it is
unclear whether this relationship holds over time or if they also have additional roles. For instance, genes
expressed along the dorsal-ventral (DV) axis of the \textit{Drosophila} embryo are responsive to the concentration of
transcription factor Dorsal (Dl), homolog of Nuclear Factor kappa-B (NF \textit{kB}). However, levels of Dl steadily increase
from one nuclear cycle (nc) to the next in this syncytial embryo, and Dl is required only early but not late in nc14
to support expression of genes in ventral regions like \textit{snail} (\textit{sna}), an important regulator of patterning and
mesoderm morphogenesis. Furthermore, two enhancers control \textit{sna} gene expression, \textit{sna.prox} and \textit{sna.dis}, and
while both require Dl early input only \textit{sna.prox} requires input late to remain active. Here we studied Dl's dynamic
action as it was unclear why this factor continues to build if expression of high-threshold targets like \textit{sna}s already
supported. Using a photosensitive degron to assay Dl’s temporal role, we focused on \textit{sna} expression, specifically
the \textit{sna.prox} enhancer, using MS2-MCP live imaging. Surprisingly, our data demonstrate that Dl binding sites
within the \textit{sna.prox} enhancer function as a molecular damper to limit activity of the \textit{sna.dis} enhancer and, in this
way, downregulate \textit{sna} gene expression total output when Dl levels are maximal. Dl can act both to define the
spatial extent of target genes as well as to modulate their levels of expression, changing its role over time and
suggesting that other morphogens also are multifunctional and dynamic. This study was supported by funding
from NIH R35GM118146 to A.S.

Program Abstract #439
Timer genes that regulate segmentation in \textit{Drosophila} can also largely explain segmentation dynamics in
\textit{Nasonia vitripennis}
Shannon Taylor, Peter Dearden
\textit{Genomics Aotearoa and Department of Biochemistry, University of Otago, New Zealand}\nSegmentation is a crucial developmental process and a paradigm for understanding patterning. In \textit{Drosophila} each
body segment of the embryo is specified simultaneously by the pair rule gene regulatory network (GRN), a well-
understood process. Simultaneous patterning is unusual however, many insects pattern their trunk sequentially,
specifying each segment in a posterior segment addition zone. Recent modeling work appears to reconcile these
two types of segmentation. The \textit{Drosophila} pair rule GRN can be broken into two GRNs. The first establishes the
initial pair-rule pattern, while the second reads this pattern out. These two GRNs are activated by the timer genes
\textit{caudal}, \textit{Dichaete}, and \textit{odd-paired}. The spatial and temporal sequence of the expression of these genes is thought
to control whether an insect segments simultaneously or sequentially. We have tested these ideas in \textit{Nasonia},
which combines both simultaneous and sequential patterning. Two separate GRNs act during Nasonia
segmentation, as evidenced by 1) altered regulatory relationships, and 2) segmental expression of new genes.
Expression patterns suggesting an early pair rule network correlate with \textit{Dichaete} expression, while late network
patterns correlate with odd-paired. In the posterior, where segments appear sequentially, the spatial sequence of
these genes is characteristic of sequential segmentation. However, we were unable to obtain wild-type gene
phasings by simulating the dynamics of timer gene expression using the *Drosophila* GRN. In addition, there is evidence both from Boolean network analysis, and our gene expression patterns, that the topology of the late network has changed. Altogether, our work is consistent with the timer gene hypothesis, but more work is required to uncover the precise regulatory changes allowing unique features of *Nasonia* segmentation, and whether these changes constrain other functions of these networks. Funder: Genomics Aotearoa

**Program Abstract #440**

**From gastrulation to left-right patterning in veiled chameleon (*Chamaeleo calyptratus*).**

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Squamates represent the largest order of reptiles, with over 10,000 species. However, little is known about the earliest events in their development, since at the time of oviposition most squamate embryos are well into organogenesis. Veiled chameleon (*Chamaeleo calyptratus*) embryos are, however, pre-gastrula at oviposition, making it possible to study early morphogenetic events in this clade. Left-right (L-R) patterning in reptiles has long been an enigma. The ancestral mode of L-R patterning in deuterostomes involves motile cilia in the L-R organizer. However, avians, a sister taxon to squamates, have lost motile cilia in their embryonic organizer, and break L-R symmetry through asymmetric cell movements, tightly linked to gastrulation. Developing chameleon embryos also lack motile cilia, suggesting that the loss of motile cilia may be a synapomorphy of all reptiles. However, veiled chameleon development and L-R patterning stand apart from avians. Chameleons develop slowly, with gastrulation taking about 70 days, and several days dedicated to L-R patterning. *Shh* and *Fgf8*, markers typically expressed asymmetrically in chicken, are symmetrically expressed in chameleon. Furthermore, we find evidence for expression of both *Nodal1* and *Nodal2* in veiled chameleon, despite *Nodal1* being lost in other squamates and avians examined thus far. We also observe unique patterns of *Nodal1/2* and *Lefty* expression, suggesting potential adaptations to the shape of the embryo and the gastrulation processes. Reptile embryos are slightly domed, and unlike avian and mammalian embryos, do not form a primitive streak, but instead use a combination of involution and ingestion to form a blastopore, and go through gastrulation. Through live imaging, we are tracking these processes in chameleon embryos to understand the breaking of symmetry in context of gastrulation and evolution of L-R patterning. Supported by Stowers Institute for Medical Research and SDB Emerging Research Organisms Grant.

**Program Abstract #441**

**Discovery of genes required for body axis and limb formation by global identification of retinoic acid regulated enhancers and silencers**

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Identification of direct target genes for transcription factors is hampered by the large number of genes whose expression changes when the factor is removed and numerous binding sites in the genome. Retinoic acid (RA) receptors bound to RA directly regulate transcription through RA response elements (RAREs) of which there are thousands in the mouse genome. Here, we focused on identification of direct RA target genes in the embryonic trunk during body axis and limb formation. Here, we performed genomic ChIP-seq (H3K27ac and H3K27me3) and RNA-seq studies on E8.5 mouse embryonic trunks from wild-type and *Aldh1a2*/* mouse embryos, lacking RA synthesis, to globally identify RA target genes involved in trunk development. We define candidate targets genes whose mRNA levels are altered by genetic loss of RA that also present nearby RA-regulated epigenetic marks associated with conserved RAREs, suggesting they have important downstream functions. This approach was able to identify many previously reported RA target genes known to control embryonic trunk development (including all three known RA target genes from RARE knockout studies: *Hoxa1*, *CdX1*, and *Fgf8*), plus we identified numerous new candidate RA target genes that may control trunk development. CRISPR knockout studies on several of these new candidate RA target genes validated them as being required for body axis and/or limb formation. *Nr2f1*, *Nr2f2*, *Meis1*, and *Meis2* gene family members all have RARE enhancers, and double knockouts of each family demonstrated requirements for body axis and/or limb formation, thus validating our method for identifying RA target genes important for development. Our approach is generally applicable to determine tissue-
specific target genes for any transcriptional regulator that has a knockout available. Research reported in this publication was supported by NIAMS of the National Institutes of Health.

Program Abstract #442
BMP and WNT signaling crosstalk to promote patterning of the developing large airways of the respiratory tract.

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Tracheobronchomalacia and Complete Tracheal Rings are congenital malformations of the trachea and causes of morbidity and mortality in infants and children. We demonstrated that Wls (a cargo receptor mediating Wnt ligand secretion) is essential for the patterning of the mouse large airways. Deletion of Wls from developing trachea epithelium results in the absence of cartilage replaced by poorly organized muscle. RNA Sequencing studies revealed effects of Wls on gene expression of BMP signaling molecules. We hypothesize that BMP signaling promotes tracheal cartilage formation downstream of WNT signaling. Results: Epithelial deletion of Wls diminishes BMP signaling in ventral tracheal mesenchyme, as determined by p-Smad1/5/8 staining. Deletion of Bmp4 from tracheal mesenchyme causes phenotypes ranging from tracheal atresia to poorly developed trachea. The latter is characterized by a lack of cartilage and ectopic muscle in the ventral side, recapitulating the phenotype observed after epithelial deletion of Wls. Ectopic and increased number of muscle cells is partially caused by differential cell proliferation at expenses of cartilage progenitors. While Bmp4 deletion decreases Col2a1 in vivo, Bmp2 and non-canonical Wnt5a promote expression of Sox9 and Col2a1 in vitro. Mesenchymal deletion of Bmp4 in developing mouse trachea, caused downregulation and abnormal localization of Wnt/β-catenin target genes and attenuators Notum and Axin2. Both genes were induced in vitro by Bmp2 and canonical Wnt3a. Further, inhibition of BMP signaling by DMH1 prevented Wnt3a-mediated induction of Notum and Axin2. Bmp2 induces the activation of mouse and human Notum promoters. Conclusions: Bmp4 is a target of epithelial-induced WNT signaling that promotes cartilage formation and prevents ectopic muscle in developing trachea. By promoting expression of genes required to attenuate Wnt/β-catenin activity, Bmp4 mediates tracheal patterning. Partially supported by NIH-NHLBI R01HL144774 to DS.

Program Abstract #443
Early gestational alcohol exposure disrupts key gene pathways during embryonic development

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Multiple factors influence the severity of prenatal alcohol-related birth defects in humans and animal models of Fetal Alcohol Spectrum Disorders (FASD). Our lab uses both transgenic mice with single gene mutations and RNA sequencing of closely-related mouse strains to probe the contribution of genetics. Mice with mutations in apoptosis genes Bax and p53 are protected against alcohol, while mice with mutations in Shh pathway or cilia genes show increased rates of alcohol-induced defects. RNA-seq was conducted on embryonic tissue from two sub-strains of mice: 1) C57BL/6J mice with a known Nnt mutation that are highly sensitive to alcohol, and 2) C57BL/6NJ mice that are more resistant. Strain differences were assessed in embryos before and 6 or 12 hr after alcohol exposure during gastrulation (E7 in mice, 2nd – 3rd week in human pregnancy). Before alcohol, 80 genes differed between the strains that primarily were related to inflammation. After alcohol, prenatal alcohol was more likely to upregulate cell death pathways and the cell’s response to stress and hypoxia in the 6J’s vs. 6N’s. Cell proliferation, morphogenic, and holoprosencephaloy pathways were downregulated in the 6J’s, contributing to the increased likelihood of craniofacial defects in this strain. In the 6N’s, inflammation pathways were more likely to be upregulated. Finally, a subset of primary cilia-related genes was differentially regulated between the strains at baseline and after alcohol; prenatal alcohol and certain genetic ciliopathies share some phenotypic and mechanistic overlaps. These data demonstrate that genetic variation can contribute significantly
Program Abstract #444

*Caenorhabditis elegans* PIEZO Channel Coordinates Multiple Reproductive Tissues to Govern Ovulation

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PIEZO1/2 are newly identified excitatory mechanosensitive proteins; they are non-selective ion channels that exhibit a preference for calcium in response to mechanical stimuli. Dysfunction of PIEZOs cause a variety of genetic diseases, including the dysplasia in cardiovascular, respiration, and connective tissues. However, the cellular and molecular mechanisms of PIEZOs in these diseases are less understood. To further understand the function of these proteins, we investigated the roles of *pezo-1*, the sole PIEZO ortholog in *C. elegans*. *pezo-1* is expressed throughout development in *C. elegans*, with strong expression in reproductive tissues. A number of deletion alleles as well as a putative gain-of-function mutant caused severe defects in reproduction. A reduced brood size was observed in the strains depleted of PEZO-1. *In vivo* observations show that oocytes undergo a variety of transit defects as they enter and exit the spermatheca during ovulation. Post ovulation oocytes were frequently damaged during spermathecal contraction. Given that PIEZO is an ion channel and may regulate spermathecal contractility through Ca\(^{2+}\) signaling pathways, we tested the genetic interactions between *pezo-1* mutants and several cytosolic Ca\(^{2+}\) regulators with RNA interference (RNAi). Indeed, *pezo-1* mutants are affected upon depletion of known cytosolic Ca\(^{2+}\) regulators. Lastly, loss of PEZO-1 revealed an inability of self-sperm to properly navigate back to the spermatheca after being pushed out of the spermatheca during ovulation. Mating with males rescued these reproductive deficiencies in our *pezo-1* mutants. Reduced brood sizes were observed in each auxin-inducible tissue-specific degradation strain, suggesting PEZO-1 may act in different reproductive tissues to coordinate the reproduction. These findings suggest that PEZO-1 acts in different reproductive tissues to promote proper ovulation and fertilization in *C. elegans*. This work was supported by the NIH Intramural Research Program.

Program Abstract #445

*Cdon* mutation and fetal alcohol converge on Nodal signaling in a gene-environment interaction model of holoprosencephaly

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Many birth defects are caused by interactions between genetic and environmental risk factors, but the mechanisms of these interactions are largely unknown. Holoprosencephaly (HPE), a failure to define the midline of the forebrain and midface, arises in ~1 in 250 conceptions. It is associated with predisposing mutations in the Nodal and Hedgehog (HH) pathways, but these mutations alone are not sufficient to cause HPE. Penetrance and expressivity of HPE are graded by genetic and environmental modifiers that work via poorly understood mechanisms. Alcohol is one of most commonly abused substances in the world. Fetal alcohol exposure is implicated in many human birth defects, including HPE. CDON is a multifunctional co-receptor, including for the HH pathway. In mice, *Cdon* mutation synergizes with fetal alcohol exposure to produce HPE phenotypes closely resembling those seen in humans. We report here that, unexpectedly, Nodal, not HH, signaling is the point of synergistic interaction between mutation of *Cdon* and fetal alcohol exposure. A combination of window-of-sensitivity, genetic, and in vitro findings are consistent with a model whereby CDON and another auxiliary receptor, LRP2, function to regulate the Nodal pathway, with consequent effects on downstream HH signaling during midline patterning. Brief exposure of *Cdon* mutant embryos to ethanol during this period transiently and partially inhibits Nodal pathway activity. These results illuminate mechanisms of gene-environment interaction in a multifactorial model of a common birth defect. This project was supported by NIH grants from the National Institute of Dental and Craniofacial Research and National Institute on Alcohol Abuse and Alcoholism.
Program Abstract #446
Top autism risk genes and estrogen signaling converge during forebrain neurogenesis
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Genetic studies have identified many genes carrying large risk for autism spectrum disorders. Pleiotropy complicates the evaluation of any individual gene’s contribution to pathobiology. Instead, identifying functional and spatiotemporal convergence among many disparate risk genes constrains the pursuit of etiological mechanisms. Here we leveraged unilateral mutagenesis in Xenopus tropicalis to identify points of in vivo convergence among the top ten high-confidence autism risk genes. For all ten genes, regardless of functional annotation, perturbation led to a change in forebrain size. All ten genes were co-expressed during forebrain neurogenesis at timepoints mapping to human mid-prenatal development. Finally, we identified estrogen as a suppressor of the convergent phenotype and elaborated a novel role in opposing neurogenic Sonic hedgehog signaling in Xenopus and human neurons. This work has important implications for understanding the pathobiology underlying autism and for the strong male bias. Funding: NIMH 1R21MH112158-01 and 1U01MH115747-01A1; NIGMS: R35GM127069.

Program Abstract #447
Semaphorin3f in the post-mitotic regulation of outer retinal health in zebrafish
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Interdependency between the retinal pigment epithelium (RPE) and photoreceptors (PRs) is critical for visual function. PR death results in progressive vision loss in retinal degenerative diseases such as age-related macular degeneration. Understanding the molecular signals between the RPE and PRs will elucidate how retinal health and function is regulated. Here we explore the role of Sema3fa in the post-development zebrafish retina using CRISPR/Cas9-generated loss-of-function mutants (sema3faca304). Our data indicate that Sema3fa loss does not influence the initial stages of RPE maturation, as the expression of maturation markers involved in the visual cycle, rpe65a and rgrb, are indistinguishable between genotypes by 7 days post-fertilization (dpf). However, RPE cells of sema3faca304 mutants displayed less uniformity in size and distribution in the absence of Sema3fa. We next assessed whether the loss of Sema3fa influences the physiological response of RPE cells in addition to morphology: specifically, its retinomotor response to light. We found that 8 dpf sema3faca304 mutants exhibit a stunted retinomotor response relative to the wild type siblings. Given the critical role of the RPE in PR maturation and maintenance, the impact on PRs was assessed. Gross observation of the outer nuclear layer showed disorganization of nuclei in the sema3faca304 mutants in comparison to the wild type siblings at 7 dpf. To further assess specific subtypes of PRs, we analyzed Zpr1-labeled red/green double cones and found that the loss of Sema3fa influences the cellular morphology and synaptic terminals. Current results suggest a possible regulatory function of Sema3fa in the outer retina as it maintains RPE morphology and physiology, which in turn could be critical in the maintenance of PR health and function. This project was funded by Canadian Institutes for Health Research (CIHR), the Brightfocus Foundation, and a studentship provided by Hotchkiss Brain Institute.

Program Abstract #449
Cdx factors play a critical role in establishing trunk neural crest identity and behavior
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The neural crest (NC) is a multipotent cell population that migrates extensively to produce a remarkable array of cell types including chondrocytes, melanocytes, and neurons. Substantial evidence suggests that NC cells that originate in the head and the trunk exhibit important differences in cellular behaviors, differentiation potential, and their underlying transcriptional network. Cranial NC cells migrate in broad streams and dynamically rearrange without regard to their initial position, whereas trunk NC cells form segmental, single cell chains and depend on a leader cell for directionality. However, the mechanism by which the NC is patterned along the AP axis remains a
long-standing question in the field. Skromne and colleagues (2007) demonstrated that zebrafish cdx1a and cdx4 pattern the neuroepithelium along the AP axis by repressing the hindbrain developmental program and promoting spinal cord identity. Therefore, we have evaluated the role of Cdx proteins in regionalizing the NC. Here, we demonstrate that cdx4 is expressed in pre-migratory NC cells, where it is necessary for establishing trunk-specific migratory behaviors. In cdx4 mutants, the segmental patterns of trunk NC cell migration are disrupted and cells fail to reach their ventral destinations. Further, live-imaging approaches reveal that this disruption is likely due to the loss of leader/follower cell dynamics. We also demonstrate that the defects in cell migration lead to mis-patterning of the dorsal root ganglia, which fail to form re-iterated segmental structures. Finally, we utilize cell transplantation approaches to demonstrate that the defects in NC cell migration following loss of Cdx4 function are not due to its absence from the cells that make up the somites. Therefore, we conclude that cdx4 plays a critical, and likely tissue autonomous, role in the establishment of trunk NC fate and its characteristic cellular behaviors during migration. Funding: NIH F31HD097957, T32HD05516.

Program Abstract #450
In vivo imaging reveals proliferative wave front of enteric neural crest drives migration and colonization within the zebrafish enteric nervous system
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The enteric nervous system (ENS) consists of a series of interconnected ganglia that form nerve plexuses spanning circumferentially within the muscle walls of the gastrointestinal (GI) tract. The ENS is derived from migratory enteric neural crest cells (ENCCs) that migrate caudally in chains along the gut tube. Failure of ENCCs to properly colonize the gut and differentiate leads to the pediatric defect Hirschsprung disease, in which the GI lacks enteric neurons along variable lengths. While previous research has made progress in identifying genetic factors that mediate ENCC migration, less attention has been dedicated to understanding the cellular strategies that ENCCs utilize to colonize the gut and subsequently differentiate into enteric neurons. Using the vertebrate model zebrafish, we leveraged transgenic reporters, in vivo time-lapse confocal microscopy and quantitative image-based analyses to define the cellular mechanisms that ENCCs utilize in order to form the nascent ENS. We observe dynamic cellular behaviors between ENCCs, depending upon their location along the length of the gut. We discovered that the migratory wave front of ENCCs within the hindgut exploit proliferation as a mechanism of caudal colonization. We noted that newly generated ENCC daughter cells demonstrate higher levels of spatial displacement and migrate further than their originating parent cell. Additional analysis using immunohistochemistry suggests that the migratory wave front cells in the hindgut have yet to undergo neuronal differentiation, and that it is these cells which continue migrating, while rostrally-localized differentiating enteric neurons cease to migrate and begin to spatially pattern, forming the enteric neural network. Taken together, these lineage-tracing results suggest that undifferentiated, proliferative ENCC progenitors are responsible for hindgut colonization, thereby illuminating new mechanisms by which ENS is created. Funding by CPRIT RR170062

Program Abstract #451
It takes a village to build a brain: Defining the heterogeneous glial and neural crest contributions to zebrafish forebrain development and neurogenesis
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We challenge the canonical model of zebrafish forebrain development and propose that cranial neural crest cells (NCCs) break convention by re-entering the central nervous system (CNS). In addition to characterizing the glial cells which construct the forebrain, we demonstrate that NCCs are a required cell type for normal forebrain development. We exploit the conserved process of vertebrate embryonic development in the zebrafish model system and focus on the critical stage during which neurons send axons across the midline at specific points to connect the two halves of the nervous system with a commissure. Little is known about the cells at the midline that facilitate this tightly regulated process. This investigation has revealed that three different astroglial cell morphologies persist in contact with POC axons throughout commissure formation while olig2+ progenitor cells
occupy delineated portions of the postoptic and anterior commissures. Excitingly, through use of a \textit{tfap2a/tfap2b} LOF NCC mutant, used to interrogate the role that craniofacial development plays in forebrain development, we discovered a novel requirement for neural crest cells in zebrafish forebrain development where their appropriate migration into the forebrain is required appropriate commissure spacing and axon guidance. Subsequent investigations of NCC requirement and forebrain contributions utilizing lineage tracing techniques and live cell tracking have shown that NCCs break convention by re-entering the CNS. These same NCCs then contribute to forebrain neuronal populations. Ultimately, by unraveling the coordinated efforts of migrating neural crest, neural progenitors, and glial cells that construct the relatively simple zebrafish forebrain, we can provide insight into potentially conserved mechanisms underlying brain development and disease. Funding Source: NSF award 1656310; NIH award 2R15HD060023-02A1.

Program Abstract #452

\textbf{Embryonic hyperglycemia causes a reduction in photoreceptor cells and increased oxidative stress in the developing retina}

\textbf{Kayla Titialii}
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Chronic hyperglycemia has been linked to long-term metabolic disruptions in adults, such as tissue damage in the eye. Yet, little is known about the effects of embryonic hyperglycemia on retinal development. To address this question, we investigated retinal development in both a genetic and nutritional model of embryonic hyperglycemia. The genetic model is \textit{pdx1-/-} mutant zebrafish (which does not produce insulin and is hyperglycemic). For the nutritional model we induced hyperglycemia by exposing zebrafish embryos to 50mM glucose +/- 10µM dexamethasone (G+D) from 10 to 120 hours post fertilization (hpf). At 120 hpf, larval heads were fixed, and bodies were used to quantify glucose concentration as well as genotype for \textit{pdx1}. Retinal sectioning and immunohistochemistry was performed to label and quantify cell types in the retina. Both \textit{pdx1-/-} mutants and G+D treated larvae were consistently hyperglycemic, with >2-fold increased whole-body glucose concentration compared to control-treated or wildtype larvae. In both the genetic and nutritional models of hyperglycemia, we observed a significant reduction in rod and cone photoreceptors, with the remaining displaying abnormal morphology. We also observed aberrant Müller glia morphology and increase in reactive oxygen species (ROS) in hyperglycemic larvae. Further metabolic analysis revealed abnormal expression of various enzymes related to inflammation and ROS production. In the nutritional model, the hyaloid vasculature was widened and displayed an increase in endothelial nuclei. Our results demonstrate that embryonic hyperglycemia results in abnormal photoreceptor development, perhaps via ROS production, which is accompanied by aberrant Müller glia and hyaloid vasculature development. Our work provides insight to the effects of hyperglycemia on retinal development that may have important implications for the growing number of pregnant women with diabetes. Funding: Gertrude F. Ribble Mini-Grant, University of Kentucky

Program Abstract #453

\textbf{Photoreceptor Cell Development and Survival Require Siah E3 ubiquitin ligase regulation in CDHR1a Protein Stability During Zebrafish Retina Development}

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Ubiquitin-proteasome systems (UPS) regulate orderly and precise targeting of protein degradation to maintain proper development. Recently, our lab discovered that Siah E3 ubiquitin ligases plays a role in the fusion of the optic fissure. In this follow up study, we examine a role for siah during photoreceptor development via its predicted target \textit{Cdrh1a}, a photoreceptor-specific cadherin which has been associated with inherited retinal dystrophies, such as cone-rod dystrophy. Using whole-mount \textit{in situ} hybridization and immunohistochemistry we detected colocalization of \textit{siah} and \textit{cdrh1a} gene expression and protein in the outer nuclear layer, more specifically, in the connecting cilium of rods and cones, from 3 and up to 7 dpf. We therefore hypothesized that Siah regulates \textit{Cdrh1a} during photoreceptor development. To test, we first confirmed siah-mediated targeting of \textit{Cdrh1a} for degradation using co-immunoprecipitation in cell culture. Second, we created two transgenic zebrafish lines that express Siah1 or an inactive siah1 (Siah1DRING) under the control of the heat shock promoter. Using
heat shock, we over activated Siah1 at 48 and 60 hpf, and subsequently observed a decrease in the number of rods and cones at 72 hpf. The number of retinal ganglion cells, amacrine, bipolar and horizontal cells, however, did not change. In addition, there was a significant increase in TUNEL-positive cells in the retina. Moreover, a proteasome inhibitor, MG132, as well as cdhr1a mRNA injections were able to rescue the wildtype number of rods and decrease the number of dying cells in heat shocked embryos. Lastly, injections of a cdhr1a-Siah insensitive mRNA construct, with a mutation (Lma) in the siah1 recognition motif, rescues the phenotypes more efficiently. Taken together, our results suggest that Siah ubiquitin ligases may control Cdhr1a stability and therefore regulate photoreceptor cell development and survival. This work was supported by CNPq under grant number 202970/2014-0.

Program Abstract #454
Molecular mechanisms that consolidate temporal identity in developing Drosophila motorneurons.
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The complex function of the nervous system is dependent on precise connections between hundreds of thousands of diverse neurons. During development, a small pool of neural progenitors is tasked with quickly generating this diverse set of molecularly and morphologically distinct neuronal subtypes. These neurons are then required to navigate a complex environment to locate the appropriate synaptic partners, and establish the circuitry required for behaviour. For this reason, identifying the mechanisms used by neural progenitors to generate the correct neural subtypes is critical to understanding circuit formation, and behavior itself. During Drosophila development, each neural progenitor cell, or neuroblast (NB), generates a characteristic set of diverse neuronal progeny over time. This is accomplished through the process of temporal patterning, in which each NB sequentially expresses a cascade of temporal transcription factors (tTFs), giving rise to molecularly distinct neuronal progeny in each expression window. These tTFs are only transiently expressed; little is known about their downstream effectors and how they specify and maintain the unique molecular and morphological properties of each neuronal subtype throughout larval life. Our central hypothesis, is that each tTF induces or represses a combinatorial set of downstream identity transcription factors (iTFs), which in turn drive the expression of mature neuronal genes such as those encoding neurotransmitter machinery, ion channels, cell-surface protein expression and higher-order morphological features. Investigating the downstream targets of tTFs in a distinct embryonic lineage through single-cell sequencing will resolve this gap in understanding. For this reason, we have developed a pipeline to profile individual Drosophila NB lineages using single-cell RNA sequencing. This work is made possible by funding from the NIH and HHMI.

Program Abstract #455
A multi-layered and dynamic apical extracellular matrix shapes the vulva lumen in Caenorhabditis elegans
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During tubulogenesis, epithelial or endothelial cells secrete various apical extracellular matrix (aECM) factors into the nascent lumen. There is now ample evidence that such aECM factors impact lumen shape and that their loss or dysfunction can contribute to tube collapse or leakage in human disease. However, these sugary matrices are usually hard to visualize in vivo, so their structures remain poorly understood. The C. elegans vulva has been a paradigm for understanding many aspects of tubulogenesis. Here we describe the vulva luminal matrix at a level of detail unprecedented in any other system. This matrix contains proteins similar to those found in many mammalian ECMs. We visualized fluorescently-tagged aECM proteins by confocal microscopy in live animals over the course of tube morphogenesis. We used transmission electron microscopy of specimens preserved by high pressure freezing to generate detailed views of cell and matrix ultrastructure that correlated well with the patterns seen by light microscopy. Finally, we used genetic approaches to alter cell fates or remove specific aECM components and tested effects on aECM structure and cell and tube shape. Our results reveal a beautifully complex and dynamic aECM that contains both gel-like and fibrillar elements. Different combinations of matrix
factors assemble over the 7 different vulva cell types. Our results link the extensive literature on Ras- and Notch-signaling and cell type specification in this system to unique cell biology that corresponds to each fate. Finally, we provide evidence that chondroitin and other aECM factors have both lumen expanding and lumen constraining roles, arguing against a simple "sponge" model for lumen inflation, and suggesting instead that a complex matrix scaffold assembles (and disassembles) within the lumen to determine its changing shape. This work was funded by NIH grants: R01GM58540, R01GM125959, T32 GM008216, T32 AR007465, OD010943, and ACS grant RSG-12-149-01-DDC.

Program Abstract #456
Dynamics of primitive streak regression controls the fate of neuro-mesodermal progenitors in the chicken embryo
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In classical descriptions of vertebrate development, the segregation of the three embryonic germ layers is completed by the end of gastrulation. Body formation then proceeds in a head to tail fashion by progressive deposition of lineage committed progenitors during regression of the Primitive Streak (PS) and tail bud (Pasteels, 1937b; Stern, 2004). Identification of Neuro-Mesodermal Progenitors (NMPs) contributing to both musculo-skeletal precursors (paraxial mesoderm) and spinal cord during axis formation by retrospective clonal analysis challenged these notions (Henrique et al., 2015; Tzouanacou et al., 2009). However, in amniotes such as mouse and chicken, the precise identity and localization of these cells has remained unclear despite a wealth of fate mapping analyses of the PS region. Here, we use lineage tracing in the chicken embryo to show that single cells located in the SOX2/T positive anterior PS region contribute to both neural and mesodermal lineages in the trunk and tail, but only express this bipotential fate with some delay. We demonstrate that posterior to anterior gradients of convergence speed and ingestion along the PS gradually lead to exhaustion of all mesodermal precursor territories except for NMPs where limited ingestion and increased proliferation maintain and amplify this pool of axial progenitors. As a result, most of the remaining mesodermal precursors from the PS in the tail bud are bipotential NMPs. Together, our results provide a novel understanding of the contribution of the PS and tail bud to the formation of the body of amniote embryos. Research in the Pourquié lab was funded by a grant from the National Institute of Health (RO1HD097068-02) and EMBO ALTF 406-2015 to C.G

Program Abstract #457
Cellular and Molecular Mechanisms of Sensory Organ Segregation in The Embryonic Inner Ear
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The inner ear contains multiple sensory organs separated by non-sensory epithelial domains. These organs are specified during early otic development and become gradually individualised by segregation from a common Sox2-expressing pan-sensory domain. Some of the genes and signalling pathways essential for sensory organ specification and segregation have been identified. However, the basic cellular mechanisms of segregation are still unexplored. In this study, we analysed the changes in cell fate and cell morphology during the segregation of the lateral and anterior cristae in the embryonic chick otocyst. We found that their segregation is coupled to striking changes in cell morphology. The cells at the interface of the prospective cristae and the pan-sensory domain enlarge, become elongated and progressively align before down-regulating Sox2 expression. Multicellular rosettes and phosphorylated myosin regulatory light chain staining are present throughout the otocyst, suggesting that cell rearrangements mediated by acto-myosin contractility could contribute to sensory organ segregation. We are now testing this hypothesis using genetic and pharmacological manipulations of actomyosin contractility. We have also developed a new organotypic culture method that will enable us to study in real-time the dynamics of sensory organ specification and segregation in the chick and mouse inner ear using time-lapse imaging. Funding body: BBSRC, BSDB The Company of Biologists
Program Abstract #458
Morphogenesis during the asexual reproduction in the class Scyphozoa (Cnidaria)
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There is a high abundance and diversity of the modes of asexual reproduction among the representatives of the class Scyphozoa. During some modes of asexual reproduction, the morphogenesis of main body parts goes independently of the body parts of the parent organism. During others — parental body parts are inherited by the offspring or transformed into body parts of the offspring. We summarized the data on the asexual reproduction in Scyphozoa, gained from literature and from our research on Aurelia (Semaeostomeae) and Cassiopea (Rhizostomeae). The relative orientation of the parental and offspring body axes, as we found out, varies significantly and the same body parts of the offspring can be formed from different parts of the parental organism. There are two main life forms in this group: sessile polyps and free-swimming medusae. The polyp-to-medusa transition, strobilation, goes through terminal budding, when already differentiated cells of polyp are used to build the medusa body parts. The tentacle bases expand to form the lobes of the first medusa, the polyp manubrium becomes the manubrium of the forming medusa. Our data confirms the homology between some tentacles of the polyp and the sensory organs (rhopalia) of the oralmost medusa. The manubrium, lobes and rhopalia in all subsequent medusae (in the case of polydisc strobilation) form independently from the structures of the parental polyp. Each disc of the future medusa is able to complete the development independently of the strobila. The polyp septa take part in determining the location and in the formation of the medusae structures. Remnants of septa are involved in the formation of gastric filaments of the medusae. The high plasticity of scyphozoan developmental processes can be explained by the assumption that in this group various types of asexual reproduction evolved from regeneration processes. The work was supported with RFBR Grant no. 19-04-01131.

Program Abstract #459
Studying cell sorting during Drosophila abdominal morphogenesis
Sotiroula Chatzimatthaiou, Marcus Bischoff
University of St Andrews, GB

A fundamental developmental question is how cells adopt distinct positions in greatly complex but precisely organized tissues. There have been great advances in our understanding of how different fates of cells are specified during development. How fate information determines the positioning of cells, however, is still elusive. During the formation of the Drosophila adult abdominal epidermis, diploid histoblasts replace the larval epithelial cells and eventually adopt different fates along the anterior-posterior (a-p) axis. Changing the histoblasts’ fates in clones of cells leads to cell sorting, where clonal cells position themselves according to their new fate (Lawrence et al., 1999). We combined Drosophila genetics to produce genetic mosaics and in vivo 4D microscopy to study histoblasts sorting in vivo. In a candidate screen, we have identified several candidates that potentially might be involved in the positioning of histoblasts in different regions of the abdomen along the a-p axis. Supported by: Wellcome Trust ISSF

Program Abstract #460
Stromally expressed β-catenin modulates pathways and genes that regulate key developmental processes during kidney development
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Kidney development is regulated by cellular interactions between the ureteric epithelium, mesenchyme, and stroma. Stromally-expressed β-catenin regulates gene expression in the ureteric epithelium and mesenchyme. How stromal β-catenin communicates with these cells is unknown. We hypothesize that stromal β-catenin modulates pathways and genes to mediate these cellular communications. We generated mouse models with stroma β-catenin deficiency (β-catsd) or overexpression (β-catso). Wild type, β-catsd and β-catso stroma cells were isolated at E12.5 and E13.5 by FACS. We verified stroma cell isolation by quantitative RT-PCR, then performed
RNA-Seq. A gene ontology network-based analysis demonstrated 71 biological processes were up-regulated in β-catenin and down-regulated in β-catenin at both E12.5 and E13.5. Of particular interest were eight processes that mapped to Wnt signaling, sixteen that mapped to branching morphogenesis, five that mapped to nephrogenesis, and fifteen to vascular development. Genes and biological processes identified by the RNA-seq were manually searched for candidate genes of interest related to these four main categories. This process identified genes involved in β-catenin/Wnt signaling (Wnts, Tcf, Axin), confirming disrupted β-catenin signaling in renal stroma cells. We also identified genes that regulate branching morphogenesis and nephrogenesis (Gdnf, Bmp, Fgf) and novel targets such as Thy1. Further, these results identified secreted guidance cues involved in kidney vascular development (Angpt1, VEGF, Sema3a). Select genes (Lef1, Axin2, Thy1) disrupted in the RNA-Seq analysis were validated by immunostaining, which demonstrated that the mRNA transcript levels correlated with expression at the protein level. In conclusion, our results support an essential role for β-catenin in the renal stroma by modulating key biological processes including morphogenesis, nephrogenesis, and vascular development. Funding: NSERC, CIHR, KFOC

**Program Abstract #461**

**Imaging cytoskeletal dynamics that regulate fate specification and morphogenesis to pattern the early mouse embryo**

Hui Yi Grace Lim, Yanina D Alvarez, Maxime Gasnier, Stephanie Bissiere, Nicolas Plachta

*Agency for Science, Technology and Research, SG*

How a single-celled zygote develops into a complex multicellular organism with specialized functions remains a central question in developmental biology. During the earliest stages of development, the early mouse embryo forms a blastocyst, comprising an outer trophectoderm layer surrounding an inner cell mass and a fluid-filled cavity. Trophectoderm cells later form the placenta, whereas the inner cell mass generates the fetus along with other extraembryonic tissues; however, the precise mechanisms governing the fate specification and morphogenesis events leading to blastocyst formation remain unclear. Here, we utilized advanced imaging technologies, computational image segmentation, and quantitative analysis methods to understand how the dynamic cytoskeletal networks of the embryo regulate early development. Imaging living embryos expressing markers for actin, microtubules, and intermediate filaments revealed important dynamic behaviors during the asymmetric cell divisions positioning the first inner cells of the embryo, establishing the first differences in cytoskeletal organization between cells of the early embryo. The cytoskeletal networks in inner and outer cells in turn trigger downstream processes to differentially activate fate specification pathways and promote blastocyst morphogenesis. Thus, the cytoskeleton is a dynamic regulator of cell fate and morphogenesis in early mammalian development. This study is supported by grants from A*STAR, EMBO and HHMI.

**Program Abstract #462**

**Δ9-tetrahydrocannabinol (THC) inhibits Hedgehog pathway signaling and promotes holoprosencephaly and neural tube patterning defects in Cdon mutant mice**

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The Hedgehog (Hh) signaling pathway regulates many aspects of CNS development, including patterning of the midline of the forebrain, neuronal differentiation, and axon guidance. Loss of function in the Hh coreceptor, Cdon, leads to holoprosencephaly (HPE) with strain-dependent severity. HPE is the most common developmental defect of the forebrain and midface, and Hh inhibitors are potential HPE teratogens. It has been reported that endocannabinoids are conserved inhibitors of the Hh pathway and THC (the major psychotropic component in marijuana) is a potent cannabinoid. However, the mechanism of cannabinoid inhibition of Hh signaling remains unclear. We report that THC-exposed Cdon−/− embryos display HPE phenotypes. Additionally, Shh-dependent ventral neural tube patterning is disrupted by THC in Cdon−/− mice. Furthermore, induction of Shh target genes expression by Shh or SAG is inhibited by THC treatment. THC suppresses Gli1 expression in Ptch1−/− MEFS, but not Sufu−/− MEFS, suggesting that THC modulates Shh signaling at the level of Smo or primary cilia. We found that
THC blocks Shh-induced Smo ciliary localization, without obviously altering cilia themselves. Interestingly, cannabidiol (CBD) also diminishes Shh signaling, but without dramatically altering Smo translocation in primary cilia. THC is a competitor of Bodipy-cyclopamine binding to Smo, whereas CBD is not. Collectively, these data reveal THC is likely a direct inhibitor of Smo, whereas the mechanism by which CBD inhibits pathway activity may be distinct. THC is an agonist of CB1, which is mainly expressed in the CNS. However, CB1 is not expressed in the developing mouse ventral neural tube. Therefore, CB1 is unnecessary for THC’s ability to inhibit Hh pathway activity. Taken together, marijuana use in early pregnancy may be a risk factor for HPE, with THC acting as a direct inhibitor of Smo, a key component in the developmentally essential Hh pathway. This project is supported by NIDA/NIH grants.

Program Abstract #463
Autodecapitation linked to regulative trunk development after C quadrant ablation in neogastropod embryos
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AC Clement’s meticulous cell ablation experiments laid ground for a tidy and comprehensive model of early fate specification in embryos of the neogastropod Ilyanassa (now Tritia) obsoleta. This system comes as close as any to the ideal of ‘mosaic’ development: as later confirmed by JA Render’s clonal fate mapping, early-born ectodermal regional founder cells follow their fates almost without regard to experimental perturbation. It seems that precisely oriented asymmetric cell divisions and short-range signaling act interdependently to restrict developmental pathways at early stages. In a 1956 paper Clement reported the isolation of half- and quarter-embryos at 2- and 4-cell stages, with results deviating only slightly from the mosaic model. The same article mentions another set of experiments in which three-quarter embryos were isolated by killing one quadrant-founder cell at the four-cell stage, but no report of this work was ever published. Repeating these lost experiments, I found that most three-quarter embryos (ABD, ACD, and BCD) develop as predicted by the fates of remaining cells, but a large minority do not. Instead, early on day three these pinch off and dissociate a small fragment, which goes on to form one or two eyes, a few velar cilia, and some internal tissue including muscle, while the larger fragment consistently forms a headless larva with near perfect shell and foot, a complete gut and a beating larval heart. This perfect trunk development is especially amazing in the case of partial embryos lacking the C quadrant, because ablation of just the 2c cell invariably ruins the development of both the shell and the right side of the foot. In all the self-fragmented partial embryos, the tissue conspicuously absent is the velar epithelium bearing compound cilia and purple pigment. Could the velar defect be linked to the head’s unprecedented ejection, and also to the trunk’s unexpected perfection? Thanks to funding from the GSW Foundation, we may find out.

Program Abstract #464
Spatiotemporal patterning of ABCB, ABCC, and ABCG small molecule transporters during development: A framework for signaling and protective functions
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A core feature of multicellularity is the precisely regulated movement of small molecules between cells. Regulation of small molecule movement is central to signaling, metabolic homeostasis, and protection from the environment. Small molecule transporters (SMTs) are important for the detoxification of somatic cells, but less is known about their roles in the development or protection of embryonic cells. Here I report a map of ABC transporter activity that emerges during embryogenesis of the sea urchin. Orthologs of six major drug transporters (ABCB1, -B4, -C1, -C4, -C5, and -G2) demonstrate three expression patterns in the embryo: 1) Protective and homeostatic genes ABCB1 and ABCC1 are first expressed ubiquitously, and then become enriched in the gut. 2) ABCB4 and ABCG2 are expressed exclusively in endoderm-fated cells. 3) ABCC4 and ABCC5—known to transport signaling molecules—are restricted to a ring of mesoderm in the early blastula; ABCC4 is later expressed in the embryonic niche of the primordial germ cells. Fluorescent substrates and inhibitors were used to assay the functional outputs of transporter expression in-vivo. ABCB4 and ABCG2 efflux activity is present in nascent gut tissue days before larval feeding. In contrast, ABCC4-specific substrates are significantly effluxed out.
of the ring of ABCC4⁺ cells. This mesodermal efflux overlaps with robust ABCC5 activity in sea urchin blastulae and is also observed in sea stars, suggesting a potentially conserved role for ABCC-mediated signaling within the early mesoderm. Collectively, this map of transport is the first systems-level description of SMT activity in a deuterostome embryo. It sheds light on the emergence of unique territories of transport during embryogenesis. These observations provide a novel conceptual framework for studying the function and evolution of SMT networks in signaling and protection during animal development. This work is supported by NIH F32ES029843 to CS and R01ES027921 to AH.

Program Abstract #465
Integrating Cell Size with Genome Activation and Cell Fate Decision During Early Development
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How the embryo activates the genome and determines cell fate during early development is a fundamental question. Using Xenopus embryo, we recently discovered a spatiotemporal patterning of zygotic genome activation (ZGA) dependent on cell size, which is firstly initiated in small cells at the animal pole (AP) - the presumptive ectoderm - and gradually spread to large cells at the vegetal pole (VP) - the presumptive endoderm. However, it remains unknown which genes are activated size-dependently and how cell size regulate cell fate specification. We developed a pipeline to characterize the identity of zygotic genes by metabolically labelling all nascent RNAs with 5-ethyluridine (EU) followed by nascent EU-RNA sequencing (EU-RNA-seq). To characterize genes directly regulated by cell size, we dissected and sequenced nascent EU-RNAs in cells at AP and VP, respectively, during ZGA. Interestingly, hundreds of zygotic genes are activated earlier in the AP than the VP, consistent with our previous observation ZGA patterning, suggesting that the specification of germ layers may be hierarchically orchestrated by cell size. By making miniature embryos, we have shown that reducing cell size is sufficient to induce ZGA. On the contrary, halting development with a translation inhibitor cycloheximide (CHX) also induced early ZGA in pre-MBT embryos; however, extended ChX treatment resulted in decrease in zygotic transcription in post-MBT embryos. These data suggested that sustained zygotic transcription depends on cells reaching a critical cell size, although elongating cell cycle may transiently induce ZGA. Ongoing work suggests that manipulating cell size gradient by temperature gradient controllers alters ZGA patterning size-dependently. Further, size-dependent ZGA patterning is also conserved in X. tropicalis embryos. Together, our findings suggest cell-size dependent ZGA and cell fate decision might be conserved in various embryonic systems. Funding: R35GM12874802.

Program Abstract #466
Molecular and genetic analyses reveal that mRNA decay and translational control mechanisms are important for segmentation clock gene regulation
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Muscle and skeletal stem cells are sequentially grouped into reiterated segments, called somites, during early vertebrate embryogenesis in a process called somitogenesis. Somite formation is established by a genetic oscillator called the segmentation clock, comprised of a network of genes expressed periodically in the presomitic mesoderm. Tight temporal control of mRNA production, translation, and decay are central driving forces that regulate the timing of segmentation clock oscillations, and our work explores post-transcriptional mechanisms that regulate oscillatory expression. We have previously shown that Proline-rich nuclear receptor coactivator 2 (pnrc2) promotes segmentation clock gene transcript decay in zebrafish and that loss of pnrc2 results in segmentation clock gene transcript accumulation. To identify 3'UTR cis elements required for segmentation clock gene transcript decay, we used an inducible in vivo reporter system to show that mutation of the Pumilio Response Element (PRE) and AU-rich Element (ARE) dramatically increases reporter stability. RNA binding proteins (RBPs) that bind these elements are known to influence not only transcript decay, but also translation. Despite increased levels of segmentation clock gene mRNAs, pnrc2 mutants exhibit normal protein oscillations and
undergo segmentation normally, suggesting an additional layer of translational control. Preliminary polysome profiling analysis in wild-type and pnr2 mutant embryos reveals that accumulated segmentation clock gene transcripts her1, her7, dlc, and rhov are inefficiently translated. We are currently exploring whether translational regulatory mechanisms are involved in segmentation clock function. Based on these data, we posit that 3'UTR binding proteins, like Pumilio and ARE-BPs, support segmentation clock oscillations by regulating oscillatory mRNA stability and translation. OSU Center for RNA Biology Predoctoral Fellowship (KT); OSU Dean's Enrichment Fellowship (MM); NIH (SLA)

**Program Abstract #467**

**The Conserved RNA-binding Protein Rbm24 Post-transcriptionally Controls Gene Expression in Early Eye and Lens Development**  
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Anophthalmia (no eye) and microphthalmia (small-eye) (A/M) are eye developmental defects affecting 2-6 in 30,000 live human-births. So far, only ~30 genes in humans, and several transcription and signaling-factors are linked to A/M. However, the role that post-transcriptional regulators, namely RNA-binding proteins (RBPs), play in A/M remains to be delineated. We used a bioinformatics tool, iSyTE, and predicted a new RBP, Rbm24. In non-ocular tissues, Rbm24 mediates distinct post-transcriptional control over target-mRNAs, impacting their stability, splicing, and translation. We find that in the eye, Rbm24 is expressed in both the developing retina and lens. Additionally, our initial analysis shows that Rbm24 germline-knockout mice (Rbm24<sup>GM/GM</sup>) exhibit A/M. However, the mechanism by which Rbm24 regulates eye-development is not fully understood. Therefore, I hypothesize that **Rbm24 mediates post-transcriptional control of key-transcripts in early eye-development, disruption of which causes anophthalmia and/or microphthalmia.** To understand this, I will investigate Rbm24 function in mouse and *Xenopus* eye-development by generating and characterize two independent Rbm24 conditional-knockout mouse models, specific to the optic-vesicle (Rbm24<sup>cKO<sub>ov</sub></sup>) and the lens (Rbm24<sup>cKO<sub>le</sub></sup>) respectively. Further, I will develop and characterize Rbm24-Xenopus-knockdownmodel to evaluate the downstream-effectors of Rbm24 during development. **Impact:** Together these proposed experiments will uncover new regulatory-mechanisms of Rbm24-based control of key transcription-factors in addition to the molecular basis of the pathology of microphthalmia and anophthalmia resulting from Rbm24 deficiency in early eye-development. **Funding:** National Eye Institute

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**Program Abstract #468**

**Chondrocyte polarity during endochondral ossification requires protein-protein interactions between Prickle1 and Dishevelled 2/3**  
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The expansion and growth of the endochondral skeleton requires organized cell behaviours that control chondrocyte maturation and oriented division. Phenotypic features such as midfacial hypoplasia, and shorter stature are common in patients with Robinow Syndrome and result from decreased proximal distal outgrowth of the cranial base and limbs. Robinow Syndrome is a genetic disorder caused by mutations in members of the Wnt/PCP signaling pathway. To determine the function of Wnt/PCP signaling in the growth plate, we utilized the Prickle1<sup>Bj</sup> mouse line. The Prickle1<sup>Bj</sup> allele has a single nucleotide missense mutation in the Prickle1 LIM1 domain that results in a hypomorphic protein. The Prickle1<sup>Bj/Bj</sup> mutants lack growth plate expansion resulting in shorter stature and midfacial hypoplasia and are a model for Robinow Syndrome. The lack of expansion results from the precocious differentiation and delayed terminal differentiation of growth plate chondrocytes without changing the level of proliferation or apoptosis. The angle of cell division, location of the primary cilia and the intracellular localization of Dvl2 and Dvl3 protein is randomized in the Prickle1<sup>Bj/Bj</sup> chondrocytes. We tested if the Prickle1 and Dvl protein-protein interactions were maintained in the growth plate chondrocytes. Importantly, mutant Prickle1<sup>Bj</sup> protein has decreased protein-protein interactions with either Dvl2 or Dvl3 in chondrocytes as revealed by in vivo co-immunoprecipitation and proximity ligation assays. Therefore, the Prickle1<sup>Bj/Bj</sup> midfacial hypoplasia and short stature phenotype results from randomized growth plate chondrocyte polarity that in part, requires the interaction of the Prickle1 LIM1 domain binding with either Dvl2 or
Dvl3. This work is supported by NIH/NIDCR P30-NIH-DE020740 and the Momentum Funds and Start-up funds from the University of Pittsburgh School of Dental Medicine.

Program Abstract #469
A novel hypomorphic allele in Spag17 causes primary ciliary dyskinesia in mice
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Primary ciliary dyskinesia (PCD) is a human condition of dysfunctional motile cilia characterized by recurrent lung infection, infertility, organ laterality defects, and partially penetrant hydrocephalus. As part of an ENU forward mutagenesis genetic screen in the mouse, we recovered a mutant line that developed all the phenotypes of PCD. We named this allele primary ciliary dyskinesia only (Pcdo) and whole exome sequencing identified a nonsense mutation (c.5236A>T) in the Spag17 coding sequence creating a premature stop codon at position 1746 (K1746*). Through transcript and protein analysis we found that the Pcdo variant abolished different isoforms of SPAG17 in the Pcdo mutant testis but not in the brain, although all tissues have the c.5236A>T variant. Our data indicate differential requirements for SPAG17 in different motile cilia cell types. SPAG17 is required for proper development of the sperm flagellum, and is essential for either development or stability of the C1 microtubule structure within the central pair apparatus of the respiratory motile cilia, but not the brain ependymal cilia. We identified changes in ependymal cilia beating frequency but these did not apparently alter lateral ventricle cerebrospinal fluid (CSF) flow. However, aqueductal (Aq) stenosis resulted in significantly slower and abnormally directed CSF flow and we suggest this is the root cause of the hydrocephalus. The Spag17Pcdo homozygous mutant mice are generally viable to adulthood, but have a significantly shortened life span with chronic morbidity. We did not find any evidence of primary cilia defects in the osteoblast cells or the primary embryonic fibroblasts of the Spag17Pcdo mutant animals. Our data indicate that the c.5236A>T Pcdo variant is a hypomorphic allele of Spag17 gene and Spag17Pcdo mouse is a novel and useful model for elucidating the molecular mechanisms underlying development of PCD in the mouse. This work is funded by the NIH (R35GM131875, R01GM112744)

Program Abstract #470
Creating a Dominant-Negative Tinagl1 Mutant to Establish an Asymmetrical Craniofacial Model
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Over 10,000 children are born in the United States each year with developmental defects of the mid-face or jaw. The most common are cleft lip and cleft palate, while the next-most-common group is hemifacial microsomia, where bones and muscles are asymmetric and smaller on one side of the face. These defects can lead to struggles with eating, talking, hearing, and social stigma. Preventing these defects during fetal development may become possible if the mechanisms and signaling pathways which form the face and jaw are better understood. Tubulointerstitial Nephritis Antigen-Like 1 (tinagl1) is an extracellular matrix protein that appears to be required in zebrafish embryogenesis for function of motile cilia and for cranial neural crest cells to reach or accumulate in the pharyngeal arches where they form the skeleton of the jaw and gills. My project involves over-expressing mutant Tinagl1 in genetically wild-type zebrafish to cause dominant-negative effects on development of the craniofacial skeleton. I have mutated a predicted Wnt-binding lipocalin sequence motif that contains a hydrophobic tripeptide, WWF, to one that introduces a charged residue, WDF. Wild-type and WDF mutant mRNAs will be injected into one-cell wild-type embryos. I predict that the WDF mutant mRNA will cause cilia-related defects, as well as a novel asymmetrical development of craniofacial structures seen at low penetrance with a weak WGF construct in prior work by another undergraduate. These effects are predicted to result from the mutated Tinagl1 proteins competing for Wnt binding sites with the endogenous wild-type Tinagl1, and possibly involve stochastically variable levels of the functional and dominant-negative Tinagl1 across fields of Wnt-responsive cells. This work is funded by an MCG Translational Research Program grant to BZ and a gift from the Charles Silbereisen Fund of the Vanguard Charitable Gifts Foundation.
Program Abstract #471

A novel role for the Mediator complex subunit Med23, in craniofacial development and in the pathogenesis of Pierre-Robin Sequence

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Craniofacial development is a complex and dynamic process, which requires integration of all three germ layers and their derivatives. Ectodermally derived neural crest cells are a major contributor to craniofacial cartilage and bone and peripheral nerves innervating the head and face. Defects in neural crest cell development can result in congenital craniofacial anomalies. In a phenotype driven forward genetics screen aimed at identifying novel regulators of craniofacial development, we identified an embryonic lethal mouse mutant, *snouty*, which exhibits malformation of the craniofacial prominences. The *snouty* mutation was mapped to a single nucleotide change in *Med23*, which encodes a protein subunit of the global transcription co-factor complex, Mediator. Phenotypic analyses of *snouty* mutants revealed that the number of neural crest cells were reduced compared to controls. Neural crest cell-specific deletion of *Med23 (Med23^fx/fx;Wnt1-Cre)* leads to micrognathia, glossoptosis and cleft palate. These phenotypes are similar to that observed in humans with Pierre Robin Sequence, which is linked to mutations in *SOX9*, a transcription factor that regulates cartilage development. Both *Sox9* mRNA and protein levels are upregulated in the palatal mesenchyme of *Med23^fx/fx;Wnt1-Cre* embryos compared to controls and ChIP-qPCR and luciferase assays reveal that Med23 binds to the minimal promoter of *Sox9*, repressing its transcription. Moreover, Sox9 interacts with β-Catenin and downregulates β-Catenin and its downstream target genes, molecularly underpinning the phenotypes observed in *Med23^fx/fx;Wnt1-Cre* embryos. Altogether, this data suggests a cell-autonomous requirement for Med23 in the differentiation of neural crest cells into cartilage and bone and links global transcription through WNT signaling to craniofacial development. Funding for this research is provided by Stowers Institute for Medical Research.

Program Abstract #472

RNA Polymerase I and III function in neural crest cells and neuronal development

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Ribosome biogenesis is a global, multi-step process required for cell growth and proliferation. Disruptions in ribosome biogenesis lead to a group of disorders termed ribosomopathies, which display distinct, tissue-specific phenotypes. Perturbations in Pol I and III, which are important for the transcription of rRNAs and other non-coding RNAs, may result in craniofacial anomalies, limb anomalies, or neurological defects. To understand the mechanisms underlying the tissue-specificity of these phenotypes, we examined zebrafish with mutations in the Pol I and III subunits *polr1a* and *polr1c*. RNA-Seq analysis of mutant embryos revealed alterations in genes involved in craniofacial and neuronal development. We previously showed that *polr1a* and *polr1c* are necessary for rRNA transcription and normal neural crest cell and craniofacial development, but how these mutations alter central nervous system development remains unresolved. At 24 hours post fertilization (hpf), we observed cell death throughout the neural tube in *polr1a* and *polr1c* mutant zebrafish. We hypothesized that this domain of cell death would disrupt formation of both neural crest cells and oligodendrocytes leading to craniofacial and myelination defects. Consistent with this, *olig2* expression is diminished prior to 36 hpf and oligodendrocyte progenitors show reduced dorsal migration in *polr1a* and *polr1c* mutants. *Myelin basic protein* is downregulated, but the percentage of myelinating oligodendrocytes is unchanged. Altogether, this implies that formation and migration but not differentiation of oligodendrocyte precursors are altered upon disruption in Pol I and III. Distinguishing the function of Pol I and Pol III in the presentation of craniofacial versus central nervous system phenotypes in the future will further our understanding of the tissue-specificity of Pol I and III dysfunction in human development and disease. Funding was provided by the Stowers Institute for Medical Research.

Program Abstract #473

Insm1a Facilitates Spatiotemporal Modulation of Notch Signaling-Mediated Olfactory Neurogenesis

Sriivatsan Govinda Rajan, Lynne M. Nacke, Kaelan Wong, Jocelyn Garcia, Nathan Burg, Ankur Saxena
Defining the establishment of neuronal diversity at a system-wide level can provide fundamental insights into how a complex network of neurons is assembled. We use the developing zebrafish olfactory epithelium (OE) as a model system to delineate how distinct stem cell populations give rise to ciliated and microvillous olfactory sensory neurons (OSNs). Notch signaling receptors are expressed in the developing murine OE, but the downstream molecular mechanisms through which they might regulate OSN specification and differentiation in vertebrates have remained largely unexplored. We found that, in zebrafish, multiple components of the Notch signaling pathway exhibit spatiotemporally-dynamic expression patterns in the OE during early stages of development. Temporally-specific Notch signaling knockdown using both chemical and genetic methods yielded marked increases in the numbers of both OSN subtypes and decreased numbers of a distinct progenitor subpopulation. Additionally, we observed alterations in the expression patterns of proneural genes known to be involved in OSN differentiation. Furthermore, we identified a regulatory feedback loop between Notch signaling and the transcriptional repressor Insm1a that may modulate the dynamics of early olfactory neurogenesis. Taken together, our data suggest that a Notch signaling/Insm1a feedback loop plays critical roles in coordinating OSN differentiation in vivo. By better understanding Notch signaling-mediated olfactory development, we aim to uncover conserved molecular mechanisms that may regulate neuronal differentiation and organization across a variety of intricate organ systems. Funding: National Institute of Child Health and Human Development; NSF-Simons Center for Quantitative Biology; Chicago Biomedical Consortium.

**Program Abstract #474**
**Spatial Structuring and Dynamical Stability of Olfactory Epithelium Assembly**
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Olfactory neurogenesis in zebrafish relies upon the temporally distinct contributions of multiple stem cell populations—cranial neural crest cells (NCCs) and ectodermal placode cells (PLCs)—to form sensory neurons. To understand how these populations contribute to olfactory epithelium (OE) assembly, we tracked, segmented, and quantitatively analyzed NCC and PLC movement in developing zebrafish embryos. We found that NCCs exhibit greater volatility (range of movement) and travel a greater total distance than PLCs do, highlighting key differences in cell type-specific behavior. NCCs are initially located basally to PLCs, and the majority displace apically during OE assembly. NCC initial position is correlated with future displacement, indicating that NCCs interpret their position and tend to move toward a midline in the OE. We term this process ‘condensation’. Moreover, the NCC position-displacement association is stronger along the radial axis in comparison to the apico-basal axis, suggesting that radial coordinates better characterize condensation. Based on these data, we proposed a model whereby cell motion is described as transitions between radial shells, and we applied information-theoretic methods to investigate dynamical stability. Our analyses provide new evidence of (1) a stochastic equilibrium between memory-free dynamics and dynamics that incorporate the last transition (a Markov process) during each step of condensation; (2) a collective stabilization of transitions over the entire window of condensation. Taken together, we have constructed a working model of progenitor cell movements during OE assembly: NCCs and PLCs exhibit distinct movements that contribute to a radially-patterned condensation process with dynamical stability across multiple time-scales. These findings offer new system-wide insights into olfactory assembly and progenitor cell behavior in vivo. Funding: NICHD; NSF-Simons Center for Quantitative Biology; Chicago Biomedical Consortium.

**Program Abstract #475**
**Prdm8 regulates pMN progenitor specification for motor neuron and oligodendrocyte fates by modulating Shh signaling response**
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pMN progenitor cells of the ventral spinal cord, which express the bhlh transcription factor Olig2, first produce motor neurons and then oligodendrocyte precursor cells (OPCs). Some OPCs differentiate into myelinating oligodendrocytes whereas others remain as proliferative progenitors through the lifetime of an organism. How
pMN progenitors switch from producing motor neurons to OPCs with distinct fates is not well understood. pMN progenitors express prdm8, which encodes a transcriptional repressor protein, during motor neuron formation and oligodendrocyte lineage cells downregulate expression upon differentiation. This temporal expression pattern raises the possibility that prdm8 controls the onset OPC specification and subsequent differentiation. To address how prdm8 controls pMN cell fate specification, we used zebrafish as a model system to investigate prdm8 function. Our analysis revealed that prdm8 mutant embryos have a deficit of motor neurons resulting from a premature switch from motor neuron to OPC production. Additionally, prdm8 mutant larvae have excess oligodendrocytes and a concomitant deficit of OPCs. Notably, pMN cells of mutant embryos have elevated Shh signaling coincident with the motor neuron to OPC switch. Inhibition of Shh signaling restored the number of motor neurons to normal in mutant embryos but did not rescue the proportion of oligodendrocytes. These data suggest that Prdm8 regulates the motor neuron-oligodendrocyte switch by controlling the level of Shh activity in pMN progenitors and also regulates allocation of oligodendrocyte lineage cell fates. Funding provided by: NIH R01 NS046668 Genetic Analysis of Oligodendrocyte Specification.

Program Abstract #476
The role of Claudin-3 in neural tube closure
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The neural tube is the embryonic precursor to the central nervous system, which is formed following elevation and fusion of the lateral edges (neural folds) of the neural plate. Our lab is investigating the role of the claudin (cldn) family of tight junction proteins in neural tube development. In chick embryos, depletion of Cldn3 causes neural tube defects at the final stage of neural tube development, neural fold fusion. The mechanism of chick neural fold fusion is still not fully understood. In scanning electron microscope (SEM) images, we identified a fibrous mesh between the closing neural folds of wild-type chick embryos which was absent in the Cldn3-depleted embryos. Research from the 1970s showed that the mesh is a glycoprotein cell-surface matrix, involved in temporary adhesion between the neural folds as they fuse. We hypothesize that Cldn3 is required for formation of a glycoprotein mesh which is critical for neural fold fusion. Using fluorescently-tagged carbohydrate-binding lectin proteins, I am examining the pattern of expression of glycan chains along the neural tube. I will then determine whether this pattern changes in the Cldn3-depleted embryo and how the glycoprotein chains are involved in neural tube development. My preliminary results show that subsets of glycan chains are strongly expressed at the closing neural folds and that interrupting these glycan chains through lectin binding causes neural tube defects. To follow up on the role of claudins and tight junctions in neural fold fusion, I will be live imaging transverse vibratome-sectioned neural tubes during neural fold fusion. Dynamic rearrangements of tight junctions will be analyzed by overexpressing tagged tight junction proteins in control and Cldn3-depleted embryos. This research is working towards a better understanding of the mechanisms in chick neural tube closure, and the role that Cldn3 is playing in this process. This research is funded by NSERC.

Program Abstract #477
Snx3 is important for mammalian neural tube closure via its role in canonical and non-canonical WNT signaling
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Disruptions in neural tube (NT) closure result in neural tube defects (NTDs). To understand the molecular processes required for mammalian NT closure, we investigated the role of Snx3, a sorting nexin gene. Snx3+/− mutant mouse embryos display fully-penetrant cranial NTD. In vivo, there is a decrease in canonical WNT target gene expression in the cranial neural folds of the Snx3+/− embryos as well as a defect in convergent extension of the neural epithelium. Snx3+/− cells show decreased WNT secretion and live cell imaging reveals aberrant recycling of the WNT ligand binding protein WLS and shunting to the lysosome. The importance of SNX3 in WNT signaling regulation is demonstrated by rescue of NT closure in Snx3+/− embryos with a WNT agonist. The potential for SNX3 to function in human neurulation is revealed by a point mutation identified in an NTD-affected individual that results in functionally impaired SNX3 that does not co-localize with WLS. These data indicate that Snx3 is critical.
for NT closure via its role in recycling of WLS to control the level of WNT signaling. This work was supported by NIH NICHD HD081562 to L.A.N. with co-PI’s Trevor Williams and David Clouthier (University of Colorado Anschutz Medical Campus) and NIH NICHD HD073434 to K. S. A.

Program Abstract #478
Tuba1a microtubules are uniquely important for axon guidance through midline commissural structures
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The developing brain requires precise modulation of the cytoskeleton to ensure that neurons migrate correctly and generate proper synaptic connections. One method that could control the microtubule cytoskeleton is the tubulin isotype blend a cell expresses. The α-tubulin isotype TUBA1A is the most highly expressed α-tubulin in the developing brain. Tuba1a mutations are associated with developmental brain defects, termed tubulinopathy. Tubulinopathies are characterized by cortical malformations along with defects in commissure formation. We utilized a mouse that has an asparagine to aspartic acid (N102D) substitution in Tuba1a to explore potential mechanisms behind tubulinopathy phenotypes. We developed a novel method to visualize TUBA1A protein using an internal hexahistidine epitope tag without impacting the function of α-tubulin. Using this method, we show that Tuba1aN102D is a loss-of-function mutation, and the protein is not incorporated into microtubules. Tuba1aND/ND mice recapitulate cortical neuron migration defects and axon guidance defects that are characteristic of human tubulinopathy. Tuba1aND/+ mice undergo normal cortical migration but fail to correctly form forebrain commissures. The axons form irregular bundles adjacent to midline and are disorganized. Neurons lacking TUBA1A fail to localize microtubule associated protein-1b (Map1b) to the growth cone, which is necessary for guidance cues critical to commissural formation. The failure of Tuba1aND/+ neurons to enrich Map1b to the growth cone is likely due to trafficking defects, which cause motor proteins to stall more frequently and reduce the speed of cargo trafficking. In conclusion, we show that reduced TUBA1A is sufficient to support neuronal migration, but not axon guidance across the midline. Funded by the University of Colorado Movement Disorder Center (E.A.B.), the Department of Cell and Developmental Biology (J.K.M.), and the National Institutes of Health Grant 5R01GM112893-05 (to J.K.M).

Program Abstract #479
Transient Nodal signalling in left precursors coordinates opposed asymmetries shaping the heart loop
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Left-Right asymmetry is essential for visceral organ morphogenesis, positioning and function. The secreted factor Nodal has been shown to be a major left determinant. Although it is associated with severe congenital heart defects, its role in heart morphogenesis has remained poorly understood. Here, we report that Nodal is transiently active in precursors of the mouse heart tube poles, before the morphological changes of heart looping. In conditional mutants, we show that Nodal is not required to initiate asymmetric morphogenesis. We provide evidence of a heart-specific random generator of asymmetry that is independent of Nodal. Using 3D quantifications and simulations, we demonstrate that Nodal functions as a bias of this mechanism: it is required to amplify and coordinate opposed left-right asymmetries at the heart tube poles, thus generating a robust helical shape. We identify downstream effectors of Nodal signalling, regulating asymmetries in cell proliferation, cell differentiation and extra-cellular matrix composition. At birth, Nodal conditional mutants display complete atrioventricular septal defect and malposition of the great arteries, showing the importance of the heart loop shape for the alignment of cardiac chambers and the establishment of the double blood circulation. Our work provides novel insight into how Nodal regulates asymmetric organogenesis. Fundings: Institut Imagine, Institut Pasteur, INSERM, ANR, MSD-Avenir (Devo-Decode)
Program Abstract #480
Genetic interaction between Sostdc1 and Msx1 during mouse tooth morphogenesis
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Tooth agenesis is a common birth defect that severely affects oral health and is caused by developmental arrest of tooth morphogenesis at its early stage. Previous studies have shown that both canonical Wnt signaling and Bmp4-Msx1 pathways are critical in bud-to-cap-stage transition during early tooth morphogenesis. We recently demonstrated that the Bmp4-Msx1 pathway activates the canonical Wnt signaling by regulating the expression of secreted Wnt antagonist genes Dkk2 and Sfrp2 in the early tooth bud mesenchyme. However, our rescue experiments of tooth development, by inactivating these Wnt antagonists in mice lacking the Bmp4-Msx1 pathway, resulted in only partial rescue, and the two pathways are still not well understood. In the current study, we identify that Sostdc1 plays an important role in regulating the Bmp4-Msx1 function in early tooth development. Expression of Sostdc1 mRNA is present in the dental epithelium and mesenchyme in both control and Msx1-null tooth buds. Remarkably, by deleting Sostdc1 in the Msx1-null background mice, tooth morphogenesis was rescued in the maxillary molar, but not in the mandibular molar teeth. Furthermore, we showed that tooth developmental rescue could be achieved also in the Bmp4-neural crest-specific conditional knockout (-nCKO) mice by deleting Sostdc1. We conclude that Sostdc1 regulates the function of the Bmp4-Msx1 pathway in controlling early tooth organogenesis. This work was supported by the University at Buffalo start-up funding to H.-J. E. Kwon.

Program Abstract #481
Spatiotemporal transcriptional dynamics of the cycling mouse oviduct
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The oviduct, like other female reproductive organs, responds to the steroid hormones produced during the estrous cycle, and this cyclical responsiveness is important for female fertility. Further, while the oviduct is a critical site for female fertility, how oviduct physiology is regulated at the genetic, molecular, and cellular level remains fairly mysterious. We set out to understand the underlying global transcriptional response to the steroid hormone secretion by performing RNAseq of the mouse oviduct at each stage of the estrous cycle. In addition, as the oviduct displays intriguing cellular patterning across the anteroposterior axis – at the anterior end (near the ovary), multiciliated cells are enriched while at the posterior end (near the uterus), secretory cells abound – we performed RNAseq on anterior and posterior portions of the oviduct. We have identified over 2,000 differentially expressed genes along the anteroposterior axis, and less than 100 differentially expressed genes across the estrous cycle. Genes enriched in the anterior are mainly responsible for multiciliated cell formation and homeostasis, while genes enriched in the posterior represent secretory transport genes and developmental signaling pathways. The developmental genes enriched in the posterior include Hox genes, FGF signaling, and noncanonical Wnt signaling. Interestingly, across the estrous cycle most of the differentially expressed genes are ribosomal protein genes. We are currently investigating ribosome biogenesis across the estrous cycle of the mouse oviduct. ECR is supported by an F32 from NICHD, and NIH grants to JBW funded this work.

Program Abstract #482
Wnt signaling modulates the expression of ion channels to promote trachealis smooth muscle organization in mouse developing respiratory tract.
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Tracheomalacia and Complete tracheal Rings are congenital disorders affecting the cartilage and muscle of the large airways of the respiratory tract. We demonstrated that Wnt signaling is essential for tracheal cartilage and muscle formation. Published studies demonstrated the role of ion channels in determining tracheal smooth
muscle shape and organization. Our published research showed that trachealis muscle cell organization and polarity are disrupted after the deletion of Wls, an essential component of the Wnt signaling pathway. The abnormal trachealis muscle organization is reminiscent of the phenotype observed after deletion of ion channels. RNA sequencing studies in the Wls-deficient developing trachea revealed differential regulation of genes encoding ion channels. We hypothesize that ion homeostasis impacts trachealis muscle cell polarity and that Wnt signaling influences the expression of ion channels to promote trachealis muscle formation. Results: To study the role of Wnt and ion channels on developing trachealis muscle, we utilize mouse embryonic tracheal tissue and primary mesenchymal tracheal cells. Samples were treated with ion channel (GlyH101, 4-Amino pyridine, VU590), and Wnt signaling (Wnt C59) inhibitors or KCl. 1) In ex vivo studies, inhibition of potassium ion channels or Wnt signaling, as well as KCl treatment, impair cytoskeletal organization and contractility of developing trachealis muscle. 2) Wnt ligands and ion channel blockers differentially affect contractility of primary mesenchymal tracheal cells in free-floating collagen assay. We conclude that ion homeostasis during trachea development is necessary for trachealis muscle organization. Epithelial induced Wnt signaling plays an essential role in trachealis muscle development via regulation of gene expression of ion channels influencing the cytoskeletal organization of trachealis muscle and ion homeostasis. These studies were partially supported by NIH-NHLBI R01HL144774-01A1 to DS.

Program Abstract #483
Morphogenesis of the upper lip: insights from time-lapse imaging
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Cleft lip with or without cleft palate occurs in thousands of births every year in the United States and is a leading birth defect worldwide. A possible explanation to the high rate of incidence is the complex etiology underlying these malformations. Previous studies using static imaging has provided a broad description of lip development as a multistep process that includes nasal pit invagination, outgrowth of nasal processes, and fusion of the medial nasal, lateral nasal and maxillary processes. Combining tissue culturing and time-lapse imaging, we provide a 4D view of the fusion of medial and lateral nasal processes. In this setup, we can now track individual cells and interrogate the cellular mechanisms of fusion, including the force generation components such as actomyosin contractility. The failure of the medial nasal process to join with the and lateral nasal and maxillary processes results in cleft lip, and we aim to understand the various cellular events impacting their successful fusion. Cell-cell adhesion and actomyosin contractility have major roles in embryonic morphogenesis and are indispensable in normal lip development, based on recent reports of cleft lip-associated mutations in CTNND1 and CDH1. We have utilized a mutant mouse model with conditional deletion of Ctnnd1 and Cdh1 in the epithelium, and initial screening of mutants at embryonic day 12.5 confirmed an apparent cleft lip phenotype. Subsequent interrogation of earlier timepoints revealed medial and lateral nasal processes that fail to contact and join. Analyses of non-muscle myosin II mouse mutants with disrupted actomyosin contractility showed much earlier nasal pit invagination defects and disorganized epithelia. Collectively, our live imaging approach along with mutant mouse models allow us to better understand normal lip development and probe cellular mechanisms of fusion. This work is supported by R01DE025877.

Program Abstract #484
Dachsous Cadherin Related-1 and the Septin Cytoskeleton: A Molecular and Developmental Etiology Underlying Mitral Valve Prolapse
Kelsey Moore, Reece Moore, Rebecca Stairley, Mary Kate Rumph, Connor Graham, Sameer Abrol, Diana Fulmer, Lilong Guo, Cortney Gensemer, Christina Wang, Tyler Beck, Rupak Mukherjee
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Mitral Valve Prolapse affects 1 in 40 individuals and carries a significant burden of morbidity and mortality. Our group previously identified missense mutations in the atypical cadherin gene, DCHS1, in multiple families with non-syndromic MVP. However, the distinct consequences of these mutations and intracellular functions of DCHS1 during valve morphogenesis remain unknown. Our recent biochemical studies implicate one such mechanism involving a protein complex between DCHS1, Lix-1 Like (LIX1L), and Septin-9 (SEPT9). In vivo epistasis experiments
also support a genetic interaction between Dchs1 and Lix1L. Compound heterozygotes exhibited significant leaflet enlargement at P0 and doppler echocardiography revealed valve prolapse and regurgitation by 6 months. Valve interstitial cells from compound heterozygotes were less compact and expressed significantly less SEPT9 protein compared to wild-type or single heterozygotes. We then tested the hypothesis that DCHS1-LIX1L-SEPT9 regulates the actin cytoskeleton with in vitro approaches. Cardiac fibroblasts (CFs) were isolated at P0 and characterized with immunocytochemistry, a fibrin valve construct, and actin polymerization assays. We measured cell and nuclear shape and performed atomic force microscopy to generate functional read-outs for cytoskeleton integrity and intracellular tension. Wild-type CFs displayed aligned septin-actin filaments, elongated nuclei and ECM remodeling capabilities whereas Dchs1 and/or Lix1L deficient CFs exhibited drastic reductions in cytoskeletal organization, nuclear tension and ECM-cell alignment. Delivery of a cell-permeant peptide that mimics the refined LIX1L-SEPT9 binding domain allowed for direct perturbation of this interaction and resulted in similar deficiencies. These findings suggest that DCHS1-based cell adhesions stabilize the actin cytoskeleton through LIX1L-SEPT9 during critical stages of mitral valve morphogenesis and disease inception. Funding: T32HL007260  F31HL152494

Program Abstract #485
Dysregulation of embryonic microglia population size, morphology, and activity due to perfluoroalkyl substance exposure.
April Rodd, Nathan Martin, Jessica Plavicki
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Microglia, the immune cells of the brain, play an important and underappreciated role mediating the effects of neurotoxicants during development. Following early life exposure to a contaminant of emerging concern, perfluorooctanesulfonic acid (PFOS), we observed changes in zebrafish microglial development that would indirectly contribute to neurotoxicity. Microglia brain colonization, cell morphology, and function were examined in live and fixed embryos during a critical window of microglial colonization in the developing brain with transgenic fluorescent reporters and image analysis. Exposure to high concentrations of PFOS resulted in dose-dependent decreases in microglia numbers at 3dpf. PFOS exposure also caused increased brain apoptosis, indicating a decrease or loss in the ability of microglia to migrate into the brain or proliferate in response to cell death. PFOS-treated embryos have more rounded microglia, an important change in cell morphology that indicates more phagocytosis and a more active cellular phenotype. This change in activity is not limited to morphology, as altered calcium dynamics were observed in the microglia and macrophages of exposed embryos. Together, these data demonstrate significant changes to the embryonic microglia population that can lead to significant changes to brain development. This study identifies a potential indirect cause of PFOS-mediated neurotoxicity and possible contributor to other poorly understood toxicant-induced neurotoxicity. Future work will focus on these pathologies at lower concentrations and in combination with other insults, like brain injury, to further evaluate the role of microglia in complex neurological pathologies. This research was supported by a NIH K99/R00 (ES023848/ES2384) from NIEHS and a Brown University SRP Seed Grant.

Program Abstract #486
Clearing axonal debris after Wallerian degeneration in an ex vivo zebrafish scale model
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Skin is a highly innervated tissue responsible not only for protection from foreign insults, but also our sensory interpretation of the world. Somatosensory axon endings located within the skin sense touch, temperature, and pain. Skin injuries frequently damage these fragile cutaneous axons, triggering axonal (Wallerian) degeneration, which generates large quantities of axonal debris. Efficient debris removal promotes axon reinnervation and tissue homeostasis while preventing inflammation. Yet, how different cell populations within the skin work in concert to clear this debris and allow for axon regeneration is not well understood. Our lack of understanding stems from the inability to model the complexity of vertebrate skin in cell culture, as well as the inaccessibility of the skin to live-imaging after injury in murine models. The adult zebrafish skin shares many of the complex features of mammalian skin and is highly amenable to live-imaging. We have developed in vivo and ex vivo scale wounding assays that induce axonal degeneration, allowing us to examine how multiple cell types work to clear
axonal debris and heal skin wounds. Using a scale pluck assay combined with live-imaging to observe Wallerian degeneration and responses from surrounding tissue, we found that keratinocytes can clear axonal debris. Notably, we have found that Langerhans cells, a skin-resident immune cell not previously implicated in tissue repair, can engulf and digest axonal debris after injury. Intriguingly, Langerhans cells increase in number in several peripheral neuropathies, including chemotherapy- and diabetic-induced peripheral neuropathy, and accordingly, have been implicated in mediating neuropathic pain. Combined, these methods will provide a powerful model to assess the complexity of axon-skin interactions after injury. Funding from a Washington Research Foundation Postdoctoral Fellowship (EP) and NIH R00 HD086271 (JR).

Program Abstract #487
Lymphoangiocrine signals regulate cardiac growth
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Recent studies suggested a beneficial role for lymphatics in restoring heart function after cardiac injury. Here we report the identification of a novel lymphoangiocrine signal regulating cardiac growth. This paracrine signal produced by cardiac-associated lymphatic endothelial cells (LECs) controls cardiomyocyte (CM) proliferation and survival during mouse heart development. We show that mutant embryos devoid of LECs develop smaller hearts consequence of reduced CM proliferation and increased CM apoptosis. Furthermore, we found that culturing primary mouse CMs in the presence of LECs-conditioned media increases CM proliferation and survival, a result indicating that LECs produce paracrine signals controlling CM homeostasis. We have now identified and characterized from the LEC media secretome a likely candidate fulfilling such role during cardiac growth. This study is currently funded by NIH RO1grant to G. Oliver and AHA CDA grant to X.Liu

Program Abstract #488
Desert Hedgehog-primary cilia signaling shapes mitral valve morphogenesis and disease
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Mitral valve prolapse (MVP) is a prevalent cardiac valve disease that affects 1 in 40 individuals world-wide and carries a risk for serious comorbidities such as arrhythmia, thromboembolism, and sudden cardiac death. MVP is especially detrimental to the elderly population as it is present in 11% of individuals over the age of 75, and non-surgical therapies are unavailable. One out of ten MVP patients will require surgery to repair or replace biomechanically incompetent valves. Valve pathogenesis remains poorly understood, but here we present evidence that this disease of aging can have development origins through defects in cellular signaling organelles called primary cilia. Ciliary diseases are known as ciliopathies, and are associated with higher incidents of heart valve disease. Desert Hedgehog (DHH) signaling is a cilia-dependent developmental pathway that is active in mitral valvulogenesis. Our data indicate that DHH ligand is expressed by valve endocardial cells, and influences the organization of cytoskeletal α-SMA in the ciliated valve interstitial cells. This paracrine crosstalk contributes to remodeling of the valve extracellular matrix into the thinned mature tissue. We show through in vitro and in vivo imaging, 3D reconstruction, Western blotting, RT-PCR, and other biochemical experimentation that disruption of this pathway results in myxomatous degeneration and disease progression. Mechanistically we demonstrate that valvular DHH-cilia signaling functions through a non-canonical (GLI-independent) pathway that depends on interactions between the GPCR, Smoothened, the GEF, TIAM1, and RAC1. Taken together, these data provide new understanding of valvulogenesis, support that paracrine DHH-cilia signaling regulates how extracellular matrix is organized into a 3D lattice, and shows signaling defects contribute to mitral pathogenesis. Funding provided by: NHLBI: F31HL142159, T32 HL007260, 1R01HL131546; NIH: 5P20GM103444, P30DK074038; AHA: 15GRMT25080052.
**Program Abstract #489**  
**Hedgehog Signal Transduction in Development via Membrane Sequestration of PKA Subunits**  
Benjamin Myers¹, Corvin Arveseth¹, John Happ¹, Danielle Hedeen¹, Ju-Fen Zhu¹, Jacob Capener¹, Dana Klatt Shaw¹,², Ishan Deshpande², Jiewei Xu², Sara Stubben¹,³, Isaac Nelson¹, David Grunwald¹, Ruth Huttenhain², Aashish Manglik²  
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Hedgehog (Hh) signaling plays conserved roles in animal tissue patterning and dictates the development of nearly all vertebrate organs. A fundamental pathway step involves the 7-transmembrane protein Smoothened (SMO), which stimulates GLI transcription factors to control expression of pathway target genes. Even after many years, we still do not understand how SMO activates GLI. In addition, protein kinase A (PKA) inhibits GLI *in vivo*, but whether or how PKA participates in signal transduction from SMO to GLI also remains unresolved. Using novel approaches to reconstitute SMO regulation in heterologous cells, we uncovered a mechanism directly linking vertebrate SMO activation to changes in GLI transcription, based on SMO-mediated recruitment of PKA catalytic subunits to the membrane. This process is coordinated by the essential kinase GRK2, which phosphorylates the active state of SMO to recruit PKA catalytic subunits. PKA membrane sequestration prevents GLI phosphorylation, thereby releasing GLI from PKA-mediated inhibition. Despite several apparent differences between Hh signal transduction in mammals and insects, this membrane sequestration mechanism is conserved in *Drosophila*. Our work uncovers a mechanism connecting Hh signal reception to transcription of pathway target genes, reveals an unexpected conservation in how SMO coordinates these processes in metazoans, and helps to clarify the mechanisms by which PKA and GRK2 contribute to Hh signal transduction during development. It also suggests a noncanonical route for developmental signaling cascades to influence PKA function. As a result, these cascades may gain an ability to encode new types of transcriptional responses, expanding the number of cell fate decisions they can control during embryogenesis. (Supported by NIH, ACS, and the Huntsman Cancer Institute).

**Program Abstract #490**  
**Structural and Molecular Characterization of the EVL-Deep Cell Boundary during Zebrafish Development**  
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University of Toronto, Canada  
Proper development of distinct embryonic tissues depends on cellular interactions and rearrangements during morphogenesis and their misregulation may result in profound developmental defects that ultimately impact the adult organism. Boundary formation between prospective tissue layers requires cellular rearrangements where tissue-specific cells interact each other without intermixing to define the boundary. During zebrafish epiboly, epithelial enveloping layer cells (EVL) move from the animal to the vegetal pole to surround and enclose the embryo, while mesenchymal deep cells beneath the EVL, intercalate to thin and spread vegetally but they never mix with the EVL cells. This resembles the ectoderm-mesoderm boundary in frog embryos, where Eph receptors and their ephrin ligands facilitate the adhesive and repulsive movements of tissue-specific cells without intermixing. However, the function of Eph/ephrin signaling at the EVL-deep cell boundary during zebrafish development is not known. Our lab showed that EVL and deep cells form transient contacts via dynamic filopodia. Therefore, my aim is to determine the function of Eph/ephrin signaling in filopodia formation and its potential role in boundary formation during morphogenesis. Pharmacological inhibition of Eph/ephrin signaling and live imaging revealed that epiboly was delayed and that there were prolonged filopodial interactions between EVL and deep cells, and EVL-deep cell boundary formation was impaired. Currently, I am utilizing mutant EphB receptor to block forward signaling to understand its role(s) in the boundary formation. Global and tissue-specific chimeric expression of mutant receptor subtypes will reveal spatio-temporal regulatory properties of the forward signaling. My work will answer fundamental questions on cellular rearrangements and their function in boundary formation, which is common in between zebrafish and higher order species due to evolutionary conserved protein functions.  
Funding: NSERC.
Program Abstract #491

Understanding the role of CCM3 in endothelial development and disease in Zebrafish
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Cerebral cavernous malformations (CCMs) are focal dilations in the cerebral vasculature leading to haemorrhaging, strokes and in extreme cases death. Of the three proteins associated with CCMs, CCM1/2/3, CCM3, a scaffold protein highly conserved through species, is the least understood and proposed to have the most detrimental effects. Though various models have been used to study endpoint vascular defects, not much is known about the earliest cellular events which eventually lead to CCMs. We use the zebrafish as a vertebrate model to understand the role of Ccm3 in early vascular development and disease progression. With CRISPR/CAS9 we generated a ccm3a/b double mutant. ccm3a/b\(^{-/-}\) embryos exhibit cardiac edemas, loss of blood flow, and are lethal. Time lapse imaging was used to characterise defects in lumen formation, blood flow, and membrane dynamics. To explore the mechanism of Ccm3 function, BioID was used to determine the potential interactome of Ccm3. Cellular Ccm3 resides mostly in the striatin interacting phosphatases and kinase (STRIPAK) complex. We generated CRISPR/CAS9 mutants of these components of the STRIPAK complex, consisting of largely unstudied genes, to assess their role in vascular development and their relationship to Ccm3. We discovered that Mob4, a gene of previously largely unknown function, is involved in cardiovascular development and endothelial integrity. We also know that CCM disease progression is linked to RhoGTPase activity. We determined Cdc42 is implicated in Ccm3 function: ccm3a/b KO embryos show aberrant Cdc42 activity and KO/KD of cdc42 leads to transient cerebral haemorrhages in embryos, though cdc42\(^{-/-}\) mutants are eventually viable and fertile. Altogether, we have established a model to study early changes in Ccm3 deficient endothelial cells and probe mechanisms of function of Ccm3 \textit{in vivo}. All work is conducted under grants by CIHR.

Program Abstract #492

Zebrafish, genes, and human kidneys: gene mapping in a zebrafish mutant may help elucidate pathways involved in Polycystic Kidney Disease
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Polycystic Kidney Disease (PKD) is a common cause of end-stage renal disease. Questions remain about the precise molecular mechanisms leading to cyst formation in PKD. Early kidney development in zebrafish is similar to that of mammals making zebrafish a useful model for understanding aspects of PKD. In collaboration with the Ekker and Sussman labs at Mayo Clinic, we are working to characterize a mutant zebrafish (spinner) that develops cysts in the pronephros during development. While trying to identify the genetic cause of the kidney cysts in spinner zebrafish, we identified a region of the genome that appears to be deleted in homozygous mutants. This region contains at least seven genes, none of which has been previously implicated in PKD. Expression of each of these seven genes is absent in spinner mutants but present in the transcriptomes of wild-type zebrafish. We are using a genome editing approach to mimic the effects of the deletion in the genome and to attempt to narrow down the causative gene by creating smaller deletions in the region. Zebrafish embryos are injected with pairs of Transcription Activator-Like Effector Nucleases (TALENs) corresponding to each of the 7 genes of interest. To identify whether we have successfully deleted the genomic region, DNA markers from the region are analyzed using PCR. We hope to develop a better understanding of the causative genes and spinner mutation in relation to PKD. This work was funded by the University of Wisconsin-Eau Claire Office of Research and Sponsored Programs, the Vicki Lord Larson and James Larson Collaborative Research Grant, and Mayo Clinic.

Program Abstract #493

Cell reintegration failure: a novel model for tumorigenesis
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Epithelial tissues are the origin of most cancers. Epithelia are formed of sheets of cells that shape body compartments and organs, and form chemical and mechanical barriers. The loss of epithelial cell polarity and the displacement of cells from epithelial sheets is considered a pathway to tumorigenesis and cancer progression via...
the ‘epithelial-to-mesenchymal transition’ (EMT). Work in a number of animal systems shows that epithelial cells can be born protruding from a tissue sheet, then reincorporate into it. This cell behavior, termed cell reintegration, is driven by a conserved adhesion machinery at lateral cell-cell junctions. Disruption of the lateral adhesion machinery leads to reintegration failure and cell misplacement. We observe that misplaced cells lose the hallmarks of epithelial polarity and accumulate in tumor-like masses. Hence, we propose cell reintegration is a gatekeeper to prevent tumorigenesis. Through genetic manipulation of the reintegration adhesion machinery, we have developed a novel model for tumorigenesis in Drosophila. Using genetic and biophysical manipulations, we are probing the factors that influence reintegration and tumor progression. Research supported by NIGMS R01GM125839.

Program Abstract #494
Keratin 13 Deficiency Causes White Sponge Nevus in Mice
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White sponge nevus (WSN) is a benign autosomal dominant disorder characterized by the formation of white spongy plaques in the oral mucosa. Keratin (KRT) 13 is highly expressed in the mucosa, and mutations in this gene have been commonly associated with WSN patients. It remains unknown whether there is a relationship between KRT13 mutations and WSN and what the underlying mechanism are. Here, we use mouse genetic models to demonstrate that Krt13 is crucial for the maintenance of epithelial integrity. Krt13 KO mice show a WSN-like phenotype in several tissues including the tongue, buccal mucosa, and esophagus. Transcriptome analyses uncover that Krt13 regulates a cohort of gene networks in tongue epithelial cells, including epithelial differentiation, immune response, stress-activated kinase signaling, and metabolism. We also provide evidence that epithelial cells without Krt13 are susceptible to mechanical stress experienced during postnatal life, resulting in unbalanced cell proliferation and differentiation. These data demonstrate that Krt13 is essential for maintaining epithelial homeostasis and loss of Krt13 causes WSN-like phenotype in mice. The work was supported by grants from NIH (Skin Diseases Research Center Core Grant P30AR066524, UW Carbone Cancer Center Grant P30CA014520, and R01GM129259) BJL was supported by the Department of Veterans Affairs Merit Review Award I01BX003315; HC was supported in part by the Gary S. Wood Derm. Research Bascom Endowed Professorship

Program Abstract #495
Drosophila Chitinase-like proteins participate in tissue morphogenesis, cell migration, immune response, and CO2 sensitivity
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Chitinase-like proteins (CLPs) are members of the family 18 glycosyl hydrolases, which include chitinases and the enzymatically inactive CLPs. A mutation in the catalytic site, conserved in vertebrates and invertebrates, allowed CLPs to evolve independently with functions that do not require chitinase activity. CLPs normally function in inflammatory responses, wound healing, and host defense, but when they persist at excessive levels at sites of chronic inflammation and tissue remodeling disorders, they correlate positively with disease progression and poor prognosis. Research has focused on the expression patterns of CLPs and their relevance as potential biomarkers of disease or as targets for therapy, but little is known about their physiologic function. Drosophila melanogaster has six CLPs, termed Imaginal disc growth factors (Idgfs), encoded by ldgf1, ldgf2, ldgf3, ldgf4, ldgf5, and ldgf6. We characterized the physiological roles of the ldgfs by deleting each gene using the CRISPR/Cas9 system and assessing loss-of-function phenotypes. Flies lacking all six ldgfs have low viability and fertility. Germ cells form in reduced numbers, and most are lost before reaching the gonad. 100% of adult flies have abdominal cuticle defects, many have ectopic wing veins, and larvae have disorganized denticle belts. Dark cuticle patches appear on the abdomen, indicative of melanotic clots, which normally occur at sites of wounds or pathogen invasion. Eggs laid by sextuple mutants display severe defects in dorsal appendage morphology. Thus, ldgfs function in numerous processes. Given the parallels between ldgfs and their human orthologs, conserved disease pathways,
and sophisticated genetic tools, *Drosophila* is an excellent *in vivo* model for characterizing CLP/Idgf function. By defining the normal molecular mechanisms of CLPS, we will gain insight into the deviations that tip the balance from a physiological to a pathological state. Funding sources: NIH 2 R01 GM079433

**Program Abstract #496**

**Sarcomere formation in skeletal muscle is sensitive to Mylpf dosage**

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Regulatory light chains (RLCs) bind to the lever-arm of the sarcomeric Class II myosin heavy chains and influence myosin function during muscle contraction. RLC knockouts in Drosophila, zebrafish, and mouse have demonstrated critical functions; however, the role of RLCs during early stages of skeletal muscle development are not well understood. We generated knockouts in the two zebrafish RLC genes, *mylpfa* and *mylpfb*. The two *Mylpf* genes are highly similar in sequence and are both expressed in fast-twitch skeletal muscle, with *mylpfa* being the more abundantly expressed gene. In *mylpfa* mutants, trunk muscle strength is dramatically reduced and fin muscles are paralyzed. Myosin II extracted from *mylpfa* mutants functions less efficiently *in vitro*, moving actin filaments (F-Actin) at 75% the velocity of myosin extracted from wild-type siblings, suggesting that reduced myosin function can partially explain the observed muscle weakness. Additionally, *mylpfa* mutant sarcomeres are sparse and loosely packed. While *mylpfb* mutants have no discernable phenotype, loss of *mylpfb* strongly enhances the *mylpfa* mutant muscle phenotype. Myofibers in *mylpfa;mylpfb* double mutant embryos completely lack sarcomeres and instead contain discrete patches of F-Actin or Myosin II. MYLPF is also vital for muscle structure and function in humans. We identified 19 persons in 8 families around the globe with distal arthrogryposis who had congenital distal limb contractures, scoliosis, and short stature. These individuals were either homozygous (10 persons) or heterozygous (9 persons) for exceedingly rare variants in the coding sequence of *MYLPF* that may cause hypomorphic gene function. Similarly, we observe that a zebrafish distal muscle, the posterior hypaxial muscle, is most sensitive to loss of *mylpf*. These findings reveal that normal sarcomere formation requires full *Mylpf* dosage and suggest that reduced *MYLPF* function in humans leads to disease in distal limb muscle. Supported by NIH funding.

**Program Abstract #497**

**In search of a pro-regenerative macrophage**

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Macrophages orchestrate the healing response following injury and are essential for both tissue regeneration and scar-formation. The many roles of macrophages in wound healing, from promoting angiogenesis to clearing tissue debris, have been documented over the past two decades. However, if we could identify what roles are different for macrophages during regeneration compared to scar formation, we would find pathways that create regeneration-permissive environments. This study uses a comparative mammalian model of regeneration and scar-formation to identify similarities and differences in the acute macrophage response after injury. The African Spiny Mouse (*Acomys cahirinus*) is able to regenerate complex tissue of the external ear pinnae after 4mm ear punch injury. *Acomys* close cousin, *Mus musculus*, forms a scar after the same injury. We use *in vitro* macrophage activation assays to identify signaling pathways specific to *Acomys* macrophages. We show two of these mediators, PDGFa and IL1α, are able to promote a matrix turnover phenotype in fibroblasts that mimics regenerative fibroblasts in vivo. Finally, using single cell RNAseq, we identify acute populations of macrophages in *Acomys*, compared to *Mus*, with unique gene expression signatures that include PDGFa and IL1α. These data allow us to pinpoint macrophage pathways that are active during regeneration and not scar-formation as well as pathways that are active during scar formation and not regeneration. Results from this study characterize both a fibrotic macrophage and a regeneration-specific macrophage population and help us test for pathways that may promote tissue regeneration over scar-formation. Funding provided by NIH NIAMS 5R01AR070313, University of Kentucky VP Postdoctoral Fellowship
Program Abstract #498
A metabolic shift to glycolysis promotes zebrafish tail regeneration through TGF-B dependent dedifferentiation of notochord cells to form the blastema

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Mammals are poor at tissue regeneration, often resulting in permanent damage or complete loss of tissues, organs, and extremities following injury. In contrast, fish maintain a high capacity for regenerating complex tissues after injury throughout their lifetime. Studying these processes should provide insights into the pathways necessary to trigger therapeutic regeneration in humans. We utilize the tail of embryonic Danio rerio (zebrafish), which is an ideal model of appendage regeneration due to its easy manipulation, relatively simple mixture of cell types, and superior imaging properties. We show that tail amputation triggers an obligate metabolic shift to glycolysis in cells comprising the notochord during the repositioning of these cells near the amputation site. Inhibition of glycolysis does not affect the health of the embryo but blocks fin regeneration due to failure to form a normal blastema. To gain a better understanding of the molecular pathways that are regulated by metabolic signaling, we performed single cell RNA-sequencing on regenerating tails under normal conditions and in the absence of glycolysis. Strikingly, we detected a transient cell population in the single cell analysis that represents notochord sheath cells undergoing a TGF-B dependent epithelium-to-mesenchyme (EMT) transition to blastema cells. We further demonstrate that the metabolic switch to glycolysis is required for TGF-B signaling and blocking either glycolysis or TGF-B receptors results in aberrant blastema formation through the suppression of essential EMT mediators such as snai1. These studies not only provide new insights into tissue regeneration, but also cancer biology by demonstrating that the shift to glycolysis in the Warburg effect is not only utilized by rapidly proliferating cells, but is a cell signaling trigger that promotes EMT. This research was supported by the Intramural Research Program of the National Human Genome Research Institute (ZIAHG200386-06).

Program Abstract #499
Localized and tissue-wide changes during regeneration revealed by single-cell analysis in Drosophila

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Regeneration of damaged tissues is a complex process that requires the surviving cells to respond, proliferate, and repattern to replace what was lost. Drosophila imaginal discs are capable of regenerating following blastema formation, which is marked by local cell proliferation and increased cellular plasticity. It remains not well understood what occurs within individual cells during regenerative growth, including what genetic programs promote cellular reprogramming and whether repatterning recapitulates early development. Genetic methods in Drosophila have enabled the study of imaginal disc regeneration in situ following the ablation of a defined portion of the disc by the spatially-restricted expression of a pro-apoptotic gene during a short period of larval development. We used single-cell transcriptomics to profile cells collected from developing and regenerating wing discs. We harmonized these data sets to generate a combined cell atlas, allowing us to assign cells to different domains in the tissue including the regeneration blastema. By investigating gene expression changes at single-cell resolution, we observed diverse cellular responses at different distances from the site of tissue damage. We characterized unique transcriptional profiles of an inner and outer blastema, as marked by the gene expression of upd3 and Ilp8. In addition, we observed a global response to tissue damage where cells from the regenerating discs express many marker genes that are normally expressed only earlier in development. In contrast, many genes expressed locally around the site of damage were regeneration specific. Our analysis of the single-cell data has uncovered a number of genes that are important for promoting proper regeneration including predicted transcription factors and secreted proteins. These studies will lead to a better understanding of the cellular and molecular basis of regeneration. This work is funded by the NIH (R35 GM122490).
Program Abstract #500
Human pluripotent stem cell modeling of the ATOH1 lineage reveals a heterochronic shift in transcriptional regulators in the developing human cerebellum
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Development is regulated by conserved transcriptional programs across species, but little is known about divergent mechanisms that create human-specific characteristics. To model human cerebellar development, we devised a directed differentiation method to recapitulate the temporal sequence of differentiation of the human cerebellar ATOH1 lineage from human pluripotent stem cells. This progenitor lineage gives rise to waves of distinct excitatory neuronal subtypes including the cerebellar granule cells; a cell-type implicated in childhood disorders from autism to medulloblastoma. By cell-type specific translational profiling, we found that by day 28 in culture, the in vitro-derived human ATOH1 lineage matches the in vivo human cerebellum at 13-17 post coitus weeks. Genes detected were characteristic of a granule cell progenitor state. In an unexpected finding, the expression of several genes, which in the mouse are classically associated with differentiated neurons, were detected in this progenitor population. Immunohistochemistry on human fetal cerebellar sections confirmed a spatial shift in the expression of NEUROD1 and NeuN (RBFOX3) to the outer external granule cell layer (EGL), where progenitors reside. By contrast, in the mouse, these factors were limited to postmitotic cells only as previously reported. Ki67 co-labeling showed that the human EGL contains fewer cells in the cell cycle than its mouse counterpart, and that a large proportion of the human Ki67+ cells co-labeled with the “differentiation markers”. Thus, our platform unravels a heterochronic shift in the expression of “differentiation factors” within the human EGL in vivo. We hypothesize that this species-specific molecular divergence keeps the human ATOH1 pool in a quiescent state for longer, allowing the EGL to exist for up to two years post birth in humans but only two weeks in mice. Funding: NIH, The Renate, Hans, and Maria Hofmann Trust, The Rockefeller University.

Program Abstract #501
Characterization of p63 positive basal cells in Vomeronasal organ
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The Vomeronasal organ (VNO) is a part of accessory olfactory system (AOS). The VNO plays a primary role in the detection of pheromones, chemical factors that triggers a spectrum of sexual and social behaviors. The vomeronasal epithelium (VNE) is believed to share multiple features with the epithelium of the main olfactory epithelium (MOE), however it has been less characterized compared to the latter. Sox2 positive cells have been previously identified, in VNE, as the stem cell population that give rise to downstream neuronal progenitors and neurons. On the other hand, in MOE p63+ve horizontal basal cells (HBCs) had been identified as a second pool of stem cells that becomes active in response to injury. Currently no available studies in literature describe the existence of HBCs or HBC like cells in VNE. Based on these premises we explored whether cells with features similar to the HBC are present in VNO and how these develop. Our experimental approach includes immunolabeling and inducible in vivo Cre genetic lineage tracing. Our data suggest that p63 positive cells are present in VNO. We characterized these vomeronasal HBCs (vHBCs) from embryonic, early postnatal and adult stages. Preliminary data from Cre inducible mouse models showed that HBCs give rise to sustentacular cells and vomeronasal sensory neurons even without injury. In conclusion, for the first time we describe spatial and temporal expression pattern of vHBC stem cells in VNO. Future studies include characterizing injury models in VNO and to test whether HBCs in the VNO can regenerate. This project is funded by NIDCD 1R01DC017149-01A1, NIH 1R01HD097331-01 and 1R15HD09641101 grants.

Program Abstract #502
Reprogramming of human retinal pigment epithelium to retina progenitors
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Some amphibians spontaneously regenerate lost retinal tissue by reprogramming cells of the retinal pigment epithelium (RPE) to neural retina progenitors (NRPs). Conversely, mammalian RPE responds to retinal injury by adopting a fibrotic program, culminating in scar tissue formation and vision loss. To explore the possibility for reprogramming human RPE to NRPs, we engineered an hiPSC line that contains coding sequences for a P2A self-cleaving peptide and cyan fluorescent protein fused to the endogenous NRP-specific gene VSX2. Retina organoids differentiated from the engineered line undergo normal retina development and correctly label NRPs with cyan fluorescence. We differentiated the reporter line to mature RPE for use in a screen for identifying RPE reprogramming factors, including FGF2, TGFβ receptor inhibitor SB-431542, WNT inhibitor XAV-939, NAC, and EGTA, selected based on their roles in animal models of RPE reprogramming. Following the screen, the optimal composition of a cocktail for inducing NRP formation was estimated, simultaneously taking into account the factors’ observed abilities to dampen fibrosis-associated genes, induce NRP-associated genes, and repress RPE identity. The predicted optimal cocktail induced NRP formation in RPE derived from four independent hiPSC lines. RNA sequencing of reprogrammed RPE revealed robust epithelial dedifferentiation and activation of neurogenic gene programs. Moreover, we observed broad activation of eye field transcription factors, suggesting that RPE was reprogrammed to a multipotent state resembling the optic vesicle. Importantly, we observed a repression of fibrotic and contractile gene sets. Collectively, these observations demonstrate the capacity for human RPE to support direct reprogramming to NRP cells under the influence of a defined treatment of small molecules and growth factors. Funding sources: NEI EY026816 to KDRT, Sigma Xi GIAR to PTL and MLR, Prevent Blindness Student Fellowship to PTL.

Program Abstract #503
Inflammasomes and Autophagy in Endometrium during the course of Embryo Implantation
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Implantation of embryo in the endometrium is a rate limiting factor for reproduction in Eutherian mammals. The success of initiation of pregnancy depends on genetic and cellular interactions between the embryo and the endometrium all of which should be executed properly in a correct temporal frame for pregnancy. Amongst the various processes, immunomodulation in the endometrium is essential to accommodate the semi-allogenic blastocyst and according to the current theory, pregnancy is an anti-inflammatory state. However, we discovered that initiation of embryo implantation is actually an inflammatory condition which switches anti-inflammatory state upon establishment of placenta in the mouse. We uncovered a specific activation of IL1β in the mouse endometrial decidual cells at the site of implantation. This expression of IL1β was also associated with activation of NLRP3 and ASC positive inflammosomes and LC3B positive autophagosomes in the decidual bed at the time of implantation and early pregnancy. This activation inflammation is also observed in human endometrial cells during the course of in vitro decidualization In vivo. Single cell RNA-Seq data of human feto-maternal interface revealed a cell type specific activation of inflammatory molecules during early pregnancy. Together our results show that regulated inflammation is one of the regulatory process in initiation of pregnancy in mammals. We would like to acknowledge ICMR for institutional funds and DST-INSPIRE for doctoral fellowship.

Program Abstract #504
Analyzing mitochondrial single amino acid variants for mito-nuclear incompatibilities among Danionin species
Trevor Chamberlain
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Barriers to successful interbreeding arise as mechanisms of selection act on diverging species. One such barrier is postzygotic isolation, in which mating and fertilization occur, but embryos remain inviable. A suspected source of species isolation lies in incompatibilities between the mitochondrial genome (mtDNA) of one species and the nuclear genome of another. Members of the cyprinid subfamily Danioninae, which includes zebrafish, comprise a model phylogeny for studying mechanisms of postzygotic isolation by examining hybrid and nucleocytoplasmic hybrid (cybrid) embryonic development. To begin to study mito-nuclear incompatibilities, we sequenced mtDNA from Danio rerio, D. aesculapii, D. kyathit, D. albolineatus, D. margaritatus, and Devario aequipinnatus to identify
candidate sites of incompatibility due to single amino acid variants (SAVs) among mitochondrial proteins. We performed analyses to predict functionally differing sites across species, which show increased levels of divergence in the Nd5 and Nd6 protein subunits of OXPHOS complex I in Danio albolineatus and all three Cox proteins of complex IV in Devario aequipinnnatus. We also examined mitochondrial proteins for increased rates of amino acid divergence above neutral and found sites that experience positive selection, specifically within the ATP synthase subunit Atp8 and complex IV factor Cox1. Using the high degree of mitochondrial protein conservation among vertebrates, we also used 3D protein modeling to identify interacting nuclear-encoded gene candidates for further functional variant and positive selection analyses. These studies will determine co-evolving mitochondrial and nuclear genes that underlie mechanisms of postzygotic isolation during speciation, and provide targets for mitochondrial fitness assays in cybrids and hybrids. Funding was provided by Hatch/USDA award from the College of Agricultural and Life Sciences at UW-Madison and NIH grant T32GM007133.

Program Abstract #505
Assessing the mid-blastula transition within an interspecies model system across Danionin species.
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Mechanisms underlining animal oocytes’ ability to be nuclear reprogrammers can be harnessed by somatic cell nuclear transfer (SCNT) to study molecular events pivotal to embryonic development. A derivative method called interspecies somatic cell nuclear transfer (iSCNT) has been developed in which a somatic cell nucleus is transferred into an enucleated donor oocyte obtained from a relative species - generating a nucleo-cytoplasmic hybrid (cybrid). Molecular boundaries involve promoting functional interactions between the donor nucleus and the host egg. Phylogenetic parameters dictate these boundaries. Understanding these parameters are imperative to drive the use of iSCNT in translational applications. The model developmental system, zebrafish (Danio rerio), along with an array of members from the Danionin family, including (from proximal to distal relatedness to Danio rerio) D. kyathit, D. albolineatus, D. margaritatus are to be used as a “model family” for testing nucleo-cytoplasmic incompatibilities of iSCNT cybrids at the key developmental timepoint, the mid-blastula transition (MBT). Normal developmental staging of zebrafish is recapitulated by all cybrid embryos in early developmental timepoints prior to MBT. Following MBT (4 hours post-fertilization (hpf)), the D. kyathit cybrids continued to exhibit no changes in cell cycling. D. albolineatus and D. margaritatus cybrid embryos exhibited elongated cell cycles and subsequent delays in developmental staging (16 and 17 minutes per cell cycle, respectively). Developmental defect was observed in 75% of D. margaritatus cybrids by 12 hpf. The other cybrid groups survived up to 48 hpf. The more proximally related D. kyathit cybrids demonstrated indistinctive development to zebrafish, while the more distally related species, D. albolineatus and D. margaritatus, exhibited developmental delay, at the MBT, and predominant developmental defect in D. margaritatus. Funding: Hatch/USDA

Program Abstract #506
Sex Determination in Xenopus
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The mechanism of sex determination varies greatly among species. Amphibians use both ZZ, ZW and XX, XY systems. Amphibians belonging to the Xenopus genus do not have diverged sex chromosomes, but instead have sex determining loci on autosomes. Xenopus uses a ZZ, ZW system, where the female is the heterogametic sex. Both X. laevis and X. borealis are tetraploid and are closely related but have different sex chromosomes; chromosome 2L for X. laevis and chromosome 8L for X. borealis. In contrast, in X. tropicalis, which is diploid, its sex determining locus is on chromosome 7. Of the three species, only X. laevis contains the dm-w gene, which is located on the W chromosome; it is believed that dm-w determines female sex by inhibiting dmrt1, although this has not been proven as its only function. Neither X. borealis nor X. tropicalis contain dm-w, and it is unknown whether there is a single sex-determining gene responsible for female sex in these species. In this poster I will present our work investigating the mechanistic aspects of how sex chromosomes and genetic sex determination evolve and work in Xenopus, including CRISPR-Cas9 deletion of W-specific genes and the effects of transgenic
overexpression of dm-w in all three species. The long term goal is to create knock-out and knock-in lines of X. laevis, X. tropicalis, and X. borealis to develop a comparative framework with which to better understand how the genetic basis of sex determination evolved, and how this system varies among closely related species. This project is funded by the NIH R01HD084409, P40OD010997.

Program Abstract #507
Dynamic changes in cell type specific transcriptome during sex determination in the mouse
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Sex determination in the gonads is regulated by a complex genetic network where the gonad which is in a bi-potential state which upon activation of genetic program can develop either in to an ovary or in to a testis. In mouse this occurs between a narrow time frame termed as window of sex determination. According to current knowledge, mammalian sex determination is initiated in the somatic cells by the Sry and Sox9 aid inSertoli cell differentiation and the rest of the cells follow this decision. In absence of Sry and/or Sox9 signaling, in XX embryos a mutually antagonist set of genes must be activated in the somatic cells for ovarian development. However the genetic networks that operate in other cell types to regulate sex determination is not well explored. Bulk RNA Seq of XX and XY embryonic gonad during the window of sex determination identified several male and female biased genes. We segregate the male and female biased genes in the four different cell types based on their levels of expression. Unexpectedly, we found that sex determination involved changes in gene expression simultaneously in multiple cells types. We also identified sex biased, cell type specific genetic networks and transcription factors which could be novel candidates in the process of sex determination. Our results suggest that a smaller group of cell type specific genes, rather than larger groups of genes in selected pathways regulated during sex determination. These observations provided a cell type specific frame work of genes and pathways that potentially govern sex determination and gonad development. We are thankful to the funding agencies, Indian Council of Medical Research (ICMR), Govt. of India and UGC for the research fellowship.

Program Abstract #508
Lunatic Fringe-dependent glycosylation of Deltalike3 regulates oscillatory Notch activation in Mus musculus and Gallus gallus models of vertebrate segmentation
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Notch signaling is a highly conserved pathway that regulates the development of many organ systems in vertebrates. During somitogenesis, oscillatory Notch activation regulates a segmentation clock that synchronizes mesodermal stem cell differentiation to promote development of axial skeleton precursors. Oscillations in the expression of Notch1, Dll1, and Lfng in mammals are required for clock function and minor disruptions in the oscillatory expression of these genes results in abnormal somitogenesis. A Notch ligand, Dll3, that does not have oscillatory expression and can only cis-inhibit Notch signaling is also required for normal segmentation. How DLL3 regulates the clock is largely unknown. We hypothesized that oscillatory Lfng expression results in oscillatory glycosylation of DLL3 altering its capability to cis-inhibit Notch signaling. We find that Dll3 and Lfng have distinct effects on Notch activity and that loss of Dll3 is epistatic to loss of Lfng. Preliminary data shows that increased expression of unglycosylated DLL3 results in a reduction of NOTCH1 protein and increased localization of DLL1 to the cell surface. We also investigate how glycosylation of DLL3 affects its binding affinity for NOTCH1 and DLL1, altering their localization to the cell surface. To examine the importance of DLL3-mediated cis-inhibition in other vertebrate clades, we annotated a novel DLL3 ortholog in chickens (cDLL3) and find it is expressed in the PSM during somitogenesis. We characterized cDLL3’s subcellular localization and the glycosylation of its EGF repeats. A future goal is to generate mouse models that express unglycosylatable DLL3 in embryonic mesodermal tissue to study how DLL3 glycosylation regulates the segmentation clock and axial skeleton development in vivo. Work funded by 5R21HD095150.
Program Abstract #509
Investigation of Notch Signaling in Cone Fate Specification in Vertebrate Retina
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In the vertebrate retina, cone photoreceptors are crucial for high acuity color vision. Several retinal diseases lead to loss of cones and there is a need to identify the normal developmental genesis of these cells to inform the development of stem cell-based therapies. Cone genesis has previously been shown to be repressed by Notch signaling, however, the mechanism behind this phenotype is unknown. It has recently been identified that cone photoreceptors are formed from multipotent retinal progenitor cells (RPCs) that first generate genetically-defined, restricted RPCs with limited mitotic and fate potential to preferentially form cones and horizontal cells. Here we use cell type specific inhibition of Notch signaling to localize the primary role of Notch signaling in the formation of cone restricted retinal progenitor cells from multipotent retinal progenitor cells. Our data supports a model in which Notch signaling regulates the formation of restricted RPCs from multipotent RPCs, and is not involved in later steps of cone formation. We also proved that cell cycle promotion is not a primary effect of Notch signaling but an indirect effect of progenitor cell state transitions that leads to depletion of the multipotent progenitor cell population. Acknowledgement: Funding for this project was provided by NSF CAREER 1453044 (to M.E.) and supported by NIMHD 3G12MD007603-30S2 (to CCNY).

Program Abstract #510
Salinity-dependent invasion of skin-derived ionocytes into hair cell-containing mechanosensory organs
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Sensory hair cell function in the inner ear critically depends on the ionic composition of the surrounding endolymph. Homologous to those of the inner ear, aquatic vertebrates possess hair cells in lateral line neuromasts, mechanosensory organs that detect changes in water motion. Surrounded by a gelatinous matrix called the cupula, these hair cells are located superficially on the skin of the zebrafish head and trunk, exposed to possible fluctuations in ambient ion composition. While active maintenance of ion balance in the cupula has been suggested, little is known about the cellular and molecular mechanisms ensuring proper ion composition surrounding lateral line hair cells. Here, we identify a new population of cells that contributes to the lateral line and that is potentially involved in ion homeostasis. Intriguingly, we find that individual pairs of cells frequently invade the neuromast from the surrounding skin. Using in vivo imaging, we show that after entering, these initially motile cell pairs reside in a stereotypical position and develop a long apical extension towards the cuticular plate. Molecular characterization of this new cell population by scRNAseq and in-situ hybridization reveals the presence of multiple genes encoding ion channels that are shared with ionocytes, specialized cells involved in body fluid osmoregulation. In agreement with this finding, the number of these cells in the neuromast negatively correlates with media salinity. Finally, preliminary experiments suggest that CRISPR/Cas9-mediated knockout of transcription factors involved in ionocyte development also negatively affects the presence of these cells in the lateral line. Taken together, our study provides a detailed description of a skin-derived ionocyte population capable of invading a mechanosensory organ, contributing to our understanding of ion homeostasis in the neuromast and potentially the inner ear. (Funding: Stowers Institute for Medical Research)

Program Abstract #511
LIM homeodomain (LIM-HD) transcription factor, Lhx2 regulates meiotic progression in mouse fetal ovary
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Meiosis is a process by which sexually reproducing organisms generate gametes which is essential for formation of the embryo. In mammals, the entry of germ cells in meiosis is temporally regulated in a sexually dimorphic manner. In the mouse, the germ cells in the ovary enter meiosis at embryonic day (E)13.5 while the male germ
cells undergo mitotic arrest to enter meiosis at postnatally. Several molecular players have been identified that regulate this sexually dimorphic entry of germ cells in meiosis, herein we report the involvement of the LIM homeodomain (LIM-HD) transcription factor Lhx2 in this process. We discovered that several LIM-HD genes are expressed in gonads of several species (including human) during window of sex determination; amongst this Lhx2 has higher expression in XX gonads just preceding to the timing of entry into meiosis. RNA-seq of mouse gonads knockouts for Lhx2 revealed altered expression of genes in regulation of DNA methylation and germ cell meiosis. We found that expression of genes involved in meiotic machinery were disrupted in the gonads of XX mice during development, the germ cells were suspended in prophase I in mutant ovaries. This block in meiosis is downstream to acquisition of meiosis competency by Dazl but upstream to Stra8 possibly in a BMP2 dependent manner. Taken together our results show that, Lhx2 cell autonomously regulates the entry of XX germ cells in meiosis during development. We thanks to Department of Biotechnology (DBT) for funding the project to DM and junior Research Fellowship (DBT-JRF) to AB. NS and DD are grateful to Indian Council of Medical Research–Senior Research Fellowship (ICMR-SRF) and UGC-JRF respectively.

Program Abstract #512
The interplay between environmental signals and position of cells is decisive for T-cell lineage commitment
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Progenitor cells derived in the hematopoietic tissue move toward the thymus to specify as T-cells and then differentiate into two fundamentally distinct sublineages, which are defined by their expression of an αβ or a γδ T-cell antigen receptor (TCR). How they take separate lineages in the thymus is mainly explained by the emergence of cell-intrinsic mechanisms that mutually reinforce or antagonize a given fate choice. To what extent environmental input and position of cells influence the lineage choice is poorly understood. To address this issue, we chose to use medaka (Oryzias latipes) as a model system because the ease of in vivo imaging of the entire thymus and genetic perturbations allowed us to perform a systems-level study. Here, we implemented various genetic perturbations to either manipulate the expression level of environmental cues, alter developmental signals, or to impair the correct positioning of developing T-cells. Our findings revealed that while the Notch signal provided by thymic niche is required for the specification and commitment of both T-cell sublineages, a niche-specific production of interleukin-7 biases developing T-cells towards the γδ fate. On the other hand, the position of developing T-cells within the thymus has an impact on their fate choice. For example, when developing thymocytes through lack of chemokine receptor Ccr9b lose their ability to position within the thymus correctly, they were biased towards the γδ T-cells at the expense of αβ lineage. In conclusion, we propose a central role for environmental cues and intrathymic cell positioning in T-cell lineage decision process. This work was supported by the Deutsche Forschungsgemeinschaft (DFG) [grant number: BA5766/3-1].

Program Abstract #513
Twist1a limits cardiomyocyte differentiation in zebrafish
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Differentiation of an appropriate number of cardiomyocytes is crucial for the proper formation of the heart, as too few or too many cells can lead to a dysfunctional organ. However, the mechanisms that control the initiation and progression of myocardial differentiation are not yet fully understood. Here, we show that the bHLH transcription factor twist1a can limit myocardial differentiation in the zebrafish embryo. Reducing the function of twist1a with morpholinos results in an increased number of cells expressing myocardial markers. Conversely, overexpression of twist1a diminishes the formation of differentiated cardiomyocytes. Together, these data suggest that twist1a acts to constrain myocardial differentiation. This role of twist1a contrasts with that of hand2, a bHLH transcription factor that promotes the progression of myocardial differentiation and the formation of an appropriate number of cardiomyocytes. Interestingly, twist1a knockdown results in a substantial recovery of
cardiomyocyte production in hand2 hypomorphs, suggesting an antagonistic relationship between twist1a and hand2 in this context. In addition, twist1a knockdown in hand2 null mutants does not have a significant effect on cardiomyocyte formation, suggesting dependence of Twist1a function on the presence of Hand2. Our ongoing experiments aim to investigate further a model in which antagonism between Twist1a and Hand2 insures timely and effective differentiation of an appropriate number of cardiomyocytes.

Program Abstract #514
**Fatty-Acid ß-Oxidation regulates the differentiation of Drosophila myeloid progenitors**
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Stem cells generate progenitor cells, which further differentiate into more mature cell types. Although the role of metabolism in regulating stem cells is very well documented, its role in progenitor homeostasis is yet to be elucidated. To investigate the metabolic demands of progenitor cells, the blood progenitors in the hematopoietic organ of Drosophila larvae; the lymph gland were chosen. Blood progenitors found in Drosophila larval lymph gland belong to myeloid lineage and are multipotent. These progenitors are proliferating in early developmental stages and become G2/M arrested in late larval stages. The proliferating progenitors are marked by a high level of glycolysis whereas the G2/M arrested progenitors switch onto Fatty-Acid ß-Oxidation (FAO) as the preferred metabolic state. The disruption of FAO leads to the loss of G2/M arrest and abrogated differentiation of blood progenitors. Likewise, upregulation of FAO leads to precocious onset of quiescence and differentiation of blood progenitors. Further detailed analysis reveals the altered histone acetylation profile in FAO mutants. Restoration of histone acetylation rescues the blood progenitor quiescence and differentiation defects in FAO mutants. Our genetic and molecular analysis reveals that the transcription of rate limiting enzyme of FAO, CPT1/whd is regulated by Jun-Kinase (JNK). The ROS-JNK axis has been known to regulate the differentiation of these progenitors. Thus, our study unravels how the metabolic circuitry integrates with cellular signaling to commemorate the blood progenitor differentiation. **Funding:** Wellcome Trust DBT India Alliance: Lolitika Mandal, CSIR, India: Satish Kumar Tiwari

Program Abstract #515
**Analysis of vertebrate Delta like-3 genes and proteins: Implications for cellular localization and function**
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The Notch signaling pathway regulates cell-cell communication pathways, cell fate, cell death, embryonic development, and adult tissue differentiation in metazoans. In mice, the Delta-like 3 (DLL3) protein inhibits Notch signaling and is localized to the secretory system. In contrast, the zebrafish DLL3 orthologues, DeltaC and DeltaD, activate Notch and are found on the cell surface. Thus, DLL3 has evolved from an activatory ligand in the common vertebrate ancestor to an inhibitory ligand in mammals, concomitant with altered subcellular localization. Evolutionary analyses of vertebrate DLL3 sequences were performed using neighbor joining and maximum likelihood approaches. Comparison of DLL3 genomic sequences found that reptile sequences are more similar to fish and amphibian orthologues than birds or mammals. To investigate function, we utilized Gibson assembly to clone the A. carolinensis dll3 cDNA, an amniote reptile, transfected several mammalian cell types and anole satellite cells and examined its localization in immunofluorescently-labeled cells by confocal microscopy. Our analysis demonstrates that A. carolinensis DLL3 localizes in the cell secretory system in both mammalian and reptile cells. These data show that the A. carolinensis DLL3 protein sequence is evolutionarily closer to fish and amphibians, but it shares the mammalian DLL3 cellular localization, and possibly, function as well. This was funded in part by SOLS to JWR.
Program Abstract #516

Prodomain-mediated Regulation of Vg1-Nodal Signaling during Animal Development

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Developmental signaling proteins are secreted asymmetrically in the early animal embryo in order to generate diverse tissues. Vg1 (a.k.a. GDF1/GDF3) and Nodal form obligate heterodimers to induce mesendoderm formation in animal embryos. In zebrafish, vg1 is maternally deposited and its protein is found in the endoplasmic reticulum (ER) prior to zygotic expression of nodal at the yolk margin. We examine how Vg1 and Nodal prodomains influence the formation of the Vg1-Nodal signaling gradient. First, we find that glycosylation sites and an exposed cysteine thiol of the Vg1 prodomain mediate retention in the ER. ER retention of Vg1 may ensure that Vg1 participates in heterodimers with Nodal. Second, by developing a secreted synthetic protease that can cleave the Vg1 prodomain, we find that prodomain cleavage is not required for Vg1-Nodal secretion and can occur in a non-cell autonomous manner. Third, while prodomains of Nodal and Vg1 remain associated with secreted heterodimers, cleavage of the Vg1 prodomain is necessary for receptor activation in target cells. These findings reveal how Vg1 and Nodal prodomains regulate the generation of functional signaling in vivo. Funding from NIH DP1-HD094764 and Allen Discovery Center for Cell Lineage Tracing

Program Abstract #517

Developmental Bisphenol A Exposure Induced Hypomethylation In Primordial Germ Cells And Sperm In Medaka Fish

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DNA methylation is a major epigenetic modification that undergoes dramatic changes in two epigenetic reprogramming windows during development: first in preimplantation embryos and second in primordial germ cell (PGC) specification. In both windows, DNA methylation patterns are reprogrammed genome-wide, and the majority of inherited methylation marks are erased, generating cells with broad developmental potential. We recently reported that the reprogramming of genome methylation in medaka is similar to human and mouse, suggesting that medaka may serve as a suitable biomedical model for comparative studies focused on the epigenetic and transgenerational inheritance of phenotypic traits. Here we characterized DNA methylation alterations in response to bisphenol A exposure during the window of epigenetic reprogramming and in the sperm using whole genome bisulfite sequencing of genome. Medaka embryos were exposed to BPA (10 μg/L) for 12 days from the day of fertilization, during which PGCs undergo genome-wide demethylation. Embryonic BPA exposure caused hypomethylation in PGC genome, some of which persisted in the sperm in adulthood. The methylation differences between control and BPA-exposed group were higher in PGCs than in sperm. The majority of DMRs established in PGCs were removed during spermatogenesis except for 20% DMRs, which were represented in sperm. Taken together, our results suggest that BPA induces hypomethylation genome-wide and the BPA-induced epimutations can escape reprogramming in PGCs and are retained by sperm. Our results provide insights into epigenetic mechanisms underlying epigenetic inheritance of environmentally induced phenotypes. Acknowledgements: Supported by National Institute of Environmental Health Sciences (NIEHS R21 #ES027123) to RKB.

Program Abstract #518

Role of gabra1 during developmental behavior.

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Mutation within the genes that encode for the different subunits of the gamma-aminobutyric acid type A receptor (GABA<sub>A</sub>R) are known to result in many neurological disorders, including epilepsy. The GABA<sub>A</sub>R interacts with the major inhibitory neurotransmitter, GABA, resulting on hyperpolarization of neurons. In a recent publication we show a case study of a patient diagnosed with multiple congenital anomaly characterized by a severe seizure disorder (1). Using whole exome sequencing, we identified a mutation in the GABRA1 gene, which encodes for the
To determine the mechanisms by which mutations in GABRA1 induce seizure phenotypes during early development, we developed a zebrafish paradigm of gabra1 deficiency. We utilized morpholino technology to transiently knockdown gabra1, followed by high throughput behavioral analysis of gabra1 morphants. Knockdown of gabra1 resulted in behavioral deficits characterized by an overall decrease in total distance and total swim speed at 5 days post fertilization. Moreover, this hypoactive phenotype is accompanied by changes in gene expression of other GABAAR subunits. Importantly, gabra1 morphants are still able to respond to pentylenetetrazole stimuli, suggesting that defects in gabra1 expression alter the normal composition of the GABAAR resulting in a unique channel conformation and function. Collectively, these data demonstrate that gabra1 plays a key role in the developmental behavior of zebrafish. This work was supported by NIH NINDS Grant no. 5K01NS099153-02, the Bridges to the Baccalaureate Program grant no. 2R25GM049011-16, the RISE program Grant no. R25GM069621-11, and the BBRC Grant no. 2G12MD007592 from NIMHD. 1. Reyes-Nava NG, Yu H-C, Coughlin CR, Shaikh TH, Quintana AM. Abnormal expression of GABAA receptor subunits and hypomotility upon loss of gabra1 in zebrafish. Biol Open [Internet]. 2020 Apr 15 [cited 2020 Apr 14];9(4). Available from: https://bio.biologists.org/content/9/4/bio051367

Program Abstract #519

Understanding ALS using the Drosophila Tripartite synapse.

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Amyotrophic Lateral Sclerosis (ALS) is a progressive motor neurodegenerative disorder. ALS patients have a life expectancy of 3-5 years, with no known cure. Research has focused primarily on the death of motor neurons, while the other two cell types that define the tripartite synapse, namely muscle and glia are believed to contribute to the onset and/or progression of the disease, but are not well studied. We are attempting to understand the molecular and cellular basis of the disease by using Drosophila melanogaster as a model organism. As a first step, we modulate the levels of fly orthologs of human genes that cause familial ALS independently in neurons, glia and muscles. Then we measure changes in lifespan and motor function in these flies. We use, as a sensitized genetic background, a point mutation in the ALS8 causative gene VAP33A(VAP). Fly lines carrying the VAP^{PS8S} mutation (VAPnull;vap-promoter>VAP^{PS8S}) have a short life span, VAP^{PS8S} positive cytoplasmic inclusions and motor defects that aggravate with age. In this background, we used the UAS-Gal4 system and modulated the activity of seven Drosophila genes, namely VAPB, FUS, TBPH, SOD1, VCP, Alsin and SETX. We find that each cell-type in the synapse shows differential sensitivity to the modulation of genetic activity for each gene. Our data strongly suggests that ALS mutations contribute to the disease, from each cell type. Most interestingly, overexpression of a mutant form of VCP^{P152S}, in some cell types, but not in others, leads to a distinct improvement of lifespan and motor function. This suggests that the VCP linked Ubiquitin Proteasomal System is involved in the progression of the disease. We expect to uncover novel genetic interactions between ALS genes within a cell type and uncover interactions of ALS genetic sub-networks with anterograde and retrograde signals at the tripartite synapse. Funding: Department of Biotechnology (DBT) & Indian Council for Medical Research (ICMR), Govt. of India.

Program Abstract #520

Wnt pathway mutants show differential hair cells regeneration in the zebrafish lateral line

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Mechanosensory hair cells in the mammalian ear mediate hearing. Damage to these specialized cells leads to irreversible hearing loss. In aquatic vertebrates, hair cells are also found in the lateral line system, which senses variation in water current. The lateral line is composed of neuromast mechanosensory organs, which consist of hair cells, supporting cells and mantle cells. In zebrafish, hair cells of the lateral line have demonstrated a robust ability to regenerate following injury. Previous studies have suggested that hair cells are replaced via the division and differentiation of their surrounding support cells. The potential role of the Wnt signaling pathway in hair cell regeneration has been suggested using pharmacological modulators of the Wnt pathway. In the current study, we examine a set of Wnt signaling mutant zebrafish lines to define the pattern of hair cell regeneration following
damage. The binding of the Wnt ligand to its receptor, LRP5, induces a cascade of events leading to the activation of the transcription factor Lef1. Mutations in LRP5, Lef1 and a negative regulator of Wnt signaling, Kremen1, were used to study the role of Wnt signaling. These Wnt pathway mutant lines show distinct patterns of proliferation of supporting cells post injury and subsequent numbers of regenerated hair cells. These results suggest that disrupting Wnt signaling at distinct points in the pathway leads to differential patterns of hair cell regeneration. An understanding of the role Wnt signaling plays in hair cell regeneration will be of importance in future investigations of hearing loss reversal. This project was completely funded by Dr. McGraw’s start-up funds from the University of Missouri-Kansas City, School of Biological and Chemical Sciences.

Program Abstract #521

Polycomb Repressive Complex 1 genes regulate tissue regeneration in the planarian Schmidtea mediterranea

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E3 ubiquitin ligases mediate the conjugation of ubiquitin onto proteins, a modification that is an essential regulator of cellular processes like transcription and protein degradation. The specificity of ubiquitylation is a critical step conferred by the action of hundreds of E3 enzymes. However, the biological functions of the E3s are not well understood, especially in the context of regeneration. Planarians are an excellent model to study signaling pathways that regulate regeneration owing to their ability to regrow lost tissues from pluripotent stem cells. We used RNAi to analyze the role of RING E3 ubiquitin ligases in planarian regeneration by inhibiting 88 RING genes enriched in stem and progenitor cells. We found nine genes that were essential for regeneration and homeostasis, including rnf2, a Polycomb Repressive Complex 1 (PRC1) gene that ubiquitylates histone H2A (ub-H2A). rnf2 RNAi caused aberrant regeneration and a decrease in the levels of ub-H2A. Intriguingly, inhibition of canonical PRC1 (cPRC1) genes cbx and phc did not affect levels of ub-H2A, but caused striking phenotypes of a dorsal lesion, located anterior to the pharyngeal feeding organ, and the mis-localization of the pharynx. Marker gene analysis after phc RNAi indicated that a population of progenitors that specify the pharynx were not maintained. To examine the molecular basis of the diverse phenotypes, we are performing RNA-seq after inhibition of rnf2 or phc. Future work to profile the changes in ub-H2A during regeneration should provide insight into the enigmatic function of ubiquitylation as an epigenetic regulator of cell differentiation. We demonstrate that PRC1 is a critical regulator of stem cells and regeneration and find surprisingly diverse roles for the complex depending on if the E3 activity of PRC1 or cPRC1 activity is perturbed. [J.M.A. was supported by the ARCS Foundation and M.B. by NIH IMSD R25GM058906; this work was funded by NSF IOS-1350302 and NIH R01GM135657 to R.M.Z.]

Program Abstract #522

Investigating the roles of the RINGs involved in regulating Notch signaling and PRC1 function during planarian regeneration

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Ubiquitination is a post-translational modification that affects many cellular processes, including endocytosis, transcription and protein degradation. The specificity of ubiquitylation is mediated by the E3 ubiquitin ligases, for which biological roles remain poorly understood. To gain insights into the role of ubiquitination in stem cell-based tissue regeneration, we are studying the function of E3 ligases in the planarian Schmidtea mediterranea using RNAi. Here, we examined the largest class of E3s, the RING-type; we screened 88 RING E3s expressed in stem and progenitor cells and chose to further investigate genes involved in Notch-signaling and Polycomb Repressive Complex 1 (PRC1) factors. Notch signaling requires the action of two E3s, Mindbomb (mib) and Neuralized (neurl), to endocytose Delta and activate Notch in a neighboring cell. We identified five mib and five neurl planarian homologs; upon inhibiting all genes individually we did not observe obvious changes in neuronal marker gene expression, indicating possible genetic redundancy. To elucidate these roles, we plan to perform combinatorial RNAi knockdowns for co-expressed genes. In addition, our screen uncovered a critical role the for PRC1 member, rnf2, which led us to further investigate PRC1. PRC1 function is divided into canonical and non-canonical activities based on which Polycomb Group Ring Finger (PCGF) protein is present in the complex. We identified two pcgf
genes in *S. mediterranea* which contrasts with *C. elegans* and *Drosophila*, wherein PRC1 is absent or there is a single *pcgf* gene, respectively. To understand the evolutionary relationship of the planarian *pcgf* genes, we are performing phylogenetic analyses. Moreover, expression and RNAi studies should determine how PRC1 E3 ligase function varies based on *pcgf* ortholog inclusion, as in mammals. [J.M.A. was supported by the ARCS Foundation and M.B. by NIH IMSD R25GM058906; this work was funded by NSF IOS-1350302 and NIH R01GM135657 to R.M.Z.]

Program Abstract #523
An RNAi screen identifies *p21 activated kinase 1 (pak1)* to be required for rescaling of body axis during regeneration
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Regeneration is a phenomenon which involves one or more of the spatially and temporally regulated processes of wound healing, tissue growth, tissue rescaling and remodeling. Planarian flatworms which can heal and restore body tissue from virtually any injury are a good model to understand these processes. Protein phosphorylation is widely used as a switch to regulate most signaling pathways in different biological contexts. However, dynamics of protein phosphorylation during regeneration remains to be explored. Here, we performed an RNA interference screen to identify kinases and phosphatases important for regulating protein phosphorylation status during regeneration. The screen identified 4 kinases and 2 phosphatases to be required for different but seemingly specific processes during regeneration. *p21-activated kinase 1 (pak1)* was identified from the screen as a gene involved in tissue patterning during regeneration. *pak1* RNAi animals failed to properly rescale the anterior posterior (AP) axis as observed by expanded expression domain of *wnt1*. At later stages, these animals regenerated with a small indented blastema with ventrally located supernumerary photoreceptors suggesting a role in patterning of dorso-ventral (DV) axis. Furthermore, we show that a merlin homolog (*mer*) is required for the *pak1* phenotype. This data suggests that PAK1 inhibition of Merlin is necessary during rescaling of the AP axis post amputation. Since, *pak1* RNAi results in abnormal patterning along both AP and DV axes, we hypothesize that PAK1 acts as hub, integrating information from multiple signaling pathways. This work was supported with aid from the Stowers Institute for Medical Research (SIMR) and Howard Hughes Medical Institute (HHMI).

Program Abstract #524
Investigating the role of heterotrimeric G protein subunits in planarian regeneration and behavior
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Regeneration is the regrowth of tissue after injury. Many metazoans have a limited ability to regenerate, but freshwater flatworms called planarians display robust regeneration of all tissues. Planarian regeneration is powered by a population of pluripotent stem cells and dynamic gene expression that re-establishes body polarity. However, the signaling mechanisms that alert planarian cells to injury and induce the proper cellular responses for regeneration remain less clear. G protein-coupled receptors (GPCRs) drive the development and regeneration of the planarian germline. However, potential roles for GPCR signaling in somatic regeneration and stem cell biology are unexplored. Screening the vast planarian GPCR gene complement presents challenges. Instead, we investigated downstream signal transducers that work with GPCRs, called heterotrimeric G proteins. Here, for the first time, we characterize the heterotrimeric G protein complement in planarians and show that two G protein subunit genes highly expressed in nervous tissue – an alpha subunit (Gα12) and a beta subunit (Gβ2) – promote whole-body regeneration. To understand how these genes promote regeneration, we are determining which aspect of planarian regeneration requires their expression. Additionally, to explore the cellular and molecular mechanisms through which these subunits act, we are identifying the cell types that express these genes as well as potential downstream effectors. We further show that additional heterotrimeric G protein subunits regulate aspects of planarian behavior. Our results support the intriguing notion that heterotrimeric G protein subunits play functionally distinct and specific roles. This work increases our understanding of how an animal coordinates robust regeneration and will inspire further analyses to investigate GPCR signaling in planarian biology. This work
was supported by funding from the McKnight Foundation, the Alfred P. Sloan Foundation, and the University of Georgia.

**Program Abstract #525**
The Planarian Early Growth Response Transcription Factor-2 (Egr-2) is Required to Regenerate Proper Intestinal Morphology
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Compared to humans' limited regenerative capacity, some animals can repair damaged digestive organs, re-establishing morphology and physiology after injury. However, mechanisms that orchestrate tissue-specific transcriptional programs at early stages of gastrointestinal regeneration are currently unknown. Planarians are freshwater flatworms that can completely regenerate their intestine due to a population of adult pluripotent stem cells, called neoblasts, that proliferate and differentiate into new tissue after injury. Here, we identify Early Growth Response 2 (Egr-2) as an injury-induced transcription factor and specific regulator of planarian intestinal regeneration. Egr proteins are conserved, stimulus-responsive transcription factors whose roles in digestive tract regeneration are poorly characterized. We found that egr-2 is upregulated in wound proximal regions and is enriched during regeneration in regions where intestinal remodeling and neoblast differentiation occurs. RNAi-mediated egr-2 knockdown impaired regeneration of a single, properly fused gut branch at the midline. By performing RNA-Seq on egr-2 knockout animals, we have identified >100 dysregulated genes that are normally expressed by intestine or neoblasts in uninjured animals. Current experiments are focused on characterizing the roles of these potential targets, and on determining whether egr-2 regulates remodeling of pre-existing intestine or coordinates neoblast dynamics. Our results identify planarian Egr-2 as the earliest known injury-responsive transcription factor with an intestine-specific role in regeneration. Identification of conserved egr-2-dependent transcripts and the cellular events they coordinate will expand our understanding of organ regeneration. This project was supported by funding from NIH/COBRE GM103636 (Project 1 to DJF), the Oklahoma Medical Research Foundation (to DJF) and the OMRF John and Mildred Carson PhD Scholarship Fund (to VM).

**Program Abstract #526**
Establishing a model system to probe the effects of age on regenerative growth
Alexander Stockinger, Martin Fahrenberger, Simon Haendeler, Madeleine Zillner, Gabriele Andreatta, Arndt von Haeseler, Florian Raible

Max Perutz Labs Vienna, Austria

Across the animal kingdom, regeneration – the capacity to replace missing cells, tissues or limbs – is typically lost with progressing development and age. Our molecular understanding of how this transition is orchestrated – and how it could potentially be reverted – is still rudimentary. The marine annelid *Platynereis dumerilii* offers a unique perspective on this process: In this worm, caudal regeneration is possible only in juvenile animals, whereas sexually mature individuals lose this capacity. Regenerative growth involves a group of *bona fide* stem cells molecularly related to germ cells that undergo rapid proliferation and differentiation into cells of the missing tissues, including those of the central nervous system. Moreover, successful regeneration depends on the presence of hormones synthesized in the heads of juvenile animals. Our past work has given us unique entry points into the molecular machinery underlying annelid regenerative growth, and the timing of the regenerative-non-regenerative transition. By combining classical experimental paradigms, the manipulation of hormone levels, bulk RNA sequencing and single cell profiling, we are dissecting the modulation of stem cells and stem-cell-derived differentiation programmes during posterior regeneration to understand the impact of age on regeneration. Funding: Austrian Science Fund (FWF) projects I2972, F78, DOC72.

**Program Abstract #527**
Role of tubulin isotypes in nematode resistance to a microtubule inhibitor
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Parasitic nematodes infect over a billion people and are gaining resistance to the most widely used drug albendazole (ABZ). Since these parasites are difficult to study, *C. elegans* is used as a model organism. Driscoll et al. (1989) found that in *C. elegans*, this class of drugs targets BEN-1 beta-tubulin and knockouts cause resistance. However, *ben-1* is non-essential and there is no phenotype in the absence of the drug, suggesting that this gene acts redundantly with other beta-tubulins. Understanding the role of different *ben-1* isotypes in drug resistance is important for determining the most effective method of controlling nematode populations. We examined the two major beta-tubulin isotypes that are redundant with each other for viability, *tbb-1* and *tbb-2*, to determine their redundancy with *ben-1*. If the worms were susceptible to ABZ, the exposure to drug after hatching showed that worms are shorter in length, while maternal exposure showed a decrease in hatching. We found that *tbb-2 ben-1* is Unc and *tbb-2* is more sensitive to ABZ than wild type, implying that *tbb-2* is redundant with *ben-1*. *tbb-1* does not show these interactions. The second part of this project uses mutagenesis screening to analyse mutations that cause or modify resistance. We isolated 16 resistant strains among 8000 mutagenized haploid genomes. Based off the findings of Driscoll et al., most or all of the mutations should be found in *ben-1*. While the *ben-1* homolog is implicated in ABZ-resistant parasitic nematodes, other genes appear to modify resistance. To find additional genes that mutate to affect resistance, the mutagenesis was repeated in a strain with additional copies of *ben-1(+)*, biasing the screen again *ben-1* alleles. We are currently characterizing two candidates. Funding source: NSERC.

**Program Abstract #528**

**Interspecies transcriptome analyses identify genes that control the development and evolution of limb skeletal proportion.**

**Aditya Saxena**  
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The remarkable diversity of limb proportions allows kangaroos to hop, bats to fly, and humans to walk upright. Despite our knowledge of genes that are necessary for the elongation of all limb bones, the genetic mechanisms that make some bones long and others short within a skeleton or between homologous elements of different species remain unclear. What genes control differential growth? How is modularity in skeletal scaling encoded? Did diverse skeletal proportions emerge by tuning the expression of genes in a common bone elongation “toolkit” or by genes found only in a subset of skeletal elements? To address these questions, we employ comparative genomics in homologous growth cartilages of the mouse & a bipedal desert rodent, the lesser Egyptian jerboa. Jerboas have disproportionately long metatarsals of the feet and ‘mouse-like’ arms. We find selective enrichment of pathways controlling cell size, cell-cell adhesion, and cytoskeletal organization in fast growing growth cartilage of jerboa metatarsals. We also discover co-option of *Shox2, Pax1* and *HoxB13* expression in jerboa metatarsals and identify open chromatin regions likely to be responsible for these novel expression patterns. The genes we identify provide a framework to understand the modular genetic control of skeletal growth and the remarkable malleability of vertebrate limb form. **Funding sources- National Science Foundation grant number 1548562. National Institutes of Health under award number R01AR075415, Searle Scholar Award from the Kinship Foundation, a Pew Biomedical Scholar Award from the Pew Charitable Trusts, Packard Fellowship in Science and Engineering from the David and Lucile Packard Foundation.**

**Program Abstract #529**

**Development and evolution of self-organizing pigmentation patterns in monkeyflowers**

**Yao-Wu Yuan**  
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Many organisms exhibit visually striking spotted or striped pigmentation patterns. The classical reaction-diffusion model predicts that such spatial patterns can form when a local autocatalytic feedback loop and a long-range inhibitory feedback loop interact. At its simplest, this self-organizing network only requires one self-activating activator that also activates a repressor, which inhibits the activator and diffuses to neighboring cells. However, the molecular activators and inhibitors fully fitting this versatile model remain elusive in pigmentation systems, and virtually nothing is known about how modulation of the properties of these hypothetical molecules affects pattern evolution in nature. Here, I present the monkeyflower (*Mimulus*) system that is ideal to address these
questions, for its tremendous diversity of flower pigmentation patterns and amenability to rigorous genetic analysis and functional interrogation. We found an R2R3-MYB activator and an R3-MYB repressor that regulate anthocyanin pigmentation in *Mimulus*. Experimental perturbation and mathematical modeling demonstrate that the properties of these two proteins correspond to an activator-inhibitor pair in a two-component, reaction-diffusion system, explaining the formation of dispersed anthocyanin spots in monkeyflower petals. Furthermore, genetic analyses and biochemical experiments suggest that subtle changes in the properties of this simple activator-inhibitor system led to the evolution of longitudinal stripes in one species from randomly dispersed spots in other species. This work is supported by an NIH grant R01GM131055.

**Program Abstract #530**

The Evolution of the Echinoderm Pigment Cell

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Pigments are known to serve diverse biological functions such as camouflage and sexual selection. Larvae of the sea urchin *Strongylocentrotus purpuratus* have pigmented mesenchyme cells which play roles in immunity and gut patterning. These cells contain the transcription factor Glial Cells Missing (SpGcm), which activates the expression of many pigment-cell specific genes, including those implicated in pigment biosynthesis such as Polyketide synthase 1 (SpPkS1) and Flavin-containing monooxygenase 3 (SpFmo3) and immune related genes such Macrophage inhibitory factor 5 (SpMif5). We have previously shown that the knockout of SpGcm, SpPkS1 or SpFmo3 results in pigmentless larvae. In the echinoderm phylum, larval pigment cells are unique to echinoids, and the molecular mechanisms underlying the evolution of this multifunctional cell type remain poorly understood. We have found that the embryos of the sea star *Patiria miniata* express orthologs of these genes despite being naturally pigmentless. Orthologs of genes downstream of SpGcm have nearly identical expression patterns between the two species throughout development. Despite this similarity, PmGcm is expressed in a different cell type, suggesting the regulatory network upstream of Pks1, Fmo3 and Mif5 has diverged even though the expression pattern of these downstream genes has not. We have found that this cell population in the sea star is distinct from other mesenchyme cells and that Delta/Notch signaling is implicated in the specification of Pks1+ cells in the sea star as it is in the sea urchin. Given the many similarities this sea star cell population shares with pigment cells, it may represent an ancestral echinoderm pigment cell. Current work is focused on identifying the causal factors upstream of the pigment orthologs with the goal of constructing a gene regulatory network to explain the appearance of larval pigment in echinoderms. This work was supported by the NIH under award number 1R01GM132222.

**Program Abstract #531**

The role of BMP signaling in early development of the spiralian *Capitella teleta*

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BMP (bone morphogenetic protein) signaling has long been thought to play a key role in specification of neural ectoderm. In vertebrates (deuterostomes) and insects (ecdysozoans), delimitation of neural fate to one side of the animal relies on inhibition of BMP signaling during dorsoventral axis formation. However, studies outside model organisms have shown a diversity of roles for BMP signaling during neural development, raising the question of how homologous centralized nervous systems are across Bilateria. Preliminary evidence from spiralians (e.g. molluscs and annelids) shows that BMP signaling is not necessary for neural fate specification, but that BMP does still play a role in neural development. Here we investigated the role of BMP signaling in the development of the annelid *Capitella teleta* by injecting mRNA encoding a dominant negative BMP receptor (dnBMP-R1) to knock down BMP signaling. In situ hybridizations and antibody staining showed asymmetric reduction of multiple tissues including trunk mesoderm, foregut, and brain, with specific loss or reduction of the left side. Injected animals still formed a ventral nerve cord and a dorsal-ventral axis in the trunk. Overall, these results show a different role of BMP signaling in neural development in annelids, and support the separate origin of different centralized nervous systems. This was funded by an NSF grant to Dr. Néva Meyer.
Program Abstract #532

The Evolution of Nociceptor Neurons in Sharks
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Neural Crest Cells (NCC) are a group of multipotent stem cells that give rise to many structures throughout the embryo, like neurons, Schwann cells, melanocytes, etc. Trunk NCC are the ones that give rise to the somatic sensory neurons along spinal cord, inside the Dorsal Root Ganglia (DRG). These somatic sensory neurons are responsible for sense for pain, touch, and temperature. However, the evolution of nociceptive (pain detection) neurons is not well known and understood. While some studies state that lamprey have pain receptors, it appears that sharks have lost these nociceptors and thus the ability to sense pain. Recently, an unbiased classification of all the mouse DRG neurons from single cell transcriptomics was generated. Using this data, we are searching for the expression of nociceptor neurons in the DRG of sharks using three approaches. First, by using immunohistochemistry to label nociceptors in the DRGs of sharks. Second, looking at the genome of sharks to identify specific nociceptor sequences. Third, by isolating shark DRG and analyzing its transcriptome to find pain receptors profiles. Our preliminary data shows that sharks express robust levels of pain receptors in the trigeminal ganglion and little to none in trunk DRGs. Based on our findings, sharks express few nociceptive neurons compared to mammals. Funding resources acknowledged: NIH 1R15HD092170-01 & NIH MARC GM008395.

Program Abstract #533

Mitotic kinases choreograph receptor storage and redistribution during asymmetric division
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Trafficking of integral membrane proteins impacts signal processing and cell fate specification. Until recently, it was thought that trafficking was shut down during cell division. Thus, remarkably, the regulation of trafficking during division remains poorly characterized. Here we delineate the role of mitotic kinases in receptor trafficking during asymmetric division. Targeted perturbations reveal that Cyclin Dependent Kinase 1 (CDK1) and Aurora Kinase promote storage of fibroblast growth factor receptors by suppressing endosomal degradation and recycling pathways. As cells progress through metaphase, loss of CDK1 activity permits differential degradation and targeted recycling of stored receptors, leading to asymmetric induction. Mitotic receptor storage, as delineated in this study, may facilitate rapid reestablishment of signaling competence in nascent daughter cells. However, mutations that limit or enhance the release of stored signaling components could alter daughter cell fate or behavior thereby promoting oncogenesis. This work was funded by the NSF and AHA.

Program Abstract #534

A conserved molecular cascade initiates a trophectoderm program in human, cow and mouse embryos prior to blastocyst formation.
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Current understanding of cell specification in early mammalian preimplantation development is mainly based on mouse studies. The first lineage differentiation event occurs at the morula stage with outer cells initiating a trophectoderm (TE) program to become the earliest placental progenitors. Notably, recent gene expression analyses suggest that the mechanisms regulating early lineage specification in the mouse may differ in other mammals, including human and cow. Here, we examined evolutionary conservation of cell dynamics and a molecular cascade initiating TE segregation in mouse, cow and human embryos using a comparative embryology approach. We discovered that the expression pattern of key TE lineage-associated factors shows a high degree of conservation among all three species. Specifically, at the morula stage outer cells acquire an apico-basal cell polarity, with expression of aPKC and PARD6B at the surface-free domain, nuclear expression of the Hippo signaling pathway effectors, YAP1 and WWTR1, and restricted expression of the transcription factor GATA3,
suggesting initiation of a TE program. Furthermore, we demonstrate that inhibition of aPKC, by small-molecule pharmacological modulation and TRIM-Away protein depletion, impairs TE initiation at the morula stage. Altogether, our comparative embryology analysis provides novel insights into early lineage specification in human preimplantation embryos and suggests a similar mechanism initiating a TE program in mouse, cow and human embryos. Fundings: Fondation de l’Université de Nantes, Fonds Wetenschappelijk Onderzoek Flanders (FWOAL722), the Wetenschappelijk Fonds Willy Gepts (UZBrussel, G142), Comparative Biomedical Sciences Departmental - Royal Veterinary College, the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001120), the UK Medical Research Council (FC001120), and the Wellcome Trust (FC001120) and by the Rosa Beddington Fund.

Program Abstract #535
Axon-like projections direct the self-renewal versus differentiation cell fate decision in Follicle Stem Cells of the Drosophila ovary

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Periods of quiescence and proliferation are important for maintaining heterogeneity of stem cell pools. Constitutive or excess proliferation associated with continuous feeding or inappropriate diet drives stem cell pools toward clonality, accelerating tissue aging. The quiescence to proliferation transition is thus a critical process for promoting health of stem cell pools. Here, we investigated the temporal and mechanistic relationships between cellular events that occur during the quiescence to proliferation transition of Follicle Stem Cells (FSCs) in the Drosophila ovary. We find that feeding-dependent Hedgehog (Hh) signaling is essential for initiation of the quiescence to proliferation transition, with FSCs arrested in a quiescence-like state in the absence of key Hh effector proteins. Growth of axon-like projections from FSCs also depends on Hh, as proliferation alone is not sufficient to promote their growth. We identify sickie, a known axon growth regulator, as necessary and sufficient for inducing FSC projection growth. Manipulation of sickie expression reveals critical functions for FSC projection networks, including establishment of a somatic cell barrier in the FSC niche plane to organize germline cyst architecture during follicle formation, targeting of projections to germ cells or somatic cells within the FSC niche, and regulation of FSC self-renewal versus differentiation decisions. This work is funded by NIH.

Program Abstract #536
The conserved histone deacetylase, HDA-1, functions in cell cycle-dependent and independent roles to promote invasive differentiation

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Cell invasion occurs naturally during development; however, it also contributes to tumor progression. Although understudied, data from different cancer subtypes suggests that a dichotomy exists between invasive and proliferative behavior. Our lab uses the C. elegans anchor cell (AC) to study this proliferative-invasive switch during uterine-vulval development. During the L3 larval stage, the AC invades into the vulval epithelium to form the mature vulva. We have shown that the AC exists in G0/G1-cell cycle arrest during invasion, which requires nhr-67 (tailless/Tlx). Loss of nhr-67 results in mitotic, non-invasive ACs. nhr-67 maintains AC arrest by regulating the expression of the cyclin-dependent kinase inhibitor, cki-1. Induced expression of CKI-1 can restore invasion in an nhr-67-depleted background, suggesting that the G1/G0 state is required for invasive activity. Differentiation of invasive behavior is regulated by chromatin modifiers that act downstream and/or parallel to G0/G1 arrest. We have found that the histone deacetylase, hda-1, promotes AC invasion by positively regulating pro-invasive gene expression; however, whether hda-1 regulates invasion by controlling cell cycle arrest is unknown. Using genetic approaches and high-resolution imaging, we explored the regulatory relationships between HDA-1 and key transcription factors that regulate G1/G0 arrest and invasion. We find that hda-1 participates in a feed-forward circuit with nhr-67 and hlh-2/E to maintain the AC in G1/G0 arrest. Interestingly, induced expression of CKI-1 fails to restore invasion in hda-1-deficient ACs. Additionally, we find that the NuRD complex subunits, let-418 and chd-3, act redundantly to facilitate AC invasion. These results suggest that hda-1 functions pleiotropically during
invasion, to both maintain the post-mitotic state and regulate downstream effectors of invasive differentiation. Funding provided by the American Cancer Society, NIH, and Damon Runyon foundation.

**Program Abstract #537**

**The lysine methyltransferase SETD2 regulates early neural crest development**

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The neural crest is a population of migratory, embryonic, multipotent cells that are unique to vertebrates. While a complex network of transcription factors that govern neural crest cell fate and differentiation has been assembled, epigenetic and post-translational mechanisms that regulate the expression and activity of these factors are less well defined. Previous work from our lab demonstrated that cytoplasmic non-histone protein methylation and NSD3, a lysine methyltransferase that di-methylates lysine 36 of histone H3 (H3K36), are essential for neural crest specification and migration. Interestingly, our results showed that SETD2, the lysine methyltransferase solely responsible for H3K36 tri-methylation, is also upregulated in neural crest cells. *In situ* hybridization revealed that SETD2 is expressed throughout the chick blastoderm as early as gastrula stages, and in premigratory neural crest cells where it is downregulated prior to migration. Likely due to the early onset of its expression, SETD2 morpholino knockdown does not significantly alter premigratory *Sox10* expression or neural crest migration; however, both are disrupted by expression of a dominant negative SETD2 construct, indicating that SETD2 activity is essential for early stages of neural crest development. These results indicate that SETD2-mediated lysine methylation, which may target H3K36 or non-histone proteins, is essential for neural crest development, further demonstrating that lysine methylation is an important mechanism regulating early neural crest development. Funding: NSF IOS-1354809.

**Program Abstract #538**

**Signaling pathways that reinforce ventricular chamber identity in zebrafish**

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In the embryonic vertebrate heart, chamber-specific functions are influenced by ventricular and atrial cardiomyocytes that have distinct molecular, morphological, and electrophysiological properties. Specification of cardiac chamber lineages occurs during or shortly after gastrulation, and chamber-specific differentiation pathways initiate prior to heart tube assembly. However, a period of plasticity remains after cardiomyocyte differentiation occurs, in which ventricular and atrial characteristics are reinforced by chamber-specific transcription factors. In zebrafish, our prior studies have shown that the FGF signaling pathway is required upstream of Nkx transcription factors to enforce ventricular identity: inhibition of FGF signaling after cardiomyocyte differentiation induces ectopic expression of the atrial myosin heavy chain gene *amhc* in ventricular cardiomyocytes, accompanied by downregulation of the ventricular myosin heavy chain gene *vmhc*. Here, we investigate whether additional signaling pathways interact with FGF signaling during ventricular identity maintenance. Intriguingly, we observe ectopic ventricular *amhc* expression in response to loss of function of the BMP antagonist Chordin or constitutive activation of the BMP receptor Alk6, suggesting that inhibition of BMP signaling plays a role in reinforcing ventricular characteristics. We also find ectopic *amhc* expression in the ventricle when the endocardium is absent or highly dysmorphic, suggesting that endocardial-to-myocardial signaling may reinforce ventricular traits. Our ongoing studies are examining the interface between FGF signaling, BMP signaling, and endocardial-myocardial communication to determine how these pathways interact to control ventricular identity maintenance. We anticipate that comprehension of the network of signals regulating stability of chamber identity will inspire future strategies for directed differentiation of ventricular and atrial cardiomyocytes.

**Program Abstract #539**

**Epigenetic modulation of single cell transcriptome during palatogenesis**

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The roles of epigenetics in palatogenesis and cleft palate remain largely unknown. We report here that conditional ablation of an epigenetic modifier Ezh2 (enhancer of Zeste homolog 2) in palatal epithelia causes defective histone methylation during palatogenesis, leading to cleft palate. We dissociated embryonic palatal cells from both normal control and mutant embryos and conducted single cell RNA-sequencing analyses. In total 18 distinct cell populations are determined in the developing mouse palate. Epithelial deficiency of Ezh2 results in 27 differentially expressed genes (DEGs), including 22 upregulated and 5 downregulated DEGs in the palatal epithelium, and 55 DEGs (including 50 up and 5 down) in the palatal mesenchyme. These DEGs play crucial roles (e.g., Msx1, Shox2 and TGF-β2) in the epithelial-mesenchyme interactions which regulate the vertical and horizontal outgrowth of palatal shelves. These results reveal significant roles of Ezh2-mediated histone methylation in transcriptomic modulation of palatogenesis. This work is supported by the National Institutes of Health (R01DE026737 to C.Z.) and Shriners Hospitals for Children (a postdoctoral fellowship to B.S. and a research grant to C.Z.).

Program Abstract #540
CeLaVi: A web-based tool for interactive Cell Lineage Visualisation
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Recent innovations in genetics and imaging are providing the means to reconstruct cell lineages (genealogical relationships between every cell of an organism) either by tracking cell divisions using live microscopy or by tracking the history of cells using molecular recorders that accumulate mutations during development. A cell lineage on its own, however, is simply a description of the branching events. A major goal of current research is not only to describe this process of cell divisions, but to also describe the identities (cell types) and spatial distribution of the cells they produce. The results produced by this research will require specialised tools to integrate these diverse data. Here I will present CeLaVi, a web-based visualisation tool that allows users to interrogate the entire cell lineage or a population of cells interactively, whilst visualising the spatial context, the identity and the properties of the cells. The user interface consists of two interactive spaces that are connected in real time: the ‘lineage viewer’ and the ‘3D viewer’. I will show CeLaVi’s functionalities, which include exploring the cell lineage tree by collapsing/expanding individual branches; exploring the spatial distribution of the cells in 3D; visualising the spatial distribution of cell clones by selecting an ancestral node in the lineage viewer and visualizing its progeny in 3D; selecting cells in the 3D viewer to display their lineage history; colouring cells in the 3D viewer to indicate lineage distances to selected cells. For this demonstration I will use cell lineages of three different systems: the nematode Caenorhabditis elegans, a limb of the crustacean Parhyale hawaiensis and an in silico generated organoid. CeLaVi is built using HTML5, Ajax, jQuery, CSS and the visualisation libraries D3.js and Plotly.js. It been tested on the most common web browsers and different OS. This research has been funded by the Human Frontiers Science Program (HFSP).

Program Abstract #541
Chromatin accessibility and single cell transcriptomics expand the gene regulatory network of sea urchin gut development
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Gene regulatory networks (GRNs) have been employed as an approach to study various developmental processes and to visualize them as the interactions of the genes involved. The developmental GRN of the embryonic gut of sea urchin Strongylocentrotus purpuratus already has a solid foundation with crucial components of it uncovered. Thus, the ParaHox genes SpLox and SpCdx were shown to have a crucial role in the embryonic development of the digestive system of S. purpuratus. However, the upstream control of these genes is still unexplored. Novel sequencing approaches allow to investigate the components of a GRN at unprecedented resolution. The use of
Program Abstract #542

Tracking periocular mesenchyme derived anterior segment development using single cell transcriptomic analysis

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Periocular mesenchyme (POM) is a subgroup of neural crest cells, responsible for forming anterior structures, including the anterior segment of the eye. Despite the importance for the development of a healthy eye, knowledge about this cell group is limited. Particularly, only very few genetic markers and their respective roles are known. The purpose of this study is to identify formerly unknown markers of POM cells, to further understand their role in eye development and the genetic interactions required for POM function. Larval eyes of transgenic zebrafish Tg(Foxc1b:GFP) were collected every 24 hours between 48hpf and 144hpf. GFP+ cells were sorted via FACS cell sorting and processed with the 10x genomics single cell transcriptome kit. We collected a total of more than 12,000 Foxc1b+ cells from eyes only, including one biological replica for each individual time point. Transcriptome analysis with Loupe Cell Browser and Monocle3 showed, that these cells were organized in re-occurring clusters during zebrafish eye development. These clusters are partly localized to different structures within the eye, mainly the cornea and retinal pigment epithelium. Additionally, we found several markers with specific expression within these spatially limited areas with apparent importance for general eye development, as proven by genetic knockout. Our results provide new insights into general POM development as well as novel targets for the analysis of anterior segment formation. We identified new candidate genes for anterior segment development, that might also be associated with anterior segment disorders. This new knowledge might further enable clinicians to increase their genetic screening for anterior segment related diseases and treatment of ocular diseases such as glaucoma. This research was funded by a Career Starter Research Grant of the Knights Templar Eye Foundation, awarded to the first author.

Program Abstract #543

Single Cell and Reporter Analysis of Skeletal Muscle Interstitial Cells Identifies Unique Non-myogenic Mesenchymal Cell Populations

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Non-myogenic mesenchymal cells (NMMCs), a heterogenous group of interstitial muscle-associated cells known to include pericytes and fibro-adipogenic progenitors (FAPs), are a vital component of the skeletal muscle niche. NMMCs, such as FAPs, are required for skeletal muscle development and regeneration, are implicated in the pathologies of several muscle disorders. Since NMMCs are a heterogenous population of cells that lack clear definitions in identity and function, we performed lineage/reporter analysis on tissue sections, as well as, flow cytometry and single cell RNA sequencing (scRNA-seq) of fluorescently labeled cells sorted from the skeletal muscles of Prx1Cre; R26-tdTomato mice. Whole embryo and tissue-based reporter studies demonstrate that tdTomato labeled cells originating from the lateral plate mesoderm contribute directly to osteoblasts, chondrocytes, tenocytes, and muscle-associated interstitial cells within developing limbs. Further, both reporter assays and flow cytometry indicate that these tdTomato+ cells do not contribute to the myogenic, hematopoietic,
or endothelial cell lineages during skeletal muscle development, homeostasis, or regeneration. Our scRNA-seq analyses indicate that these tdTomato+ muscle-associated interstitial cells are indeed NMMCs and can be categorized into nine cell clusters including pericytes, vascular smooth muscle cells (vSMCs), interstitial tenocyte-like cells, and FAPs. We further determined that the greater FAP population, as defined by Pdgfra and Sca1 (Ly6a) positivity, in fact includes multiple sub-populations with unique transcriptomic signatures and functions, including novel populations of pro-osteogenic cells and neuromuscular junction capping cells. Through the identification and classification of these unique FAP subsets, we are establishing a foundation and growing our understanding of their precise impact on skeletal muscle development, regeneration and disease pathologies. Funding; NIH R01AR057022, AR063071

Program Abstract #544

orb2, a novel microcephaly gene, regulates centrosomes in Drosophila neural stem cells

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To ensure normal neurodevelopment, neural stem cells undergo repeated rounds of asymmetric cell division to maintain a population of self-renewing stem cells and generate the differentiating cells required for neurogenesis. Failure to maintain the balance of self-renewal versus differentiation during development can result in microcephaly. In Drosophila melanogaster neuroblasts (NBs), asymmetric cell division is regulated through functionally unequal, or asymmetric, centrosomes. Centrosomes are microtubule organizing centers, which function to build and orient the mitotic spindle along a fixed polarity axis to direct cell division. In human and Drosophila neural stem cells, asymmetric centrosome activity ensures error-free mitosis along a defined polarity axis. Centrosome asymmetries are generated through the asymmetric enrichment of the various proteins that comprise the pericentriolar material (PCM), which dictates the microtubule-organizing activity of the centrosome. However, the mechanism underlying regulation of centrosome activity is poorly understood. Through 3' UTR analysis of RNAs that localize to the centrosome, we identified the CPEB protein, Orb2, as a potential regulator of centrosome activity in Drosophila NBs. We examined a requirement for Orb2 in regulating NB centrosome activities and the requirement of Orb2 for proper neurodevelopment. Our preliminary data reveal that orb2 is a novel microcephaly gene. We found that 60% of orb2 mutant brains have a volume of >2 standard deviations below the WT mean. Additionally, orb2 mutant NBs show aberrant, centrosome-associated phenotypes including loss of centrosome asymmetry (60%, p-value<0.01), centrosome amplification (20%) and spindle morphology defects (30%). Additionally, orb2 mutant brains have a reduced number of neural stem cells (p-value<0.001). These data are consistent with a role for Orb2 in neurodevelopment and centrosome regulation. Funding: 5K22HL126922 awarded to DAL and GM008490 awarded to BVR

Program Abstract #545

The rehabilitation of Freddie Kruger: Evaluating regeneration in the Texas Blind Salamander

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Regenerative capacity is highly variable within the animal kingdom, even within the same order, family, or species. Amphibians are an excellent example of this variability. Literature demonstrates that only three of the six studied Urodele families exhibit robust regeneration in response to limb amputation; and within these three families, clear species differences in capacity have been documented. Studying these differences between closely related species provides a useful means to understand the mechanisms required for regeneration. The Texas Blind Salamander (Eurycea rathbuni) is an endangered cave dwelling species of the Plethodontidae Family. Although six other Plethodontidae species exhibit regenerative capacity; it is unknown if the Texas Blind Salamander can regenerate a limb, and literature is scattered with observations of cave dwelling species that have lost regenerative capacity in specific structures and organs, such as heart regeneration in Astyanax mexicanus. At the Marcos Aquatic Resources Center, wild Texas Blind Salamanders are caught for conservation purposes. One female colony member (Freddie Kruger) was captured with only a single intact limb. The left forelimb was a short stump which impeded the animal’s locomotion, particularly in terms of climbing. To potentially aid in
rehabilitating this individual, improving quality of life, and assess for regenerative ability within the species, we amputated the left limb stump to attempt to stimulate the animal’s innate regenerative abilities. We simultaneously amputated tail tips from other individuals within the colony to also assess for tail regeneration within this species. We observed that adult Texas Blind Salamanders are able to regenerate complete limbs and tails. Funding source: The Eunice Kennedy Shriver National Institute of Child Health and Human Development of the Nation Institutes of Health, Grant Number: 1R15HD092180-01A1

Program Abstract #546
apolipoprotein b orthologs function non-cell autonomously during adult stem cell differentiation in planarians
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Unlike many animals, planarians are programmed to undergo whole organism regeneration. Neoblasts, the adult stem cells, are the only cell types that can proliferate to repair and rebuild a proportioned new individual. The highly branched intestine of planarian digests and distributes nutrients throughout the animal, and is the primary site for lipid droplet production, storage, and secretion via lipoproteins. Previously, we showed that RNA interference (RNAi)-mediated knockdown of an intestine-enriched transcription factor, nkx2.2, caused dramatic reduction in new tissue production during regeneration. This suggests that the intestine may regulate neoblast proliferation or differentiation, consistent with neoblasts’ proximity to the intestinal branches. Here, we identified two potential downstream effectors, apolipoprotein b (apob) orthologs, whose expressions were dramatically reduced in the absence of nkx-2.2. As expected, apob expression was highly enriched in the intestine, however, we detected robust protein labeling in many cell types outside the intestine. When we abrogated apob function by RNAi, we observed reduced viability in intact animals and delay in establishment of body axis polarity and regeneration of brain and pharynx in amputated fragments. Using flow cytometry, we showed that the cell fraction containing post-mitotic early progeny of neoblasts was expanded. Together, these results suggested a defect in cellular differentiation in the apob knockdown animals. Additionally, we demonstrated that neoblasts in the apob knockdown animals had reduced neutral lipid content. Since apob is a key component in the production of lipoproteins for neutral lipid trafficking, we propose that apob functions non-cell autonomously to modulate neoblast differentiation, possibly by mobilization of neutral lipids and/or other cargoes in lipoproteins to facilitate stem cell differentiation. This project was supported by NIH GM103636 (DJF), HHMI (PAN), and OMRF (DJF).

Program Abstract #547
Tail Regeneration in the American Alligator (Alligator mississippiensis): First Characterization of Appendage Regrowth in an Archosaurian Reptile
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Among amniote vertebrates, reptiles retain the capacity to regrow entire appendages after injury, which is lost in mammals and birds. While tail regrowth has been extensively studied in lepidosaurian reptiles such as lizards, in this study, we present the first anatomical and histological evidence of regeneration in an archosaur, the American alligator (Alligator mississippiensis). All animals were wild caught juveniles or sub-adults with an average total body length of 133.4 ± 29.2cm (n = 3). Although the time period required for tail regrowth is unknown, the average length of the regenerated tail was 15.7 ± 7.3cm. We identified the following differences between the original and regenerated tail: i.) scales were compacted and lacked both an organized pattern and dorsal scutes, ii.) the caudal vertebrae were replaced by a ventrally positioned, unsegmented endoskeleton, as determined by gross dissection, radiographs, and magnetic resonance imaging, and confirmed by immunohistochemistry to be
composed of cartilage, iii.) the regenerated tail lacked skeletal muscle, and iv.) regenerated soft tissue consisted of adipose and fibrous connective tissue with type I and type III collagen fibers, supplied by a dense network of newly established axons and blood vessels. We observed the following differences between tail regeneration in the alligator compared to lizards, including: i.) lizards are able to regrow functional skeletal muscle groups, and ii.) lizards regrow a tail that is radially organized around a central endoskeleton, whereas the alligator regrows a tail with a clear dorsal-ventral axis, as seen in urodele amphibians. Further analysis of conserved versus divergent features of regeneration in amniote vertebrates will be highly instructive in efforts to activate regenerative mechanisms in humans for future clinical therapies. This work was funded by the College of Liberal Arts and Sciences at Arizona State University to KK.

Program Abstract #548
Axolotl spinal cord regeneration is dependent on brain signals
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Axolotl is a salamander with the amazing ability to regenerate many body organs, including its central nervous system. After suffering from spinal cord injury, axolotls can regenerate its spinal cord to fully recapitulate the pre-injury structure and regain complete functionality. Ependymal cells of the spinal cord are central towards this regenerative ability. They remain pluripotent into adulthood and quickly proliferate upon injury to regrow the ependymal tube. We observed that upon injury by removing a segment of spinal cord, only the anterior stump regrows, while the posterior stump remains dormant. Remarkably, blocking the connection to the brain prevents the anterior stump from regrowth. The data thus far suggest the brain provides certain signal that is essential for ependymal cells to proliferate, perhaps via cerebrospinal fluid or brain derived axons.

Program Abstract #550
Heart Regeneration in a Basal Chordate
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The adult human heart is a notoriously regeneration-incompetent organ, but the heart can regenerate in adult fish, amphibians, and neonatal mammals. The phylogenetic distribution of heart regeneration is not known, neither is it known to which extent invertebrate species can regenerate their hearts and/or major vessels after damage. In vertebrate models, 30 to 60% loss of cardiomyocytes can still sustain regeneration of a functional myocardium via dedifferentiation and proliferation of surviving cardiomyocytes. We have recently discovered that Ciona robusta, an invertebrate chordate, can regenerate its heart in post-metamorphic individuals, which was previously thought not to be possible based on the classical regeneration literature. Upon genetic ablation using the Nitroreductase-Metronidazole chemical genetic system expressed in the cardiopharyngeal lineage, we found that the vast majority of cardiomyocytes die as evidenced by caspase activity and loss of Mhc2 staining. Despite this heavy loss of cardiomyocytes, the heart is able to recover and regenerate Mhc2+ cardiomyocytes within 3-4 days. This suggests that the Ciona heart may be able to regenerate de novo from non-cardiac cellular sources. It is yet unclear what the origin(s) of these new cardiomyocytes is(are), whether a few surviving cardiomyocytes are able replenish the entire heart, or other cells from distinct lineages such as mesenchyme or the blood transdifferentiate into functional cardiomyocytes when the heart is ablated. I will present evidence with the initial characterization of this phenomenon at the cellular and molecular level. This work is funded by the Leducq Foundation (15CVD001).

Program Abstract #551
Dynamics of transcription during germband formation and segmentation in Tribolium castaneum
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The red flour beetle, Tribolium castaneum, is an established model organism with noted differences to other well-characterized models in developmental biology: the vinegar fly Drosophila melanogaster and the zebrafish Danio rerio. T. castaneum, unlike the near-simultaneous segmentation of Drosophila, adds segments to the posterior of
the germband sequentially. This is similar to zebrafish and both Danio and Tribolium are thought to control segmentation through a genetic oscillator. Unlike Danio, however, the segment addition rate in Tribolium varies, with abdominal segments added more rapidly than thoracic and gnathal segments and segmentation slowing to a stop approaching 24 hours after egg lay. The regulators of segmentation rate and the oscillator of Tribolium are not well-characterized. In order to identify potential regulators of germband segmentation and formation we performed an RNASeq experiment on pools of flour beetle embryos laid within short one hour time windows that included late blastoderm to germband elongation stages. We found low levels of expression for most Tribolium genes throughout the period examined, but highly dynamic expression with more than half of genes significantly differentially expressed between the blastoderm to germband transition. This in contrast to less than a quarter of genes differentially expressed between the middle and late period of germband extension. We then profiled transcription factor (TF) gene expression using a kmeans clustering algorithm. We found TF genes clustered with expression peaks either during blastoderm or late germ extension stages, with two exceptions of TF genes with peak expression at both stages or one cluster with peak expression during germband formation. This last cluster contained members of the segmentation oscillator and known regulators such as caudal. Support from NSF IOS 1755124 to L Nagy and T Williams.

Program Abstract #552
How the Beetle got its Stripes: A Look into the Mechanism and Eve-olution of the Tribolium castaneum Segmentation Oscillator.
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Vertebrates, annelids, and arthropods, while in evolutionarily distant clades, all possess a segmented body plan. In many species, segmentation is driven by a cycle of periodic transcription factor gene expression. The best studied of these transcription factors is even-skipped (eve). In the beetle Tribolium castaneum, eve is part of an oscillator that generates segments sequentially. By contrast, in Drosophila melanogaster, eve is part of a hierarchical transcription factor cascade that leads to nearly simultaneous segment development. The stripe-specific enhancers of eve are among the best studied of all known enhancers, while little is known about the transcriptional regulation of eve in beetles. Will stripe-specific enhancers found in D. melanogaster eve enhancers be preserved in a sequentially segmenting insect, or will other types of periodicity enhancers be identified? Here we report our work to describe eve enhancer function in T. castaneum as well as to understand to what degree eve’s regulation is conserved within other Coleoptera. We used computational tools to identify clustered regions of putative enhancer binding sites surrounding the T. castaneum eve locus. With this information we built enhancer:mCHERRY reporter constructs of regions with high predicted transcription factor binding and are analyzing their expression in flies and beetles. Lastly, we identified sequence conservation among coleopteran eve enhancers that may be significant for eve function. Funded by: NSF IOS grant 1755124 to L Nagy and T Williams.

Program Abstract #553
Endodermal contributions revise the placodal origin of the vertebrate pituitary
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The pituitary is a master endocrine gland that regulates basic body functions such as growth, metabolism, and reproduction. Along with the nose, lens, and ear, the pituitary constitutes one of the major cranial placodes - epithelial thickenings of the head ectoderm. Although the vertebrate pituitary is thought to derive solely from an ectodermal placode, non-vertebrate chordates have a pituitary-like structure derived from endoderm, making the evolution of the vertebrate pituitary a long-standing mystery. Here, we use three independent methods of lineage tracing in zebrafish – Cre-based recombination, endodermal transplantation, and endoderm-specific transgenic lines – to show a significant contribution of endoderm to the pituitary placode. Time-lapse imaging shows that the anterior endoderm buds, detaches, and fuses with the ectodermal placode to generate a pituitary of dual endoderm/ectoderm origin. We confirm by RNA in situ hybridization and single-cell RNA sequencing that
endoderm-lineage cells contribute to all the major hormone-producing cells of the adult pituitary. Equal competence of the endodermal and ectodermal epithelia in generating endocrine cell types of the pituitary suggests that it is the inductive signals (likely from the hypothalamus), and not the germ layer origins, that drove evolution of the pituitary. As opposed to the de novo emergence of the pituitary from an evolutionarily novel type of ectoderm, we propose that the fish condition reflects the incorporation of ectodermal cells into an ancestral endoderm-derived proto-pituitary during the origin of vertebrates.

Program Abstract #554
Genetic interactions between pam-1 and wee-1.3 during embryonic development and oocyte maturation in C. elegans
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The puromycin-sensitive aminopeptidase, PAM-1, is necessary for timely meiotic exit and proper establishment of the anterior-posterior axis in one-cell C. elegans embryos. We have identified a mutation in wee-1.3 as a suppressor of pam-1. WEE-1.3 is a cell-cycle regulator that is involved in oocyte maturation. While only 2% of embryos laid by pam-1 mutant worms survive, the presence of the wee-1.3(lz5) suppressor restores the hatch rate to approximately 50%. To test for interactions, we examined phenotypes associated with pam-1 and wee-1.3 mutants. pam-1 mutants fail to polarize the anterior-posterior axis during the one-cell stage. However, when the suppressor mutation in wee-1.3 is also present, polarization is significantly improved. Signs of polarity such as localization of the PAR proteins and an asymmetric cell division are restored when the suppressor is present. WEE-1.3 is known to regulate oocyte maturation. Worms treated with wee-1.3(RNAi) become sterile within 24 hours due to precocious oocyte maturation. However, pam-1 worms treated with the same RNAi are protected against this sterility and lay a comparable number of embryos to pam-1 mutants alone. In addition, precocious oocyte maturation is not observed after wee-1.3(RNAi) treatment in pam-1 worms when oocyte maturation markers were examined. Currently we are looking for potential interactions during meiosis and examining the levels of WEE-1.3 in pam-1 worms to test if WEE-1.3 may be a target of the aminopeptidase. Taken together, these data suggest that pam-1 and wee-1.3 interact during axis polarization and oocyte maturation. This work is funded by NIH 2R15GM110614-02.

Program Abstract #555
Fluorescently tagged knock-in allele allows live imaging and degradation-mediated manipulation of planar cell polarity in zebrafish
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Planar cell polarity signalling (PCP) coordinates the uniform orientation, structure and movement of cells within a plane of a tissue during development. PCP activity is based on the asymmetric localization of its core components on cell membranes. In order to understand how asymmetric component localization is translated into polarized cell behaviour, and to study endogenous PCP activity in real time in zebrafish embryos, we have used CRISPR/Cas9 gene editing to target a superfolder GFP linker cassette (sfGFP-l) onto the N-terminus of Vangl2 protein, a core and specific PCP regulator. Fish homozygous for this vangl2 knock-in allele, and fish trans-heterozygous for vangl2 knock-in and vangl2 loss-of-function alleles are viable and fertile, demonstrating that the sfGFP-l-Vangl2 fusion protein is functional. Our analysis of sfGFP-l-Vangl2 localization has revealed robust plasma membrane localization prior to the onset of gastrulation, which is earlier than in previous immunohistochemical studies, and an anterior polarized localization of Vangl2 in neuroepithelial cells, which has been demonstrated for PCP components using non-endogenous approaches. These observations validate the functionality of our endogenously labeled vangl2 allele and highlight its sensitivity and utility. To manipulate Vangl2 protein levels in the early embryo, we have combined our sfGFP-l-Vangl2 line with zGrad, a GFP-specific protein degradation system. Strikingly, zGrad-dependent modulation of sfGFP-l-Vangl2 protein levels phenocopies vangl2 mutant phenotypes. Furthermore, defects observed upon zGrad expression in sfGFP-l-Vangl2 heterozygote animals suggest that zGrad-induced protein degradation may bypass genetic compensation. In the future, conditional
zGrad strategies will permit temporal and tissue-specific analysis of PCP function across diverse embryonic, juvenile and adult contexts. Funding sources: Sigrid Juselius Foundation, SickKids’ Research Institute Restracomp Award and CIHR.

**Program Abstract #556**

**Distinct activities of Scrib module proteins organize epithelial polarity**

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Cell polarity is critical for the structure and function of epithelial tissues. In most animal cells, polarity arises from mutual antagonism between the Par complex and the Scrib module, which define the apical and basolateral domains, respectively. Numerous studies have delineated the molecular mechanisms of apical specification by the Par complex proteins. However, despite their discovery over 20 years ago, little is known about the molecular functions of the Scrib module proteins, Scrib, Dlg and Lgl and how they specify basolateral polarity. Using the Drosophila follicle epithelium, we demonstrate independent and distinct activities of Scrib, Dlg and Lgl. We find a linear hierarchy for localization to the basolateral membrane, but that previously proposed Scrib-Dlg interactions are dispensable for polarity. We show that in contrast to mammals, cortical Scrib localization does not require S-palmitoylation, but rather an independent stabilizing activity of Dlg. While Scrib, Dlg and Lgl are required for polarity, together they are not sufficient to specify a basolateral domain. Unlike Lgl, Scrib and Dlg do not directly inhibit the Par complex kinase, aPKC, but restrict apical expansion by enabling the aPKC-inhibiting activity of Lgl. Our data suggest that Scrib and Dlg ‘protect’ Lgl by limiting aPKC phosphorylation at specific residues. Our current work is focused on understanding this Lgl protection mechanism. We aim to untangle the relationships between Lgl spatial and biochemical states and their regulation by Scrib and Dlg. From these experiments, we will begin to define the elusive molecular functions of the Scrib module. Funding - NIH Grants GM068675, GM090150 and GM130388 to D.B. and AHA Fellowship 20PRE35120150 to M.J.K.

**Program Abstract #557**

**Cholesterol stimulates SHH release from Human PDAC cells via CDON**

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Sonic Hedgehog (SHH) is a tightly-regulated, membrane-associated morphogen and a known driver of tumorigenesis, especially in pancreatic ductal adenocarcinoma (PDAC). Following processing, SHH remains at the plasma membrane of the SHH producing cell, thereby limiting its distribution and signal strength. In PDAC, the release of SHH from tumor cells is necessary to promote a tumor permissive microenvironment. Mechanisms regulating SHH release from tumor cells to signal in distant stromal cells are not well known. Identifying the mechanisms that stimulate SHH release from the tumor cell membrane may provide new avenues to reduce signaling between the tumor and its surrounding environment, which may restrain tumor development. Previously, our lab demonstrated that the Drosophila transmembrane protein Boi sequesters Hedgehog (Hh) at the membrane of the Hh-producing cells of the fly ovary. In response to dietary cholesterol or in the absence of Boi, Hh is constitutively released to promote epithelial stem cell proliferation. Here, we investigated the conservation of this mechanism between Drosophila and mammals by exploring the role of the human Boi homolog, CDON, in SHH-producing PDAC cells. Our results support functional similarity between Boi and CDON in regulating SHH release is modified by dietary cholesterol. Moreover, our results show that CDON role in promoting tumorigenesis may be relative to the differentiation status of the tissue and/or mutation. Altogether, this study findings may potentially provide new therapeutic and/or diagnostic targets for pancreatic cancer. This work was funded by Bucks County Board of Associates, Pennsylvania Department of Health Research Formula Funds, Commonwealth of Pennsylvania Ben Franklin Technology Development Authority Keystone Innovation Starter Kit C000026964, Department of Defense W81XWH-16-1-0142, The Marvin and Concetta Pancreatic Cancer Institute, and National Institutes of Health, Fox Chase Cancer Center CA06927.
Program Abstract #558
Peptide ligand LXY30 for targeting Cancer Stem Cells
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Cancer stem cells (CSCs) are a rare sub-population of tumor cells with self-renewal and differentiation capacities often implicated in tumor initiation, chemoresistance and metastasis. For achieving complete remission, it is essential to identify and specifically target CSCs in addition to bulk tumor cells. CD44, CD133 and ALDH are reported as CSC biomarkers and cancer cells displaying high levels of CD44 and ALDH, and low levels of CD133 are deemed CSCs in breast and lung cancer. Cancer cell surface targeting peptide ligands are a potential candidate for targeted therapies due to their small size, enhanced permeability and retention in tumor sites and flexibility for synthesis. LXY30 is 9-amino acid cyclic peptide with selective binding to α3β1 integrin heterodimer on the surface of live tumor cells in both in vitro and in vivo tumor models. Herein, we investigated whether this tumor cell binding peptide LXY30 also bound to CSCs. To this effect, tumor cells from MDA-MB-231 and A549 cell lines were stained with CD44-APC-H7-antibody, CD133-APC-antibody and cells expressing CD44-high/CD133-low/ALDH-high and binding to LXY30 were detected using multi-color flow cytometry analysis. We observed that 3% of MDA-MB-231 cells and 2.86% of A549 cells were CD44-high/ALDH-high and bound to LXY30. There was no significant difference in CD133 expression. Use of α3β1 integrin as a biomarker in solid tumors and prognostic value of the ITGA3 gene encoding the α3 subunit was determined by genomic data mining of the gene in TCGA subsets which revealed that high integrin expression was associated with poor prognosis in breast invasive carcinoma and lung squamous cell carcinoma. In this study, we report that peptide LXY30 can be used for targeting CSCs in addition to bulk tumor cells. Modification of LXY30 to deliver cancer drugs for eliminating tumor cells and CSCs is a promising strategy to prevent cancer relapse and achieve complete elimination of cancer. CIRM funded this research.

Program Abstract #559
Paclitaxel Resistance in Breast Cancer is Regulated by Loss of Adenomatous Polyposis Coli
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Adenomatous Polyposis Coli (APC) is lost in up to 70% of sporadic breast cancers with a tendency towards triple negative breast cancer (TNBC). TNBC patients receive traditional chemotherapy, like paclitaxel (PTX), but tumors often develop drug resistance. PTX binds β-tubulin, stabilizing microtubules (MTs) to induce mitotic arrest and apoptosis. APC also regulates MT stability through binding β-tubulin, suggesting that APC may be involved in PTX response. Our lab created APC shRNA (APCKD) cells using the human TNBC cell line, MDA-MB-157, and determined that APC loss significantly increases PTX resistance. We predicted that varying MT stability among the cell lines would alter G2/M cell cycle protein modulators. Cell cycle analysis of PTX-treated cells by flow cytometry indicated more APCKD than parental cells in G2/M. The G2/M transition proteins cyclin B1 and CDK1, including the inhibitory and activating phosphorylation sites on CDK1, were analyzed by western blot (WB), showing increased CDK1 in APCKD cells. As the complex of cyclin B1 and CDK1 is only active when the proteins interact directly and in the nucleus, we examined the localization and protein-protein interactions. CDK1 is preferentially localized to the cytoplasm while cyclin B1 has no clear preference. Other cell cycle proteins involved in PTX sensitivity were examined by WB. APCKD cells show decreased CDK6 and increased p27 expression, while PTX increased cyclin D1 in both cell lines. Analysis of cell cycle protein modulators will further our understanding of PTX resistance in APC-deficient TNBC and help identify a potential therapeutic target. Future directions include interrogating the effects of alterations in cell cycle protein modulators on interactions between APC, PTX, and MTs. This research was supported by the American Cancer Society – Institutional Research Grant, Navari Family Foundation, the Indiana CTSI, grant #UL1 TR001108 from the NIH, NCATS, and a CTSI Core Usage grant to JRP.
Program Abstract #560
Studying consequences of cell-cell fusion using Drosophila neural stem cells as a model system
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Cell-cell fusion is a highly regulated biological event involved in important processes such as fertilization, placenta formation, muscle formation, and bone homeostasis. Misregulation of cell-cell fusion has been proposed to be involved in the initiation and progression of cancer, but direct evidence in support of this theory is currently missing. Here, we use the developing larval Drosophila melanogaster brain to define the cellular consequences of induced cell-cell fusion. We designed a protocol to induce fusions between fly neural stem cells (called neuroblasts) and adjacent differentiating cells (ganglion mother cells; GMCs) with high temporal and spatial precision. Live cell imaging revealed that proper alignment of ectopic GMC chromosomes in hybrid cells is cell cycle dependent. We further found that ectopic GMC chromosomes form an independent spindle using GMC centrioles. This ectopic spindle is able to align ectopic GMC chromosomes at the metaphase plate. Hybrid cells delay anaphase onset and often display lagging chromosomes or failed segregation, which may result in the formation of micronuclei or heterokaryons. We are currently performing experiments to identify the molecular identity and cell fate of hybrid cells. Furthermore, we will test whether hybrid cells will induce tumor formation, using classic transplantation assays. In addition to studying the role of cell fusion in cancer, this project will significantly contribute to a better understanding of the basic cell – cell fusion cell biology. This work is funded by a Research Scholar Grant from the American Cancer Society (ACS; 130285-RSG-16-253-01-CSM).

Program Abstract #561
Prostaglandin synthesis influences 3D organization of uterine glands and blood vessels in the pre-implantation uterus
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During pregnancy women consume pain medications such as non-steroidal anti-inflammatory drugs (NSAIDs) that act by inhibiting prostaglandin synthesis. These NSAIDs include over-the-counter medications such as acetaminophen, ibuprofen, and aspirin and their effects on pre-implantation events in women are controversial. Previous studies using rodent models and the NSAID – indomethacin, have shown decreased vascular permeability as the first visible effect of prostaglandin synthesis inhibition, followed by defects in decidualization. We used indomethacin to determine its effects on structural changes in three-dimensions in the uterine gland structure, 3D blood vessel organization, and vascular permeability. Using a time course we demonstrate that indomethacin, first targets the gland reorganization process, before it shows any visible effects on uterine vascular permeability. Further, we show that indomethacin treatment perturbs the amount of vasculature that is closely associated with uterine glands during pre-implantation time points. In light of this data, we hypothesize that changes in gland structure prior to vascular organization around the implantation site are essential to the establishment of pregnancy. We propose a model wherein, glandular structural changes guide pre-implantation vascular remodeling, and these changes in uterine gland structure are prostaglandin dependent and sensitive to the action of NSAIDs. Thus, prostaglandins influence changes in 3D uterine gland structure to impact vascular reorganization, which impacts pregnancy success in the mouse model. This project was funded by Basil O Connor award by March of Dimes and startup funds provided by Michigan State University to RA.

Program Abstract #562
Building Patterning-Dependent Chromatin States
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Following zygotic genome activation, Drosophila embryos undergo the MBT having established a homogeneous chromatin landscape common to all somatic cells of the embryo. Ubiquitously expressed pioneer factors, such as Zelda, establish this initial ‘ground state’ of chromatin structure. Over the next 24 hours of development, cell fate specification and differentiation will take place, driving the emergence of cell-type specific chromatin
organization. How do such patterns emerge, and how do developmental patterning systems drive these changes? To address this, we have profiled chromatin accessibility by ATAC-seq between the MBT and gastrulation comparing wild-type embryos with those deficient for all maternal patterning systems. Unlike wild type embryos, quintuple bicoid oskar capicua torsolike Toll[RM9] mutants develop with an apparent single uniform cell identity that carries a molecular signature of posterior endodermal progenitors. I demonstrate that with the exception of Bicoid, maternal systems drive patterning events largely constrained by the initial state of chromatin structure. However, distinct zygotic targets of maternal patterning systems drive regionalized chromatin accessibility patterns, resulting in emergent heterogeneity. One single factor, odd-paired (Zic1/2), accounts for ~30% of all new accessibility states that emerge in response to maternal cues. Such patterning-dependent changes in chromatin accessibility represent the emergence of ‘new’ regulatory information in the genome, occurring largely at previously silent cis-regulatory modules that are typically not bound by maternal pioneers such as Zelda and GAGA. Interfering with the temporal sequence in which pioneers reveal regulatory sites leads to misreading of regulatory cues and incorrect patterning of the embryo. This demonstrates that regulation of chromatin accessibility plays a deterministic role in the developmental program. [Funded by Northwestern University Start-Up Funds]

Program Abstract #563
Analysis of epigenetic gene regulation of neurodevelopment using a novel zebrafish epigenetic reporter transgenic line
Miranda Marvel, Aniket V. Gore, Kiyohito Taimatsu, Daniel Castranova, Andrew Davis, Brant M. Weinstein
NICHD/NIH, USA
Epigenetic factors are critical regulators of cell differentiation and specification during embryonic development. Epigenetic mechanisms, including DNA methylation, histone modifications, chromatin remodeling, and others, play crucial roles in gene regulation during development, and are especially important for the prolonged process of neurodifferentiation and neurogenesis. Large-scale genetic screens performed in fruit flies and nematode worms have been very successful in identifying epigenetic regulators in invertebrates. However, comparable screens have not been carried out in vertebrates, and mechanisms of neural tissue-specific epigenetic regulation in vertebrates are still not well understood. We have developed a novel transgenic epigenetic reporter zebrafish line that reliably reports changes in tissue-specific epigenetic silencing based on the dynamic expression of destabilized green fluorescent protein (GFPd2). Using this line, we are performing the first ever large-scale forward genetic screen in a vertebrate to identify recessive mutants that regulate developmental epigenetic silencing or activation. One of the mutants isolated through the forward genetic screen exhibits aberrant morphology and epigenetic silencing in the head. We identified the responsible mutation in a largely uncharacterized histone lysine demethylase gene. Morpholino silencing and targeted CRISPR knockout phenocopied the abnormal head morphology and epigenetic silencing of the brain in the ENU mutant, verifying the mutation induces a loss-of-function phenotype and suggesting that this gene codes for a critical epigenetic regulator of neurodevelopment. We are conducting further studies to reveal the downstream histone methylation and genetic targets of this factor and identify its specific neurodevelopmental roles. This work is supported by the intramural program of the NICHD, NIH.

Program Abstract #564
DNA methyltransferase disruption in the cranial neural crest causes cleft lip and palate in the mouse
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Orofacial clefts (OFCs) of the lip and palate are etiologically heterogeneous outcomes thought to result from gene-environment interaction. DNA methylation, an environmentally sensitive epigenetic mechanism, has been implicated in OFC biology but direct evidence of its requirement for orofacial morphogenesis has yet to be presented. Here, we applied genetic and pharmacologic approaches to interrogate the role of DNA methylation in orofacial morphogenesis. Expression of the DNA methyltransferase enzyme, DNMT1, was detected in the epithelium and cranial neural crest cell (cNCC) mesenchyme of the palatal shelves throughout palate development.
morphogenesis. While no impact of \textit{Dnmt1} deletion from the palate epithelium was observed, conditional \textit{Sox10Cre}-mediated deletion from cNCs resulted in cleft lip and cleft palate. \textit{Acute in utero} exposure to the DNA methyltransferase inhibitor 5-Aza-2‘-deoxycytidine (AzadC) at gestational day (GD)9.75 also resulted in cleft palate. AzadC exposure reduced cNC proliferation \textit{in vitro}, and both AzadC exposure and \textit{Dnmt1} deletion caused attenuated elevation and outgrowth of the palatal shelves \textit{in vivo}. These results demonstrate that DNA methylation in cNCs is required for orofacial morphogenesis and provide a model to evaluate environmental mediators of DNA methylation as potential causes and prevention strategies for OFCs. This work was supported by R03 DE027162 to RL and by the UW Hilldale Research Fellowship to CU.

Program Abstract #565

\textbf{Regulation of epigenetics and development by the Keap1-Nrf2 oxidative/xenobiotic response signaling in Drosophila}

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How environmental toxins regulate epigenetic status and developmental programs is less understood at the molecular level. The Nrf2 transcription factor and its key regulator Keap1 essentially mediate transcriptional responses to oxidative and xenobiotic stimuli, therefore are highly related with human diseases. Using the Drosophila model, we are exploring the novel molecular mechanisms and biological functions of dKeap1-CncC, the Keap1-Nrf2 homologues in Drosophila. We have revealed a novel developmental function of dKeap1 and CncC in the regulation of ecdysone signaling and metamorphosis. In addition, both dKeap1 and CncC are required for heterochromatic silencing, providing the first evidence in support of their epigenetic role in chromatin remodeling. Interestingly, these novel functions are all related with a non-classic mechanism in which dKeap1 binds chromatin and cooperates with CncC. This is in contrast with the conventional model stating that Keap1 inhibits Nrf2 activity through interacting and degrading Nrf2 in cytoplasm. Therefore, dKeap1-CncC complexes likely regulate detoxifying genes and developmental genes using different molecular mechanisms. To fully explore the biological functions of Keap1-Nrf2 family proteins, we employed polytene chromosome BiFC (bimolecular fluorescence complementation) assay to visualize the genomic loci and map the developmental genes that are exclusively targeted by dKeap1-CncC complex. In addition, a hypomorphic mutant that specifically abolishes the chromatin-binding of dKeap1 while retains its ability to inhibit CncC was generated, allowing us to characterize the developmental functions of dKeap1-CncC that are independent of their classic role in redox-control. These studies are expected to reveal novel mechanisms whereby stress response factors mediate the epigenetic and developmental adaptations to environmental toxins. This work is supported by NIH grant (1R15GM128143-1) to H.D.

Program Abstract #566

\textbf{A novel transgenic zebrafish line to study epigenetics during disease and development}

Aniket Gore, Kiyohito Taimatsu, Miranda Marvel, Daniel Castrnova, Avery Swearer, Keith Barnes, Andrew Davis, Brant Weinstein

\textit{Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, USA}

Epigenetic mechanisms such as DNA methylation, histone modification, and chromatin remodeling play crucial roles in regulating tissue-specific gene expression and acquisition of cellular identity. Although a number of molecular and biochemical approaches have been developed over the years to study tissue-specific epigenetic marks and gene expression, there are few tools available to visualize the dynamic nature of epigenetic gene regulation in live vertebrate embryos at the cellular level. We have developed a novel \textit{Tg(dazlCGI-ef1a:gfpd2)} “EpiTag” transgenic line that reliably reports dynamic tissue-specific epigenetic changes during development and regeneration. The transgenic construct used in this line contains a CpG island from the \textit{dazl} gene, a germ cell-specific gene targeted for silencing in all somatic cells. The \textit{dazl} CpG island is cloned next to a ubiquitous \textit{ef1a} promoter driving expression of destabilized GFP (GFPd2), allowing for dynamic visualization of rapid changes in GFP reporter expression in living animals. GFP expression in the EpiTag line begins at 6 hpf, peaks at 24 hpf, begins to fade by 48 hpf, and is almost undetectable by 5 dpf. GFP expression after 24 hpf can be reactivated by treatments that interfere with epigenetic silencing, validating the line as an effective “epigenetic reporter.” The
epigenetic reporter is silenced in all adult fish tissues except for reproductive tissues, consistent with endogenous dazl gene expression. We are currently using this line to carry out a pilot F3 ENU mutagenesis screen to identify recessive tissue-specific mutants in epigenetic silencing. This highly successful screen has already identified tissue-specific epigenetic silencing mutants. We have also shown that EpiTag GFP expression is reactivated during regeneration. Our findings highlight the power of the EpiTag reporter for studying dynamic epigenetic changes during development and regeneration. This work is supported by NICHD, NIH intramural funds.

Program Abstract #567
MicroRNA Influence on Craniofacial Development
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Craniofacial defects such as cleft lip/palate are among the most common congenital anomalies. The cranial and facial bones develop from neural crest cells (NCCs) and craniofacial defects are often attributed to misregulation in gene expression within NCCs. The differentiation of neural crest cells into a multipotent cell population is invoked by a complex gene regulatory network. One aspect of this network are microRNAs (miRs) that contribute to embryonic development through epigenetic control of genes involved with development. Therefore, aberrant expression of key miRs can result in developmental skeletal defects. However, no studies as of yet have researched the gene interactions of miRs that contribute to the formation of osteoblasts, the bone forming cells. Our preliminary data has revealed that miR361 promotes differentiation of embryonic stem cells (ESCs) into bone. Our ESC osteogenic differentiation protocol suggests this differentiation occurs mainly through the NC route and miR361 was detected within the NC of mouse and Xenopus embryos. Endogenous levels of miR361 within osteogenic differentiation showed a biphasic expression level of miR361 where in early ESC commitment, miR361 levels are downregulated and begin to increase during neural crest induction. To understand the transcriptional regulation that miR361 exerts within NC differentiation into the osteogenic lineage, we performed an RNA-seq on mouse ESCs overexpressing miR361. These results showed genes involved with ossification, NC development and cleft lip/cleft palate all significantly upregulated. GO analysis further identified up-regulated genes associated with the Wnt and bone morphogenic pathways (BMP) signaling pathway. Together, these preliminary results suggest miR361 exerts its pro-osteogenic effect through the non-canonical Wnt and BMP pathways. Ultimately, miR361 may serve as a potential biomarker for prenatal screening of craniofacial defects. Funding provided by: NIDCR R01DE025330

Program Abstract #568
The Reestablishment of Hemodynamic Balance After Blood Vessel Injury
Leah Greenspan, Daniel Castranova
National Institutes of Health, USA
Blood circulation must be carefully balanced between arterial and venous flow to ensure all organs and tissues receive the necessary oxygen and nutrients required for their viability. Disruption to this balanced flow due to disease or physical vessel injury can lead to severe consequences such as ischemia or hemorrhage. After damage occurs, reperfusion of vessels is critical to ensure tissue survival. However, little is still known about how vessels remodel after injury. While it is thought that hemodynamics and cell-cell signaling play a role, the precise cellular and molecular mechanisms underlying vessel repatterning remain unclear. Zebrafish are an ideal model organism to study vascular damage and repatterning in vivo due to their optical clarity, their genetic and experimental accessibility, and their conserved vertebrate vascular anatomy. To elucidate what drives the restoration of vascular networks following vessel injury, I have established three different vessel injury assays to recapitulate vessel severing, clotting, and endothelial cell death. Using high-resolution imaging of the zebrafish vasculature I can observe in detail the rewiring of vascular networks after injury. My data reveals that severed trunk intersegmental vessels (ISVs) reattach to major vessels withing 2-3 days after injury, but sometimes alter their vessel identity following reconnection. In addition, unmanipulated ISVs neighboring injured ISVs are also observed
switching flow direction. This suggests that arterial-venous identity of ISVs is plastic and that these blood vessels can adapt to changes in flow in order to restore hemodynamic balance. Future work using transgenic reporters and mutant animals will help uncover the molecular mechanisms that guide restoration of functional vascular networks in injured tissues and provide potential new targets for therapeutic approaches. Funding is provided by the intramural research program of the NICHD.

**Program Abstract #569**  
Desert hedgehog regulates fat infiltration and muscle regeneration  
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Even though skeletal muscle tissue has an amazing capability to regenerate upon injury, this process is compromised in such a way that muscle is replaced with fat and fibrosis, as seen with age, Duchenne muscular dystrophy (DMD) and neuromuscular diseases. We and others have found that the hedgehog pathway (HH), once activated, improves adult muscle regeneration, along with repressing fat formation. The role this complex pathway plays in the adult muscle tissue is not fully understood, nor which ligand is responsible for this protective effect. We evaluated the expression of the three Hh ligands (Sonic, Indian and Desert) during the regeneration process. Surprisingly, we found that Dhh was upregulated with an acute injury, induced by cardiotoxin (CTX), and repressed by an adipogenic injury, induced by Glycerol (Gly); suggesting it could act as an adipogenic break. Excitingly, upon evaluating a Dhh knock-out mouse after a CTX injury we found an increase in intramuscular fat formation and an impairment in muscle regeneration. These results suggest that Dhh is the responsible ligand through which HH signaling controls muscle regeneration and blocks fat formation. I have now begun to analyze this mouse model after an adipogenic injury, and in a DMD mouse model (mdx mouse) to evaluate its role in disease progression. understanding the role of Dhh during muscle regeneration can lead to new therapeutic targets to control fat infiltration and enhance muscle repair to prevent its degeneration in muscular dystrophies and in age.

**Program Abstract #571**  
Comparison of bone fracture healing in axolotl and mouse  
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For the treatment of complicated bone fractures, understanding of the cellular and molecular mechanisms of fracture callus formation and bone healing is needed. Axolotls are widely used as a model for whole limb regeneration, but the bone healing capacity of the axolotl is poorly understood. Thus we aimed to compare axolotl and mouse long bone fracture healing. In this study, we used 12-14 weeks old mice and adult axolotls (>20 cm snout-to-tail length, 5-8 years old), which have femurs of comparable size. We used bone-aligned plates to fix femurs prior to fracture (MouseFix, RISystem). Mouse samples were harvested 1, 2, 3, 5, and 8 weeks after surgery. In axolotl, bone healing is slower, thus, samples were harvested 3 weeks, and 3, 6 and 9 months after surgery. In addition, non-fixed fractures and regenerating amputated hind limbs were studied in axolotl. Histological analysis and immunofluorescent staining for SOX9 cartilage progenitor marker showed that cartilaginous callus is formed in axolotls at 3-6 months post-surgery, suggesting that axolotl bone fractures heal through the process of endochondral ossification. PRRX1 mesenchymal progenitor marker expression was evaluated in murine and axolotl fracture sites. In axolotl, PRRX1-positive mesenchymal progenitors accumulate at the fracture site at 3 weeks post-fracture and then are retained at the callus margin. In mouse, PRRX1-positive cell population partially overlaps with LepR-positive cells. In axolotl, cell proliferation in callus and blastema is reduced at 3 months post-fracture, and in mouse fracture – at 2 weeks post-fracture. In this work, we characterized adult axolotl bone healing and showed that despite remarkable regenerating capacity, axolotl bone fracture healing is much delayed in comparison to the terrestrial tetrapods, such as a mouse.
Program Abstract #572
Control of osteoblast regeneration by a train of Erk activity waves
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Regeneration is a complex chain of events that restores a tissue to its original size and shape. Gradients of diffusible morphogens regulate cell behavior in many multicellular systems, but it is unclear how such gradients could coordinate tissue growth across the large spatial scales of regenerating adult tissues. Feedback mechanisms can provide an effective mechanism of communication across large distance, but their role in regulating regeneration remains largely unexplored, owing to difficulties in imaging, analyzing and conceptualizing these complex systems. To overcome those barriers, we developed the regenerating scale of adult zebrafish as a quantitative platform to study tissue growth and morphogenesis. Scales are external millimeter-sized bony disks coated with a mono-layer of bone-depositing osteoblasts. After scale loss, the osteoblast tissue regenerates in just a few weeks. Scales are an ideal system to study tissue regeneration quantitatively owing to their simple organization and accessibility to live imaging. We discovered that traveling waves of Erk activity instruct hypertrophic growth of the regenerating osteoblast tissue. Using a combination of theoretical and experimental analyses, we find that Erk waves propagate across the millimeter-sized tissue as concentric trigger waves and induce patterned tissue growth, thus orchestrating scale morphogenesis. We estimate that simple diffusion would take weeks to propagate growth signals across the entire tissue, while concentric trigger waves deliver signals across the entire scale in 1-2 days. Furthermore, periodic induction of synchronous, tissue-wide, Erk activation in place of travelling waves impairs tissue growth, indicating that wave-distributed Erk activation is key to regeneration. Our findings reveal trigger waves as a regulatory strategy to coordinate cell behavior and control tissue morphogenesis during regeneration. Funding sources: Swiss National Science Foundation, NSF, NIH, Whitehead.

Program Abstract #573
Comparative Transcriptomics and Single-Cell RNA Seq Identify Divergent Gene Expression in Osteoprogenitors During Zebrafish and Rat Bone Regeneration
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Zebrafish can regenerate bony appendages following amputation, which may reflect unique genes regulating osteoprogenitor activation and regulation. We used cross-species transcriptomics and single-cell combinatorial indexing RNA sequencing (sci-RNA seq) to 1) identify genes dissimilarly expressed during rat and zebrafish bone regeneration, and 2) determine which cell populations in zebrafish expressed these genes. We modified a previously developed bioinformatics pipeline to include a robust similarity metric (dynamic time warping distance) to quantify similarity. We analyzed microarray datasets for zebrafish caudal fin regeneration and rat marrow ablation-induced bone regeneration. Of the 30 most dissimilarly expressed genes, 11 were upregulated in zebrafish while downregulated in rat. f13a1b was the most dissimilarly expressed of those genes. We performed sci-RNA seq of the regenerating zebrafish caudal fin to investigate these genes at single-cell resolution. f13a1b is expressed within osteoblasts, epidermal, and mesenchymal cells. Within the osteoblasts, further clustering identified cycling and non-cycling osteoblasts, pre-osteoblasts (col10a1+), and early-osteoblasts (sp7+). f13a1b expression is enriched in two of the osteoprogenitor populations and less abundant in later stages of osteoblast differentiation. In conclusion, we have performed comparative transcriptomics to elucidate genes that are upregulated in zebrafish relative to rats, and determined their involvement at single-cell resolution in the regenerating zebrafish caudal fin. Genes upregulated during zebrafish bone regeneration may provide drug targets to improve appendage regeneration in humans. This research was supported by the NIH, the Brotman Baty Institute for Precision Medicine, UW Department of Orthopaedics and Sports Medicine, the Institute for Stem Cell and Regenerative Medicine at UW, and the Mary Gates Endowment.
Program Abstract #574
An Inclusive Professional Development Boot Camp for Junior Science Faculty
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Starting a tenure-track faculty position is challenging. Besides setting up a laboratory, most assistant professors will need to develop teaching modules and engage in some type of institutional service. Most junior faculty do not receive training in these topics, or in the “soft skills” necessary for running their lab, during their graduate programs or postdoctoral fellowships. There are some “how-to” manuals available to read, but these lack field-specific advice and require the faculty to seek out and read them. The Society for Developmental Biology wished to help junior colleagues by providing a workshop that would encompass important skills needed for them to succeed in the tenure process. A two-day boot camp was developed for new faculty that occurs every other year prior to the annual meeting of the Society for Developmental Biology. We evaluated the program in its tenth year, looking to see if the program was succeeding, and for whom. Our findings suggest that programs like this support junior faculty successfully and without significant signs of gender bias, institutional exclusivity, or tenure-status relevance. We suggest that similar programs be launched by other professional societies.

Program Abstract #575
Trails to Research: An Intensive Introduction to Research for Tribal College Students
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In order to expand the participation of American Indian students in research, we developed intensive one-week courses for American Indian tribal college students. In these courses, students experience life science research in an immersive setting, both at Montana State University (MSU) and at tribal colleges across Montana. In these courses, zebrafish are used as a model to explore aspects of environmental health and human health. Students design experiments that test the effects of variety of substances (ingestible substances such as alcohol and caffeine, chemicals found in the environment such as herbicide components and heavy metals, local water samples etc) on the development of zebrafish embryos. In these student-designed and -managed research projects, tribal college students learn the research process from developing a hypothesis to presenting their findings. Each course is specifically designed to address student-selected topics and to meet the needs of tribal communities. In order to assess the effectiveness of our program, we created assessment tools that include surveys, discussions, and knowledge-based questions that are administered at the beginning and at the end of each course. We will present our findings from the 2018 - 2020 courses. These include courses at Aaniiih Nakoda College, Little Big Horn College, Fort Peck Community College, and Chief Dull Knife College (these are all associate’s degree granting 2-year tribal colleges), as well as courses at MSU and comparison courses conducted at urban 2-year colleges (Great Falls College and Gallatin College). Overall, our data indicate that our courses were effective in increasing students’ confidence and positive attitudes towards scientific research and boosting students’ confidence in a laboratory setting. Future assessment will determine the transfer rate to 4-year colleges among the students, who participated in these courses. NSF TCUP Targeted STEM Infusion Project.

Program Abstract #576
Incorporating Metacognition into a Biology Course to Improve Student Learning
Laura Romano
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The goal of any biology course is to not only teach content, but also the skills required for future scientists to solve real world problems. I have developed a strategy to improve student learning in an introductory biology course, particularly skills that may not have been emphasized at the high school level. Such skills go beyond “remembering” and “understanding” and correspond to those highlighted in the upper tiers of Bloom’s Taxonomy, namely, “applying,” “analyzing,” “evaluating,” and “creating.” For each unit of my course, students read a recent primary article from the scientific literature and then complete a worksheet before classroom discussion. All of the articles are related to an interesting aspect of bird biology, and the worksheets always
consist of six questions, each labeled as corresponding to a particular tier of Bloom’s Taxonomy. I have found that a consistent theme and format, as well as a more explicit approach to metacognition, not only contributes to students learning critical skills but also fosters their confidence in the ability to engage with scientific literature, which is a big focus of advanced courses such as Developmental Biology.

Program Abstract #577

Pomacea canaliculata: a new research organism to study complex eye regeneration
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Vertebrate complex eyes are comprised of cornea, iris, lens, retina and optic nerve. Although their sensitivity and image resolution are astonishing, their regenerative capacities are either limited or absent. A few amphibians and fishes can repair specific ablations of the cornea, lens or sub-populations of retinal cells, but complete regeneration after full removal of their eyes has not been observed. Histological staining, electron microscopy and transcriptome analyses indicate that the complex camera-type eyes of the freshwater snail Pomacea canaliculata are not only anatomically comparable to vertebrate eyes, but are also able to regenerate de novo after complete amputation. The combination of scRNAseq of isolated P. canaliculata retina, and extensive eye regeneration transcriptomes allowed us to identify multiple cell types present in the snail retina, and to select markers for cells potentially involved in the processes of wound healing, cell proliferation and eye regeneration. To further understand gene function through CRISPR mediated gene perturbation, we developed protocols to harvest zygotes, microinject them and culture the dividing embryos ex ovo. About 15% of the embryos injected with exogenous GFP mRNA survived and showed positive signal for up to 4 days, while indels in the region of interest were obtained after injection of gRNA and Cas9 protein. Further optimizations of genome manipulation protocols will allow us to establish transgenic reporter lines for live imaging the direct development of these embryos, mechanistically dissecting the regeneration of a complex organ that is irreplaceable in all current research organisms and testing whether eye regeneration recapitulates embryonic eye development. Funding provided by Howard Hughes Medical Institute (ASA), Stowers Institute for Medical Research (ASA), Society for Developmental Biology (SDB_Emerging Models grant) (AA), American Association of Anatomists (AAA_Postdoctoral fellowship) (AA)

Program Abstract #578

The Planarian Anatomy Ontology: A resource to connect data within and across experimental platforms
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As the planarian Schmidtea mediterranea (Smed) gains popularity as a research organism, the need for standard anatomical nomenclature has become increasingly apparent. Utilization of a controlled vocabulary streamlines data annotation, improves data organization, and enhances cross-platform and cross-species searchability. We created the Planarian Anatomy Ontology (PLANA), an extendable framework of defined Smed anatomical entities organized using relationship terms. PLANA Version 29-02-2020 contains more than 1000 terms that holistically describe Smed anatomy from the subcellular to system-level scale across all life cycle stages, in intact animals and regenerating fragments. Terms from open-source anatomy ontologies were imported into PLANA to promote ontology interoperability and comparative anatomy queries. To demonstrate the utility of PLANA for data curation, we created web-based resources for planarian embryogenesis, encompassing a molecular staging series and molecular fate mapping atlas, and the Planarian Anatomy Gene Expression (PAGE) database, which integrates published gene expression data generated using different transcriptomes and experimental platforms. Finally, we establish an open-source pipeline for continued curation and versioning of PLANA, providing a path for expansion and evolution of this community resource. This work was conducted using the Protégé resource, which is supported by grant GM10331601 from the NIGMS. We also acknowledge funding from NIH Grant R37GM057260, the Stowers Institute for Medical Research and the Howard Hughes Medical Institute.
Program Abstract #579

Xenobots: computer designed living machines built from amphibian stem cells

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Living systems are more robust, diverse, complex, and supportive of human life than any technology yet created. However, our ability to create novel lifeforms is currently limited to varying existing organisms or bioengineering organoids in vitro. Here we show a scalable pipeline for creating functional novel lifeforms: AI methods automatically design diverse candidate lifeforms in silico to perform some desired function, and transferable designs are then created using a cell-based construction toolkit to realize living systems with the predicted behaviors. Although some steps in this pipeline still require manual intervention, complete automation in future would pave the way to designing and deploying unique, bespoke living systems for a wide range of functions. This research was sponsored by the Defense Advanced Research Projects Agency (DARPA) under the Lifelong Learning Machines program, the Allen Discovery Center program through The Paul G. Allen Frontiers Group, the National Science Foundation’s Emergent Behaviors of Integrated Cellular Systems Grant, the National Science Foundation’s Emerging Frontiers in Research and Innovation (EFRI) Continuum, Compliant, and Configurable Soft Robotics Engineering (C3 SoRo) program.

Program Abstract #580

PhaRedox: An Improved Image Analysis Pipeline for in vivo Ratiometric Fluorescence Microscopy Reduces Measurement Error and Increases Throughput

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The development of genetically-encoded redox-sensitive fluorescent proteins has allowed for high-resolution spatio-temporal visualization of the cytosolic redox state of live animals at the cellular level. These redox sensors have proven to be powerful tools leading to a deeper understanding of the signaling role of ROS. However, the method requires multiple images be taken per sample, and assumes the sample to be stationary. We are interested in imaging in vivo, and so the animal may move between frames. Inter-frame movement must be manually scored, and animals that move must be excluded from analysis. Thus, inter-frame movement results in a significant time investment on the part of the researcher, as well as a reduced statistical power. To address these issues, we developed an improved image analysis pipeline for measuring spatio-temporal patterns in the pharynx of C. elegans. We chose the pharynx due to its highly stereotyped geometry and simple cellular structure. Our method calculates the centerline along the anterior-posterior axis of the pharynx, and measures intensities under it. By using a functional version of dynamic time warping, we remove much of the unwanted phase variation that comes from inter-frame movement while retaining the amplitude variation corresponding to biochemical processes inside the cell. Our pipeline, PhaRedox, requires minimal manual intervention and reduces intensity-normalized error across stationary and moving animals by up to 4-fold as compared to a previous pipeline. This work was funded by the NIH and by the Matz Biotechnology fellowship program at Northeastern University.

Program Abstract #581

Mechanics of behavior: Cytoskeletal dynamics that give rise to comprehensive search behavior in the single cell Lacrymaria olor

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Complex animal behavior arises at the intersection of form and function. Surprisingly, single eukaryotic cells such as protists are also capable of complex animal-like behavior, but how an algorithm such as search might be encoded in the morphology and cytoskeletal geometry of a single cell remains an open puzzle. Protists perform many remarkable complex behaviors, such as capturing prey or predator avoidance. One single-celled organism, Lacrymaria olor, changes morphology as it searches for prey using a microtubule machine that resembles a helical basket of microtubule filaments. Our recent work establishes that L. olor’s search strategy is encoded in the coupling of antagonistic active systems: subcellular structures that use surface cilia and the cortical cytoskeleton (Coyle et al, 2019). Here we reveal the underlying geometrical features of the cytoskeleton, and membrane and
volume constraints, that together program extension and contraction dynamics of L. olor’s neck-like protrusion. By performing force spectroscopy and controlled membrane tension experiments in live cells, we reveal the role of this dynamic force landscape and how it shapes the search phase space. Our work combines physical models with experimental data to unravel how cytoskeletal geometry encodes morphological changes, enabling efficient prey search and capture. Funding Sources: Biophysics NIH Training Grant, Center for Cellular Construction, CZ Biohub, HHMI

Program Abstract #582
Mathematical modeling of skin pattern formation in zebrafish
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Wild-type zebrafish (Danio rerio) feature dark and light stripes across their body and fins, but mutants display a range of altered skin patterns, including spots and labyrinth curves. All these patterns form due to the interactions of pigment cells, which sort out through movement, birth, competition, and transitions in cellular shape during development. The goal of our work is to help link genotype, altered cell behavior, and phenotype using mathematical modeling. Working closely with the biological data, we model the interactions of individual pigment cells through a combination of differential equations and stochastic rules. We use our models to simulate the development of wild-type and mutant skin patterns as zebrafish grow from a few weeks to a few months old. Our models help suggest the unknown cellular signals that are behind newly observed cell behaviors, and we make experimentally-testable predictions about how various Danio fish may be related evolutionarily. This work has been partially supported by the National Science Foundation through DMS-1148284, DMS-1440386, DMS-0907904, and DMS-1409742, and is currently supported by the NSF through DMS-1764421 and by the Simons Foundation/SFARI under 597491-RWC.

Program Abstract #583
Single-cell and genetic analyses reveal conserved populations and signaling mechanisms of stomach and intestinal stromal niches
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Stomach and intestinal stem cells are located in discrete niches along the gastrointestinal epithelium called the isthmus and crypt, respectively. Recent studies have demonstrated a conserved role for Wnt signaling in gastrointestinal development, reflecting their role in adult homeostasis. Although intestinal stromal cells secrete Wnt ligands to promote stem cell renewal, the source and regulation of gastric Wnt ligands are still unclear. Here we define the heterogeneity of stomach and intestinal stromal cells by performing single-cell RNA-sequencing of Bapx1-cre lineage traced stromal cells, identifying stromal cell populations with conserved transcriptome signatures in the stomach and intestine. These subepithelial ‘pericyte-like cells’ are labeled by Ng2-Cre and highly express both telocyte and pericyte markers, Wnt ligands, and they are enriched for Hh signaling. By analyzing mouse models of activated Hh signaling and through chromatin immunoprecipitation and sequencing, we demonstrate a conserved mechanism of GLI2-mediated activation of Wnt ligands in stomach and intestinal stromal cells. Ng2-Cre-specific inhibition of Wnt secretion through deletion of Wntless produces a mild phenotype, while its deletion in a broader population of stomach and intestinal stromal cells using Bapx1-Cre leads to dramatic stem cell defects in both the forestomach and intestine during development. These findings reveal cell-type-specific roles within stromal stem cell niches during gut development and regeneration. By comparing Ng2-2 and Bapx1-labeled cells through CyTOF, we further show that distinct stromal niche populations may account for the observed phenotypic differences, highlighting the redundancy of gastrointestinal stem cell niches secreting Wnt ligands. This work was supported by SickKids Foundation, the University of Toronto’s MbD Initiative/the Canada First Research Excellence Fund, CIHR; PJT 155923 and NSERC Discovery #RGPIN-2016-06093 grants.
Program Abstract #584
Potential regulatory elements of the human keratin type II gene locus identified by the analysis of chromatin distant interactions.
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Human keratin genes are clustered in two domains located at chromosomes 12 and 17. Keratin expression program is highly cell type-specific for different epithelial tissues and differentiation stages, and is governed by microenvironment signals modulating epigenetic state and activity of promoters in keratin gene loci. In mammals, switching of expression profile in complex multigene domains is typically provided by superenhancers (SE) marked with H3K27ac and H3K4me1 histone modifications and occupied with numerous tissue-specific transcription factors and chromatin architectural proteins such as CTCF and cohesin. Here, we utilized high-resolution chromosome conformation capture method, C-TALE (Chromatin TArget Ligation Enrichment) to probe chromatin spatial organization of 12q13.13 locus in human iPSCs, fibroblasts, cultured keratinocytes (HaCaT cell line) and epidermal skin keratinocytes at two distinct stages of differentiation. We found that, in skin keratinocytes, expression switching between keratin 5 (specific for basal epidermal keratinocytes) and keratin 1 genes (actively transcribed in spinous K1/K10positive keratinocytes) is followed by significant remodeling of chromatin loop profile within the locus. Both genes establish contacts with two regions located at the borders of the domain while transcriptionally active, and loose these contacts after inactivation. According to ENCODE epigenetic datasets, both regions identified are SE: they demonstrate high level of H3K2ac, presence of DNAse I hypersensitivity sites, binding of CTCF and transcription factors involved into keratin gene regulation. In all analyzed cell lines these regions interact with each other forming a chromatin hub. These data potentially denote that switching of keratin gene expression is controlled by two SE establishing long-range chromatin loops with active keratin promoters and other genomic elements within the domain. This study was funded by RFBR, project number 20-04-00778.

Program Abstract #585
Effect of cellular iron deficiency on epigenetic modifications at the Bdnf locus
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Iron deficiency (ID) is the most common micronutrient deficiency worldwide, affecting approximately 30% of pregnant women and preschool-age children. Early-life ID causes cognitive and behavioral abnormalities associated with long-term gene dysregulation in preclinical models. However, the mechanism behind the long-term gene dysregulation is not well understood. Lysine Demethylase 5B (JARID1B) is an iron-dependent histone modifier that is important for neural development by regulating brain-derived neurotrophic factor (Bdnf) gene, which plays critical roles in neuronal survival, differentiation, and plasticity. Previous studies showed that early-life ID alters JARID1B expression and histone H3K4-methylation at the Bdnf locus. We used chromatin immunoprecipitation (ChiP) assay to analyze the effect of ID on the interaction between JARID1B and Bdnf.
Neuronal cell line derived from embryonic mouse hippocampus (HT-22) was treated with deferoxamine (DFO), an iron chelator, to induce ID. Preliminary results showed that 24-hr after DFO exposure, cells showed upregulation of both genes. However, ChiP assays showed lower enrichment of H3K4me3, a histone modification associated with active transcription. Furthermore, enrichment of JARID1B occurred at the Bdnf promoter, but not at its own promoter. Collectively, these findings suggest that iron-deficient neurons acutely alter gene regulation in the opposite direction than our expectation, raising the possibility of differential responses between acute and chronic ID at a molecular level. Thank you to Choose Development! Program from the Society of Developmental Biology for funding and guidance.

Program Abstract #586
Characterizing zebrafish tissue regeneration using a novel epigenetic reporter line
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Tissue regeneration is a complex and carefully orchestrated process. In contrast to mammals, some lower vertebrates exhibit high regenerative capacities, including the zebrafish. Adult zebrafish are able to efficiently and completely regenerate many different organs and tissues, including their fins, retina, spinal cord, and heart. During zebrafish heart regeneration, epicardial cells undergo dedifferentiation and activate embryonic signaling pathways, allowing them to reenter the cell cycle and transdifferentiate into new cell types. Regenerating cardiomyocytes lose their original epigenetic marks and undergo “epigenomic reprogramming” as they activate different gene expression programs. We have developed a new zebrafish “EpiTag” transgenic reporter line that becomes activated in and selectively marks cardiac and other cells undergoing regenerative epigenetic reprogramming. The EpiTag line contains a dazl CpG island linked to the ubiquitous EF1a promoter driving destabilized GFP. Reporter GFPd2 expression is normally silenced by methylation in quiescent somatic tissues, but it can be reactivated by treatments that interfere with epigenetic silencing, validating the line as an effective “epigenetic reporter.” We find that the EpiTag reporter is also selectively reactivated in cryoinjured hearts. GFP activation is restricted to cells actively undergoing regeneration, suggesting transgene expression may be due to epigenetic reprogramming. We are currently performing additional molecular and anatomical studies to further understand epigenomic reprogramming in post-injury EpiTag-activated hearts. EpiTag reactivation is not limited only to regenerating cardiac tissues. We also see activation during fin and muscle regeneration, suggesting EpiTag reporter is a more widely useful and powerful tool to mark, visualize, and study cells undergoing epigenomic reprogramming during tissue regeneration. This work is supported by the intramural program of the NICHD, NIH.

Program Abstract #587
Loss of extreme long-range enhancers drives a human craniofacial disorder
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SOX9 is an important developmental transcription factor that plays pleiotropic roles during embryogenesis. Heterozygous mutations in the SOX9 gene cause a severe congenital disorder called campomelic dysplasia (CD) associated with skeletal dysplasia, XY sex reversal, and dysmorphic facial features. Interestingly, distinct non-coding mutations within a large gene desert surrounding the SOX9 gene recapitulate specific subsets of CD phenotypes, suggesting loss of tissue-specific regulatory elements. One class of patients with an anomaly called Pierre Robin sequence (PRS), characterised by under development of the lower jaw and cleft palate, have deletions over a megabase upstream of the SOX9 gene. Within this deletion region, we identify two clusters of extreme long-range enhancers that mediate human cranial neural crest-specific SOX9 expression at distances of up to 1.45 Mb away from the promoter. Enhancers within the two clusters exhibit highly synergistic activity and are conserved in activity down to Coelacanth fish. Using mouse models, we propose a novel mechanism for the observed specificity of PRS manifestations arising from a combination of two mechanisms: confinement of Sox9 dosage perturbation to the developing facial structures due to context-specific enhancer activity, and a heightened sensitivity of the lower jaw to reduction in Sox9 levels. Overall, we characterize the longest-range human enhancers involved in congenital malformations, directly demonstrate that PRS is an enhanceropathy, and illustrate how small changes in gene expression can lead to morphological variation. This work was supported by funding from HHMI, U01 DE024430 FaceBase2 and Wellcome Trust Sir Henry Wellcome fellowship 106051/Z/14/Z.

Program Abstract #588
Carbaryl exposure in zebrafish leads to activation of the aryl hydrocarbon receptor pathway and increased cyp1 gene expression
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Xenobiotics are foreign biological chemicals present in an environment/ecosystem. Pesticides are common examples of xenobiotics which are becoming more and more prevalent-especially in waterways. Carbaryl is a commonly used insecticide within the U.S. that acts to inhibit the acetylcholinesterase (AChE) enzyme in muscle
tissues—leading to insect death. While AChE inhibition is well documented, Carbaryl is also hypothesized to bind to the aryl hydrocarbon receptor (AhR) and activate expression of the cytochrome P450 (cyp1) genes. Zebrafish were exposed to either carbaryl or 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)—a known aryl hydrocarbon receptor agonist, and RNA was extracted after 24 hours of exposure. cDNA was synthesized and used to quantify gene expression of the cyp1 genes using qPCR. Gene expression analysis showed that both carbaryl and TCDD exposure resulted in a comparable increase in expression of both cyp1a and cyp1b genes. TCDD, but not carbaryl, exposure led to increased expression of cyp1c1 and cyp1c2 genes. The data support the hypothesis that carbaryl does activate the AhR pathway and ultimately increases expression of some cyp1 genes (cyp1a and cyp1b) in zebrafish. To confirm the increase in gene expression is AhR dependent, the next step is to see if blocking the AhR receptor eliminates the induction of cyp1 gene expression. It is unclear why TCDD exposure, but not carbaryl, resulted in increased cyp1c1 and cyp1c2 expression. The Student Development Board and Faculty Development Board at Wittenberg University have funded this research.

Program Abstract #589
Control of C. briggsae germline development by TRA-1-interacting co-factors
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Chromatin remodelers work with transcriptional regulators to control gene expression during development. Many studies have focused on the role transcription factors play in evolutionary change, but much less is known about the role of chromatin remodelers. Gli proteins form a conserved group of Zn-finger transcription factors that regulate many important developmental processes. In Caenorhabditis nematodes, TRA-1 is the sole Gli protein, and acts as the terminal regulator of the sex-determination pathway. Both the activator and repressor functions of this Gli protein can be studied in the germ line, where they regulate the sperm/oocyte decision. TRA-1 activity is influenced by several chromatin regulators that may work as co-factors. In C. briggsae, it interacts genetically with TRR-1 (part of the TIP-60 HAT complex), WDR-5.1 and the NURF complex to promote spermatogenesis. We are studying these epigenetic interactions in the germ line to learn how each co-factor works with TRA-1 to control its target fog-3. We made these control strains to allow us to alter germ cell fates: Cbr-glp-1(v429) III, Cbr-fog-1(v442) I and Cbr-glp-4 (v473ts) I. Next, we produced alleles of Cbr-tra-1 with either N-proximal or C-proximal OLLAS tags that retain wildtype function. We performed anti-H3K4me3 and anti-OLLAS ChIP-qPCR to amplify the tra-1 binding site from the fog-3 promoter at different developmental stages. The results revealed the differential expression pattern of fog-3 gene levels, low level fog-3 before the spermatogenesis and high fog-3 level after the spermatogenesis stage. This shows that the TRA-1 binding at fog-3 varies during germline development. Further work is in progress to identify the co-factors involved in TRA-1 mediated germ line developmental regulation. Acknowledgment: We thank NIH for the funding.

Program Abstract #590
Deeply conserved enhancer regulates heart and gut looping in vertebrates
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During the phylotypic period embryos from different genera show similar gene expression patterns, implying common regulatory mechanisms. To identify enhancers involved in the initial events of cardiogenesis, which occurs during the phylotypic period, we isolated early cardiac progenitor cells from zebrafish embryos and characterized 3838 open chromatin regions specific to this cell population. Of these regions, 162 overlapped with conserved non-coding elements (CNEs) that also mapped to open chromatin regions in the human genome, which we referred to as aCNEs. Most of the zebrafish aCNEs (15/21) drove gene expression in the developing heart in stable lines. Despite modest sequence identity, human orthologous aCNEs could recapitulate the spatial-temporal expression patterns of the zebrafish sequence, potentially providing a basis for phylotypic gene expression patterns and setting up the vertebrate body plan. To characterize the functional roles of aCNEs, we deleted aCNE1 in zebrafish. Our enhancer assay and 4C result showed that aCNE1 was a distal enhancer that regulated the expression of hand2 in cardiac progenitors. While most aCNE1-/- mutants were viable, nearly half of them displayed laterality defects in heart or gut looping, partially recapitulating the phenotype seen in hand2 null
mutants. In-situ hybridization and qPCR analysis indicated that the anterior expression of hand2 was downregulated and left/right asymmetric gene expression (lefty2) was delayed or disrupted in the mutants, leading to randomization in left/right axis establishment in these embryos. On-going work will investigate how the loss of aCNE1 disrupts laterality pathway and if genetic variants within aCNE1 are associated with human diseases. Together, our study suggests that a diverse repertoire of deeply conserved developmental enhancers is established prior to the phylotypic period, which later contributes to organogenesis in vertebrates. This work was funded by CIHR and HSF of Canada.

Program Abstract #591
Developing an in vivo system for PGC migration using xenotransplantation
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Primordial germ cells (PGCs) are stem cells of egg and sperm that are established early in development, well before the gonads. Although the mechanisms of PGC specification differ across organisms, their migration through the growing embryo to the developing gonads is conserved across the Metazoan kingdom, and relies upon conserved signaling pathways, such as the cxcr4-sdf-1 axis. In human, PGC migration is poorly understood; owing to the inaccessibility of embryos at the early developmental window in which it occurs and the barriers to emulating the complex embryonic environment in organoid culture. This study utilizes the accessibility and tractability of the chick embryo to create a system for PGC migration. Avian and mouse PGC migration have parallels; PGCs cross epithelia and transit through the mesentery to the gonad at similar developmental timepoints, both relying on chemokine SDF1 for guidance. Earlier steps of migration differ between birds and rodents, as chick PGCs do not pass through the hindgut but rather through the vasculature, which is accessible for cell delivery. We developed a xenograft model for mammalian PGCs and methods for 3D quantitative imaging of PGC development in the chick embryo. Using the Oct4GFP reporter and species-specific antibodies, we show that migratory mouse PGCs injected into vasculature of equivalently-staged chick embryo can engraft in its gonads. Preliminary studies suggest that PGC-like cells differentiated in culture from human embryonic stem cells are also capable of migration in this system. Current efforts are focused on optimizing the frequency of donor PGC engraftment, depleting endogenous niches, and testing the reliance of migration upon conserved cxcr4-sdf-1 signaling. This xenotransplant system will serve as a platform for elucidating the mechanisms of human and mouse PGC migration while understanding the basis of PGC heterogeneity. Funding for this project is through Dr. Laird’s grants from the NIH 2019 R01 GM.

Program Abstract #592
Adhesive forces promote effective organization, movement, and leader/trailer cell state during collective cell migration.
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Embryonic morphogenesis involves a wide array of cell migratory events. To understand this complex process we have combined confocal microscopy, genetic perturbations, and computational modeling to generate models of collective cell movement based on the observed in vivo behavior of migrating cardiopharyngeal cells (Trunk Ventral Cells, TVCs) of the basal chordate Ciona robusta. These cells migrate as polarized bilateral pairs, which consist of a committed leader and trailer, from the embryonic tail to the ventral trunk and constitute the simplest model of collective cell migration. Our model faithfully reproduces the evolution of migratory cell shape based on modulation of adhesive/protrusive forces acting within the cells. We validate the predictions generated by the model in vivo by decreasing cell adhesion through expression of dominant negative Intβ1 or increasing the protrusive activity of one cell using constitutively active RhoD/F and find that these perturbations can promote leader or trailer cell states in accordance with the model. Further, we find that Ddr and Intβ1, two collagen-binding receptors we have shown to promote TVC polarity, have distinct roles in promoting adhesion by regulating distribution of focal adhesions and myosin in the migrating cell pair. Our model also predicts that migration of cell pairs is more efficient than migration of single cells and polarization along the direction of
migration is preferable to side-by-side migration due to interaction of migrating cells with surrounding tissues. In vivo observations support this finding by showing that migration of single TVCs is less efficient than that of cell pairs. We thus gain insights into the basic rules governing collective cell migration in the context of live embryos by integrating biological observations with modeling. This work is funded by NIH/NIGMS R01 GM09603 award to LC and NIH F32 GM108369–01A1 to YB.

Program Abstract #593
Protein Kinase C δ regulates actomyosin organization in cellular protrusions during collective cell migration.
Amad Bhatti, Felix Gunawan, Saad Husainie, Adam Kramer, Jing Lu, Dorothea Godt
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Actin-based membrane protrusions are essential for cell migration, including the collective migration of the border cell cluster (BCC) during Drosophila oogenesis. Here, we show that the serine/threonine kinase Protein Kinase C δ (PKCδ) regulates the structure and behavior of the leading protrusion of the BCC, where it appears to be enriched. Examination of CRISPR-generated loss-of-function PKCδ mutants revealed that PKCδ is required for normal F-actin organization and Myosin II (MyoII) distribution, thereby regulating the shape and extension/retraction dynamics of the leading protrusion. Moreover, PKCδ prevents simultaneous formation of multiple leading protrusions. Overexpression of PKCδ caused a severe overall reduction in actomyosin in the BCC and delayed BCC migration, possibly due to disruption of MyoII-mediated detachment of the BCC from the epithelium. Rho1, a positive upstream regulator of MyoII appears to be activated in the leading protrusion precociously when PKCδ function is lost, suggesting that the timing of Rho1 activation is influenced by PKCδ activity. In summary, our analysis identifies PKCδ as an important regulator of the actomyosin network in leading protrusions during collective cell migration. Funding sources: University of Toronto Fellowship, Research Assistantship, Teaching Assistantship.

Program Abstract #594
Protein phosphatase 1 complex controls a balance between collective and single cell modes of migration
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Collective cell migration is critically important in many developmental and pathological processes. While extensive research has been conducted in a variety of models, the mechanisms underlying collective cell migration are still poorly understood. Drosophila border cells travel as a cohesive cluster during oogenesis and provide an excellent genetic model for identifying how cell collectives move inside tissues. While roles for several serine-threonine kinases and their target substrates have been established, much less is known about serine-threonine phosphatases. Here we show that Protein Phosphatase 1 (Pp1) maintains the collective cohesion and migration of border cells. Inhibition of Pp1 activity, either through the endogenous Pp1 protein inhibitor NiPp1, or by knockdown of Pp1 catalytic subunits, causes border cells to round up and completely dissociate from the cluster during migration. These defects are fully rescued by overexpressing Pp1 catalytic subunits. The Pp1-inhibited border cells switch to an overall slower individual cell motility mode, with altered protrusion number, size and lifetime. We also found that E- Cadherin and β-Catenin between cells are strongly reduced upon Pp1-inhibition. Activated non-muscle myosin II (myo-II) and F-actin are now enriched around each individual border cell rather than at the outer edges of the entire border cell cluster. Further, the Pp1-containing myosin phosphatase complex promotes proper border cell shape and collective cohesion. Overexpression of another Pp1 regulatory subunit, PPP1R15, mimics the phenotypes caused by inhibition of Pp1 activity. Thus, our work identifies Pp1 activity, through distinctive phosphatase complexes such as myosin phosphatase and PPP1R15, as a critical molecular regulator of collective cell versus single cell behaviors. Given the high degree of conservation of Pp1 complexes, this may be a common mechanism for developmental collective cell migration. Funding: NSF 1456053 and NSF 1738757.
Program Abstract #595
Evaluating the Effect of Extracellular Gaps on Border Cell Migration in Drosophila
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Elucidating the underlying mechanisms governing collective cell migration is imperative, given their role in development, wound healing, and diseases like cancer. Many cell migration studies, however, are conducted in vitro, which neglects to consider the chemical and biophysical complexities of the in vivo tissue environment. Collectively migrating cells traverse through the diverse physical architecture of a tissue, often along a concentration gradient of diffusible chemical attractants (chemoattractants). Using the border cells, which navigate through the three-dimensional cellular terrain of the Drosophila egg chamber during oogenesis, we can study the impact of physical architecture on collective cell migration in vivo. We observe extracellular gaps within the egg chamber and hypothesize that they directly affect the migration behaviors of the border cells, potentially by altering the local distributions of secreted chemoattractants. In silico, our lab has demonstrated these gaps affect the distribution of the morphogen that specifies border cell fate and preliminary results in vivo has shown that diffusible chemical signals concentrate between cells at potential gaps. Therefore, the underlying tissue architecture might affect chemical distribution. Additionally, genetically inducing a uniform concentration of the potent chemoattractant, Platelet Derived Growth Factor/Vascular Endothelial Growth Factor (PVF1), in the egg chamber stalled border cells at what may be an extracellular gap. Furthermore, we plan to express chemical signals tagged with the photoswitchable Dendra2 protein, thus allowing us to map the chemical gradients as they form in real-time. This study identifies a role for surrounding tissue architecture in affecting collective cell migration and aims to explore how this can alter the distribution of chemical cues. Funding provided by UMBC and NSF grant: NSF – IOS-1656550.

Program Abstract #596
Functional analysis of Actin-interacting protein 1 (AIP1) in Drosophila epithelia.
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As a negative actin regulator, Actin-interacting protein 1 (AIP1) enhances the cofilin-mediated actin filament (F-actin) depolymerization. Epithelial tissues are layers of polarized epithelial cells that are linked to each other through cell-cell junctions. During development, these junctions are continuously being broken down and remodeled, which requires assistance of the dynamic actin networks. Hence, having a balanced and tightly regulated actin turnover is important for maintaining epithelial integrity. The aim of this project is to uncover the mechanism by which AIP1 maintains the organization of the apical cell junction—Adherens junction (AJ), in coordination with facilitating the cofilin-mediated actin disassembly in Drosophila. My data show that AIP1 is enriched in the apical region and in the vicinity of AJs in Drosophila embryos and ovarian follicle cells. This suggests AIP1 may play a role in the apical domain of epithelial cells. Knocking down (KD) AIP1 in the follicular epithelium causes formation of abnormal F-actin aggregates. In addition, AIP1 KD disrupts the distribution of apically localized protein: In WT cells, AJ components are localized at the apical-lateral cell boundaries. However, their signal intensities are noticeably increased along the apical and lateral membrane of AIP1-KD cells, and occasionally formed small puncta in the apical region. Moreover, the normal apical distribution of Crumbs and Par-3 protein is disrupted in AIP1-KD cells, of which formed apical aggregates in AIP1-KD cells. In contrast, basolateral membrane proteins, such as Discs large, Fasciclin-3, Coracle were indistinguishable in AIP1-KD and WT cells. Together, my preliminary data suggest AIP1 is essential for maintaining the apical cell junctions, such as AJs, whereas it is less important at the basolateral membrane.

Program Abstract #597
Anchoring of cortical actin pools by the late endocytic pathway during subcellular tube guidance
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Anchoring of cortical actin pools by the late endocytic pathway during subcellular tube guidance
The actin cytoskeleton participates in a wide range of cellular processes, from endocytosis to cell migration. Nucleation and anchoring at different subcellular locations also allow cells to undergo shape changes required for morphogenesis. Terminal cells of the Drosophila respiratory system form a subcellular tube by invaginating the apical plasma membrane, and at least three distinct actin pools regulate this process: One in the basal membrane domain (1), in filopodia (2), and in the apical membrane domain (3). Disrupting any of these pools affects subcellular tube formation in similar ways, however, what mediates the crosstalk among them is not known. We report here that actin also assembles around vesicles of the late endocytic pathway, which are mostly present towards the tip of the growing subcellular tube. Actin bundles seem to bridge the apical and basal cortical actin pools, with late endosomes acting as intermediate anchoring points. We show that disturbing late endosome formation or actin nucleation at late endosomes affects the directionality of tube growth, uncoupling it from the direction of cell elongation and migration. These findings highlight a role for late endosomes in contributing to proper cell morphogenesis, besides their conventional role in membrane and protein trafficking. This work was supported by funding from EMBL and EMBO; LDRB was funded by the EMBL Interdisciplinary Postdoctoral Programme under Marie Curie Actions.

Program Abstract #598
Hemodynamic force is required for vascular smooth muscle cell recruitment to blood vessels during mouse embryonic development
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Blood vessel maturation, the investment of vascular smooth muscle cells (vSMCs) around developing blood vessels, begins when vessels remodel into a hierarchy of proximal arteries and veins that branch into smaller distal capillaries. The ultimate result of maturation is formation of the tunica media—the middlemost layer of a vessel composed of vSMCs that controls vessel integrity and vascular tone. Though many studies have implicated the role of various signaling molecules in regulating maturation, no studies have determined a role for hemodynamic force in the regulation of maturation in the mouse. In the current study, we provide evidence that hemodynamic force-dependent maturation occurs in the mouse because reduced blood flow mouse embryos exhibited a diminished or absent coverage of vSMCs around vessels, and in normal-flow embryos, extent of coverage correlated to the amount of blood flow that vessels were exposed to. We also determine that the cellular mechanism of force-induced maturation was not by promoting vSMC differentiation/proliferation, but instead involved the recruitment of vSMCs away from neighboring low-flow distal capillaries towards high-flow vessels. Finally, we hypothesize that hemodynamic force may regulate expression of specific signaling molecules to control vSMC recruitment to high-flow vessels, as reduction of flow results in the misexpression of Semaphorin 3A, 3F, 3G, and the Notch target gene Hey1, all of which are implicated in controlling maturation. This study reveals another role for hemodynamic force in regulating blood vessel development of the mouse, and opens up a new model to begin elucidating mechanotransduction pathways regulating vascular maturation. Funding sources: Missouri State University Faculty Research Grant.

Program Abstract #599
Post-transcriptional regulation of a developmental EMT program is mediated by P-bodies
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Neural crest cells undergo a tightly regulated epithelial—mesenchymal transition (EMT) to delaminate from the neural tube. Recently, we have shown that this developmental EMT program is controlled by temporally restricted expression of the Wnt antagonist, Draxin. A hallmark of Draxin function during EMT is its rapid downregulation; however, precisely how Draxin expression is regulated has been unclear. Here, using an in vivo reporter construct, we show that the rapid degradation of Draxin mRNA is mediated post-transcriptionally via its 3’-untranslated region (3’-UTR). Using an MS2-MCP reporter system and time lapse imaging of neural crest explants, we demonstrate that Draxin mRNA localizes to small cytoplasmic granules resembling processing bodies (P-bodies), which are established sites of RNA processing and decay. To further validate the identity of these granules as P-
bodies, we performed *in situ* hybridization and found that mRNAs of known P-body components are expressed during neural crest EMT. By functional and perturbation analysis of P-bodies, we describe their role during neural crest development in mediating the degradation of Draxin during neural crest EMT. Together, our data highlight a novel and important role for P-bodies in an intact organismal system — playing an essential role in neural crest EMT via post-transcriptional target degradation. *This work was funded by the US National Institutes of Health K99 DE028592 (EJH) and R01 DE024157 (MEB).*

**Program Abstract #600**

**Shared and distinct mechanisms for cannabinoid receptor, CB1R, and Myosin II in regulation of growth cone filopodia and optic axonal projections in the optic tract.**  
**Sophia Dao, Kenton Jones, John Lim, Kenneth Hanton, George Chen, Tamira Elul**  
**Touro University California, United States**

Previous studies show that the main cannabinoid receptor in the brain - CB1R- influences axonal pathfinding in developing neurons by signaling through an actin cytoskeletal regulatory pathway. However, questions remain about how CB1R coordinates with different actin regulatory factors to regulate specific features of axonal projections, especially in neurons developing in the native environment. Here, we studied how CB1R and non-muscle Myosin II modulate growth cone filopodia and optic axon projections in the optic tract of whole brains from *Xenopus laevis* tadpoles. *Xenopus* tadpoles containing small numbers of GFP optic axons were bathed in pharmacological inhibitors for either CB1R (AM251) or Myosin II (Blebbistatin) during developmental stages when optic axons navigate through the optic tract. The GFP expressing optic control and experimental axons were then imaged in the optic tract of whole brains dissected from these tadpoles. Analysis of these images showed that optic axons exposed to AM251 and Blebbistatin both formed growth cones with significantly more filopodial protrusions than control growth cones. With the AM251 inhibitor, the optic axons also made significant pathfinding errors in the optic tract, turning away from their target. In contrast, application of Blebbistatin resulted in optic axons that extended a shorter distance through the optic tract. This data suggest that CB1R and Myosin II exert shared and distinct functions in modulating growth cone filopodia and axonal pathfinding and extension in optic axons in the optic tract of *Xenopus* brains. This research was supported by the Department of Basic Sciences, College of Osteopathic Medicine, Touro University California.

**Program Abstract #601**

**Talin1 regulates endothelial cell rearrangements during vascular morphogenesis in zebrafish**  
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Endothelial cells (ECs) are anchored in a basement membrane that consists of extracellular matrix (ECM) molecules like proteoglycans and proteins such as Collagen and Fibronectin. At the cell-matrix interface, adhesive structures called Focal Adhesions (FAs) connect cells to ECM molecules. Transmembrane Integrin heterodimers formed by α- and β-subunits are the major adhesion receptors that reside at the cell-matrix interface and connect cells to the ECM. Talin1 is an endothelial adaptor molecule that can bind the cytoplasmic tail of Integrin receptors and thereby link those to the Actin cytoskeleton allowing for downstream signalling events. Ubiquitous and endothelial-specific knockout models for β1-Integrin and Talin1 have clearly shown that Focal adhesions formation and downstream Integrin signalling are essential for vascular network formation and vessel integrity. However, the precise mechanism and cellular events that are regulated by Talin1 are not fully understood. Here, we examine the roles of Talin1 in vascular development by detailed live analysis of EC morphogenesis of a novel talin1-/- mutant allele. Using high-resolution imaging of F-actin and VE-cadherin, we have identified that Talin1 is required for EC elongation and cell-cell junction linearization cell-autonomously. Interestingly, application of an Actin polymerisation agonist rescues talin1-/- EC elongation, suggesting that the compromised cell elongation process in zebrafish mutant is a consequence reduced Actin polymerisation. We are currently investigating how Talin1 promotes Actin polymerisation in order to control cell-cell junction rearrangements. This project is supported by NHMRC Project Grant (1124011).
Program Abstract #602
PAR polarity proteins direct intracellular tube expansion through apical recruitment of the exocyst complex
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Organs are comprised of various tubes with distinct cellular compositions. The smallest tubes are unicellular and can be seamless, with a lumen extending through the center of the cytoplasm. To expand their lumen, seamless tubes are thought to direct vesicles to the luminal surface. But how vesicles are targeted to the luminal surface is poorly understood. We show that seamless tube expansion in the C. elegans excretory canal relies on precise interplay between PAR polarity proteins (PAR-3, PAR-6, PKC-3, CDC-42) and the exocyst vesicle-tethering complex at the luminal surface. To study this, we devised a strategy to conditionally deplete proteins in the excretory canal using the ZF1 degron method (Armenti 2014). We tagged PARs and exocyst with ZF1 and conditionally degraded members of each complex to determine their role during lumen growth. Canal-specific depletion of PAR-6 caused severe tube outgrowth defects, whereas PAR-3 depletion caused only mild defects that later resolve. Depletion of SEC-5, a core exocyst protein, resulted in severe defects similar to PAR-6, suggesting that PAR-6 may position the exocyst. To test if PAR-6 recruits exocyst, we used heat shock to degrade PAR-6 after canal development completed, and examined exocyst localization. Indeed, PAR-6 depletion resulted in loss of apical exocyst. Interestingly, PAR-6 itself was reduced at the apical membrane in the absence of PAR-3, suggesting that PAR-3 contributes to PAR-6 positioning. In addition, depletion of the small GTPase CDC-42 caused defects similar to PAR-6, and loss of EXC-5 (a CDC-42 GEF) caused mislocalization of the PAR-6-binding protein PKC-3. These observations suggest that active CDC-42 recruits PAR-6/PKC-3 apically and PAR-3 acts independently to enrich PAR-6/PKC-3 along the membrane. The exocyst is recruited to these sites where vesicle tethering and tube expansion proceeds. These findings provide a molecular pathway for intracellular lumen extension. NIH-F32HL136038, R35GM118081

Program Abstract #603
LET-99 localization by the PAR proteins during asymmetric division of the C. elegans embryo
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Asymmetric cell division is the process in which one cell divides to give rise to cells with different fate determinants. This process is conserved throughout animals and is important for generating different cell types. To divide asymmetrically, a cell must first polarize and then the mitotic spindle must align with this polarity axis. In many organisms, the polarity axis is established by the PAR proteins. In the one-cell C. elegans embryo, PAR proteins are localized into distinct, mutually exclusive, anterior and posterior domains on the cell cortex. Scaffolding proteins PAR-3 and PAR-6, a small GTPase CDC-42, and an atypical protein kinase C PKC-3 make up the anterior PARs. The kinase PAR-1 is localized at the posterior. A key intermediate between cortical polarity and spindle positioning is LET-99, a DEPDC1 family protein, which is localized into a posterior lateral band at the cortex with highest levels between the PAR domains. Previous work suggests that PAR-1 inhibits LET-99 accumulation at the posterior, potentially via phosphorylation. However, the mechanism by which LET-99 is inhibited from the anterior is still unknown. To investigate which anterior PARs are needed to properly localize LET-99, we performed RNAi knockdowns of PAR-3, PKC-3, and CDC-42 in a YFP::LET-99 strain and measured cortical intensity at nuclear envelope breakdown. Knockdown of all of these proteins resulted in uniform YFP::LET-99 around the cortex. Because in cdc-42 embryos PAR-3 and PKC-3 are still at the anterior cortex initially, but PKC-3 is inactive, these results suggest that PKC-3 is critical for inhibiting LET-99 at the anterior. We plan to further test this idea using different pkc-3 mutant strains, to determine whether or not PKC-3’s kinase activity is needed to localize LET-99. This research is funded by NIH Grant 1R01GM68744.

Program Abstract #604
Apico-basolateral polarity establishment in the C. elegans intestinal epithelium
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Apico-basolateral polarization is essential for epithelial cells to function as selective barriers and transporters and to provide mechanical resiliency to organs. Different epithelia use divergent mechanisms to establish polarity,
which are only beginning to be understood. Here, we use the C. elegans embryonic intestine to investigate how epithelial cells establish apico-basolateral polarity in vivo. PAR-3 is the most upstream known polarity protein in this tissue, and is required to establish apical polarity at the intestinal midline (Achilleos et al 2010). We find that intestine specific depletion of PAR-3 disrupts both apical and basolateral protein localization, resulting in edematous intestines and early larval lethality. In other organisms, the actin binding protein, afadin (AFD-1), and the basolateral kinase, PAR-1, are essential for PAR-3’s localization during epithelial polarity establishment. We tagged AFD-1 and PAR-1 with GFP and a tissue specific degron tag to determine if these proteins are required for C. elegans intestinal polarization. We find that AFD-1 and PAR-1 localize to subapical junctions in the polarizing intestine, only partially co-localizing with PAR-3. Intriguingly, these proteins localize basal to the presumptive apical surface prior to the exclusion of basolateral proteins (LET-413, LGL-1) from this surface. This suggests that polarity establishment occurs in a stepwise manner with the apical surface and junctions established before the basolateral domain. In contrast to other organisms, we find that intestine specific depletion of AFD-1 or PAR-1 does not affect the initial localization of PAR-3 to the apical surface. AFD-1 depletion does result in subtle intestinal defects and slowed growth in later embryonic and larval stages, suggesting a possible role for AFD-1 in junction maturation or integrity, which we are currently investigating. This work was funded by Stanford University and the NIH: DP2 GM119136-01(JF), F32 GM129900-01(MP).

Program Abstract #605
Interplay of ubiquitination and palmitoylation in regulation of Fat-Hippo signaling
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Precise coordination of growth and morphogenesis is critical to formation of optimally functioning organs. The evolutionarily conserved protocadherins, Dachsous (Ds) and Fat constitute a signaling pathway that coordinates growth and morphogenesis by regulating the Hippo pathway and planar cell polarity (PCP) respectively. The atypical myosin, Dachs is a key downstream effector of Fat signaling that mediates both of these effects, and apical localization is critical for Dachs function. Fat regulates growth and PCP by modulating the levels and polarity of Dachs at the apical membrane. However, how Dachs localization is regulated is not well understood. A palmitoyl transferase, Approximated (App) is required for proper Dachs localization. But the exact mechanism by which it regulates Dachs is unknown. Recently, we identified a novel gene, Vamana (Vam), which encodes an SH3 domain containing adapter protein that plays a critical role in apical membrane localization of Dachs. Further, Vam functions as an adapter by physically connecting Dachs to Ds and Fat. To identify additional regulators of this signaling pathway, I conducted a genetic screen using RNAi targeted against the ubiquitin ligases encoded by the Drosophila genome and isolated a novel RING domain E3 ligase, Elgi, which when depleted results in tissue overgrowth and PCP defects, phenotypes reminiscent of mutations in Fat signaling. At the cellular level, depletion of Elgi results in significant increase in Dachs and Vam levels. Interestingly, expression of a dominant negative Elgi results in failure of Dachs and Vam to localize to the apical cortex and their accumulation in the cytoplasm in punctate structures. Similar accumulations are observed when Elgi and App are depleted simultaneously. Taken together, these results indicate that Elgi and app coordinately regulate the trafficking of Dachs and Vam, the downstream effectors to the apical membrane. Supported by NICHD R00HD092553 to JM.

Program Abstract #606
Characterization of a new function of the tRNA modifying complex TCTC during neural differentiation in Drosophila melanogaster
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Introduction: tRNAs are highly modified molecules and can actively impact the cell proliferation/differentiation program. The Threonyl-carbamoyl transferase complex (TCTC) catalyzes a threonylcarbamoyl adenosine (t6A) modification in ANN decoding tRNAs. Although this complex is widely present across different species, it is unknown whether is equally active in all tissues. Previously we observed that TCTC is differentially required in proliferating and differentiated tissues. Thus, the loss of function of any of TCTC core subunits causes cell death in...
proliferative tissues, whereas loss of Tcs5 subunit alters the. In this work we address the differential requirements of TCTC subunits within proliferative and differentiated tissues, especially the role of Tcs5 in the dynamics of the actin cytoskeleton in neurons. **Methods:** Knockdown and proteins over-expression were attained using the Gal4/UAS system in *D. melanogaster*. Immunofluorescences were analyzed by confocal microscopy. Immunoprecipitation were performed using tagged versions of TCTC subunits. **Results:** The subunits of TCTC are expressed in a similar way in proliferative and differentiated tissues. Knockdown of Tsc5 alters axon elongation in photoreceptors and neuronal arborization in neuromuscular junction in an independent process of other TCTC subunits. Both processes are associated with actin cytoskeleton regulation. On the other hand, the C-terminal end of Tcs5 is determinant for the assembly of the complex, which includes the residue T221 which is phosphorylated by Akt. **Discussion:** We propose TCTC operate in a tissue specific manner, where Tsc5 can regulates complex assembly via Akt phosphorylation, while in differentiated cells regulates actin cytoskeleton independently of TCTC. We are investigating the molecular cues for assembly/disassembly of the TCTC. **Founding:** FONDECYT 1190119, FONDAP15090007 and María Ghilardi Venegas Grant.

**Program Abstract #607**

**Shaping the Extracellular Matrix Through Kinesin-3 and Kinesin-1 Driven Polarized Secretion**

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Basement membranes (BMs) are sheet-like extracellular matrices (ECMs) found in nearly every organ in the body. In addition to providing attachment sites for cells, these structures act as a reservoir for growth factors, provide polarity information to cells, and provide mechanical support to guide tissue morphogenesis. In epithelial cells, polarized secretion of BM proteins ensures that the BM matrix assembles exclusively along the basal surface. Working in the follicular epithelium of the *Drosophila* egg chamber, our lab previously identified the GTPase Rab10 as a key regulator of sorting BM proteins into the basolateral secretory pathway. Since BM proteins are designed to form networks upon exposure to the extracellular environment, we hypothesized that additional control over the site of their secretion within the large basolateral plasma membrane may be important. Using a combination of genetics and *in vivo* live imaging, we found that Rab10-based BM secretion is concentrated on the basal-most ~1μm of the lateral plasma membrane and the basal surface. This bias requires the activity of two kinesins, kinesin-1 and the kinesin-3 Khc73, which transport Rab10+ vesicles along the polarized microtubule (MT) network towards the basal surface prior to secretion. Comparison of BM secretion defects and Rab10+ vesicle motility in different motor mutant backgrounds shows that both kinesins are needed to fine-tune the site of BM secretion. When this kinesin-based transport is lost, a network of BM proteins forms in between cells, interfering with normal cell organization and movement. These findings highlight the importance of controlling the secretion site for BM proteins, and suggest a better understanding of the role of MT motor-driven transport in epithelial cells will enhance our understanding of how polarity is established and maintained during development. NIH R01GM136961(SHB), AHA RSG-14-176(SHB), AHA 16POST27260189(ALZ), ACS PF-18-025-01-CSM(ALZ)

**Program Abstract #608**

**Dynamic cell cycle-dependent regulation of the pancreas transcription factor Pdx1**

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Cell cycle progression is tightly regulated, with multiple check points ensuring faithful transmission of genetic material. During the cell cycle, many proteins exhibit dynamic expression and localization. Previous studies of pancreatic β-cell proliferation focused mainly on the transition from quiescence to proliferation, leaving specific cell cycle stages unexplored. Proliferating β cells express lower levels of functional cell identity genes, suggesting that proliferation and optimal function are incompatible. The Pdx1 transcription factor is important for β-cell specification, function, and proliferation. However, its regulation during the cell cycle is unknown. Here we examined Pdx1 protein expression in immortalized β cells, mouse islets during pregnancy, and embryonic pancreas. We demonstrate that Pdx1 localization and protein levels are highly dynamic. During interphase and in quiescent cells, Pdx1 was not observed in constitutive heterochromatin, nucleoli, and most areas containing repressive epigenetic marks. At prophase, Pdx1 forms fiber-like structures encapsulating the chromosomes prior
to Ki67 coating the chromosome surface. Pdx1 fibers disassembled at prometaphase and reformed at the end of cell division, prior to nuclear envelope re-formation. β cells in G2 showed a lower level of Pdx1 protein expression than cells at earlier cell cycle stages, suggesting that decreased expression of Pdx1 may be important for cell cycle progression. Indeed over-expression of Pdx1 in INS-1 β cells prevented progression through G2, suggesting that optimal Pdx1 levels are required for completion of mitosis. Together, we find that Pdx1 localization and level are tightly regulated throughout the cell cycle. This dynamic regulation has implications for the dichotomous role of Pdx1 in β-cell function and proliferation. Funding: R01s (DK105689) to M.G. and D.A.S. and (DK108921) to S.A.S., VA Merit awards (I01 BX003744-01) to M.G. and (I01 BX004444) to S.A.S.

Program Abstract #609
Identifying mechanisms of thymus and parathyroid cell fate specification
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The thymus is an essential organ for T cell development, while the parathyroid regulates extracellular calcium levels. Despite their differences in location and function in the adult, each organ arises from a common primordium: the third pharyngeal pouch (3rd PP). A longstanding question is how 3rd PP cells are specified into thymus or parathyroid cell fates. Two transcription factors, Foxn1 and Gcm2, mark early thymus and parathyroid cell fates and are necessary for organ survival and function, but are not the specifiers. Early studies show that SHH promotes the parathyroid cell fate while BMP4 promotes the thymus cell fate, but also establish that additional unknown signals from the surrounding mesenchyme regulate 3rd PP cell fates. The goal of this study is to identify the cell-autonomous specifiers of thymus and parathyroid cell fates and the extracellular signals that regulate them. Our hypothesis is that the proximity of 3rd PP domains to multiple, localized extracellular signals regulates the specifiers of thymus and parathyroid cell fates, which then activate a network of genes including Gcm2 and Foxn1. I am taking a single cell RNA-seq/ATAC-seq approach to identify such specifiers and signals. I optimized the dissections and cell dissociation of the 3rd PP in E11.25 mouse embryos, when there are Gcm2 positive cells, Foxn1 positive cells, and marker negative cells. In an initial scRNA-seq experiment, I identified the heterogeneity of the 3rd PP endoderm and surrounding mesenchyme and the transcriptomes of each cell population. By identifying differentially expressed transcription factors between thymus and parathyroid clusters and differentially expressed signals between mesenchyme clusters, I generated a preliminary candidate gene list of cell-autonomous specifiers and extracellular signals that drive them. Using scRNA-seq/ATAC-seq at earlier stages, I will refine my candidate gene list and identify molecular mechanisms of 3rd PP cell fate specification.

Program Abstract #610
A two-step mechanism controlling stochastic cell fate specification in the fly eye
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Stochastic fate specification diversifies cells during development. How cells randomly choose between two or more fates remains poorly understood. In the fly eye, the random mosaic of two R7 photoreceptor subtypes is determined by stochastic ON/OFF expression of the transcription factor Spineless (Ss). Here, we interrogated how transcription and chromatin influence stochastic fate specification. We evaluated reporters and CRISPR-generated mutants to identify early and late enhancers that drive ss expression in the fly eye. Using a three-color DNA FISH assay, we found that chromatin state is dynamic at the ss locus and dependent on transcription driven by the early enhancer. A repression reporter strategy showed that the ss locus limits expression to a subset of terminal R7s. Together, our results suggest a two-step mechanism governing stochastic R7 subtype specification. In the first step, the early enhancer drives ss expression in R7 precursors to open the ss locus. In the second step, transcription ceases, chromatin variably compacts, and repression limits activation by a late enhancer to a random subset of R7s. ss remains expressed in a subset of R7s (SsON R7s) and repressed in the complementary subset (SsOFF R7s) to determine the random pattern. These data support a “prime and boost” mechanism adapted for stochastic R7 subtype patterning. Our work highlights the interplay of chromatin compaction and gene expression
state that stochastically specifies cell fates, resulting in random patterning across a tissue. This work is funded by the NIH R01EY025598.

Program Abstract #611
MiR-690 enhances osteogenic differentiation in mouse and human embryonic stem cells
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Osteogenesis is a complex and critical process for the proper development of vertebrates. The complexity of osteogenesis resides in the multitude of pathways that embryonic stem cells (ESCs) take to differentiate into osteoblasts and the intricate genetic regulatory network, which controls this lineage development. Adding to the complexity, osteogenic differentiation is induced from either mesoderm or neural crest cells; both of which can differentiate into mesenchymal cells and subsequently into osteoblasts. This overlap of differentiation into mesenchymal cells creates a convenient point at which to study osteogenesis, but reduces the focus on the intermediate stages of differentiation. Additionally, the role of microRNAs (miRNAs) in these stages appears to be crucial for the fate of specific progenitors. In this study we show that overexpression of a specific miRNA, miR-690, upregulates osteogenesis through the direct targeting of β-catenin. Additionally, the increased osteogenesis achieved from miR-690 overexpression during differentiation is time dependent, best achieved with a transfection during days 5-7 of differentiation. Furthermore, stable and transient transfection of miR-690 result in morphological differences as well as a shift in the calcification timeline possibly stemming from a shift in the overall cell population origin. Both the transient and stable transfection resulted in a change of the overall cell population origin producing more neural crest head mesenchyme/prechordal mesenchyme as well as lateral plate mesoderm, limb bud, and somatopleure. Additionally, these findings were mimicked in human ESCs yielding increased calcification and an alteration of cell fate origin. Together, our data indicate that miR-690 supports osteogenesis and plays a role in early cell fate decisions. Funding sources: Translational Centre for Regenerative Medicine Researcher Starter Grant to D. Kaniowska, and NIDCR R01 R01DE025330 to N.I. zur Nieden.

Program Abstract #612
Integrin α5 and Integrin α4 act redundantly to promote endocardial differentiation and heart morphogenesis
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Endocardium is critically important for proper function of the cardiovascular system. Not only does endocardium connect the heart to blood vasculature, it also plays an important role in heart morphogenesis, valve formation, and ventricular trabeculation. The extracellular protein Fibronectin (Fn1) promotes endocardial differentiation, but the signaling pathways downstream of Fn1 that regulate endocardial development are not understood. Here, we analyzed the role of the Fibronectin receptors Integrin alpha5 (Itga5) and Integrin alpha4 (Itga4) in zebrafish heart development. We show that itga5 mRNA is expressed in both endocardium and myocardium during early stages of heart development. Through analysis of both itga5 single mutants and itga4;itga5 double mutants, we show that loss of both itga5 and itga4 results in enhanced defects in endocardial differentiation and morphogenesis compared to loss of itga5 alone. Loss of both itga5 and itga4 results in cardia bifida and severe myocardial morphology defects. Finally, we find that loss of itga5 and itga4 results in abnormal anterior endodermal sheet morphology. Together, our results support a model in which Itga5 and Itga4 act redundantly to promote endocardial differentiation, medial migration of endocardial and myocardial cells, and morphogenesis of anterior endoderm. This work was funded by the American Heart Association.

Program Abstract #613
Mitochondria signaling maintains lymphatic endothelial cell fate
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Lymphatic endothelial cell (LEC) identity depends on the master transcription factor Prox1. We previously demonstrated that Vegfr3 is a dosage-dependent target of Prox1, and that in turn Vegfr3 signaling maintains
Prox1 expression in LECs. Prox1 and Vegfr3 are independently required for LEC fate maintenance and we proposed that a Prox1-Vegfr3 feedback loop is the primary mechanism that maintains LEC fate and determines the number of LECs budding from the cardinal vein. However, how is the temporal generation and budding of LECs progenitors regulated is not yet known. A likely scenario is that as LEC progenitors exit the cardinal vein, their metabolic status changes according to different local tissue environments and functional requirements. To evaluate this possibility, we conditionally deleted in mice the mitochondria respiratory chain complex III subunit QPC from budding LECs. We found that by mid-gestation, mutant mice are devoid of LECs due to downregulation of multiple LEC markers, particularly Vegfr3. Mechanistically, LEC gene downregulation is associated with reduced H3K4me3 and H3K27ac histone modification at key LEC regulators such as Vegfr3 and Prox1. Accordingly, we argue that by sensing the cellular metabolic status, mitochondria complex III regulates the Prox1-Vegfr3 feedback loop required to maintain LEC fate and to control the number of budding LEC progenitors. NIH#RO1HL073402-16.

Program Abstract #614
A changing signaling environment induces multiciliated cell trans-differentiation during developmental remodeling
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Multiciliated cells (MCCs) are extremely highly-differentiated, presenting >100 cilia and basal bodies. Hence, it is widely considered impossible for MCCs to undergo fate change during normal development and in regeneration. We analyzed how MCCs are lost from the airway-like Xenopus embryonic epidermis during developmental tissue remodeling and found that MCCs are first locally removed by lateral line-dependent apoptosis, but later globally removed through trans-differentiation into a mucus-secretory cell type. This resembled observations during tissue remodeling associated with chronic lung disease (e.g. COPD). We demonstrate that MCC trans-differentiation occurs in a non-pathogenic state, involves loss of ciliary gene expression, coordinated cilia retraction, cytoskeletal rearrangement, and lysosomal elimination of ciliary and basal body material. Apoptosis and trans-differentiation are both induced by a changing Notch signaling environment and modulated by Jak/STAT, Thyroid hormone and mTOR signals. Furthermore, trans-differentiation can be inhibited by Rapamycin, indicating a positive role for autophagy in MCC ciliation, similar to primary cilia. This demonstrates that even cells with extreme differentiation features can undergo direct fate conversion during normal development. Our data further suggest that the reactivation of this developmental mechanism in adults can drive tissue remodeling in human chronic airway disease (one of the most common causes of death worldwide), a paradigm resembling cancer formation and progression. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) under the Emmy Noether Programme (grant WA3365/2-1) and under Germany’s Excellence Strategy (CIBSS – EXC-2189 – Project ID 390939984) to PW.

Program Abstract #615
Chibby: a Regulator of Ciliogenesis and Kidney Development
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Genetic disorders that affect the development of cilia (ciliopathies) result in a variety of diseases. One of the most common clinical features of ciliopathies is the development of cysts within the kidney. Cystic diseases of the kidney (CDKs) occur in ~1/800 births, making them the most common life-threatening hereditary disorder. Specific manifestations of each CDK depend on the gene affected. How ciliopathies result in CDKs is largely unknown. However, multiple lines of evidence suggest Wnt signaling likely plays a role in their development. Loss of cilia in multiple models, including polycystic kidney disease (PKD) results in increased Wnt signaling, and artificially increased Wnt signaling in mouse results in the development of cysts within the kidney. However how
the Wnt pathway is regulated by cilia is largely unknown. A protein called Chibby has been shown to bind β-catenin and shuttle it from the nucleus into the cytoplasm. Additionally, Chibby binds to the polycystic disease protein Pkd2. Though loss of Chibby results in malformed cilia, the function of Chibby at the cilia and within the kidney itself is largely unknown. In *Xenopus laevis* we have found that loss of Chibby results in kidneys like those seen in PKD models. Threw single cell sequencing, and imaging of *X. laevis* kidney mutants we are setting up models for cystic kidney diseases. National Institute of Diabetes and Digestive and Kidney Diseases K01DK092320 to Rachel K. Miller National Institute of Diabetes and Digestive and Kidney Diseases R03DK118771 to Rachel K. Miller; National Institute of Diabetes and Digestive and Kidney Diseases R01DK115655 to Rachel K. Miller; UTH ealth McGovern Medical School Department of Pediatrics Startup Funds to Rachel K. Miller; National Kidney Foundation FLB1628 to Rachel K. Miller.

**Program Abstract #616**

**Monoamine-positive structures in cells of non-neural invertebrates (phylum Porifera)**

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Monoamines (serotonin and dopamine) have a wide range of signal activity in living organisms realizing their action via nerve cells. However, monoamines are functionally active even in animals without the nervous system. In particular, sea urchin larvae possess monoamine-positive intracellular structures associated with the Golgi apparatus at the base of each cilium at pre-neural stages. Monoamines in these structures regulate cilia growth and regeneration rate and affect larval locomotion (Obukhova et al., 2014). We analyzed the presence of similar structures in larvae and adults of sponges (phylum Porifera) – the ancient animals which have no nerve cells. Using immunochemical markers, we studied choanocytes (flagellated collar cells) of the three adult demosponge and one calcarean as well as the larval ciliary cells. Choanocytes of the demosponges demonstrate the presence of monoamine-positive granules (MPG) located between the flagellum and nucleus. The ultrastructural study revealed the association of MPG with the Golgi apparatus similar to that in sea urchin blastulae. No MPG was found in demosponge larvae. In choanocytes of calcarean sponge the MPG are located in lateral and distal parts of the cell. According to the TEM studies, the MPG likely corresponds to the large vacuoles with filamentous filling. In calcarean larva, the MPG occurs between the nucleus and flagellum, in a similar manner to those in sea urchin blastulae. According to genome analysis, sponges lack monoamine receptors (Riesgo et al., 2014). We hypothesize that another way of monoamine action, via posttranslational peptide modification – serotonylation and dopaminylation (Bader, 2019), may be basic in sponges. Further functional studies are necessary to confirm this suggestion. The study was supported by RFBR grants ?19-34-90084 and ?18-04-01213.

**Program Abstract #617**

**Investigation of intrinsically disordered neuronal proteins BASP1 and GAP-43 in mouse oocytes and early embryos**

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Two related proteins, BASP1 and GAP-43, are highly expressed in neurons and participate in axon guidance and synaptic plasticity. Recently we have revealed both proteins in mouse oocytes and suggested they are involved in calcium-dependent signaling pathways. While in neurons the proteins have a predominant localization at the cell periphery, particularly at the plasma membrane, in MII-oocytes and early embryo blastomeres they exhibit a dynamic intracellular distribution. BASP1 was found in the cell cortex/plasma membrane region as well as in the cytoplasm and in the nucleus (including zygote pronuclei). At the plasma membrane and in the nucleus BASP1 was colocalized with phosphatidylinositol-3,4,5-triphosphate. GAP-43 exhibited exclusively non-membrane localization. It was found associated with tubulin structures in oocytes (spindle and MTOCs), the condensed chromosomes during meiosis and mitosis and within the nucleus. GAP-43 phosphorylated on Ser41 by PKC was observed in MTOCs in colocalization with γ-tubulin as well as in the nucleus. By the use of different mAbs, several
intracellular pools of GAP-43 (probably with different posttranslational modifications) have been resolved. The absence of membrane association may suggest that GAP-43 lacks the N-terminal membrane targeting domain. It can be speculated that the N-terminally truncated form of GAP-43 (earlier discovered in brain and referred to as GAP-43-2) can be formed by the alternative translation initiation from the Met5 codon due to specific mRNA transcription. Overexpression of fluorescently labeled BASP1 and GAP-43 caused the decompaction of early embryos due to loss of blastomere adhesion. The full-length (neuronal) forms of the proteins expressed from the plasmids were localized to the blastomere plasma membrane. This suggests that formation of the cytoplasmic and nuclear protein forms is caused by the peculiarities of their mRNA transcription and translation. Supported by RFBR grant 18-04-01357.

Program Abstract #618
Inhibition of Jagged-Notch signaling reduces normal luteal angiogenesis but causes hemorrhaging of hyperstimulated ovaries
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Robust angiogenesis in the corpus luteum is critical for maintenance of pregnancy and thus mammalian female fertility. During typical angiogenesis, blood vessels sprout from preexisting vasculature and recruit pericytes to induce maturation, vessel quiescence, and regulation of endothelial proliferation and vascular permeability. In luteal angiogenesis, pericyte invasion into the corpus luteum may precede endothelial entry, and pericytes may act to both recruit and stabilize nascent vasculature. Endothelial induction of Notch signaling in adjacent pericytes help recruit and maintain pericyte coverage in some but not all tissue types. We show that in the corpus luteum, pericytes remain in a relatively undifferentiated state with no evidence of contractile function and a subset of luteal pericytes express the Notch ligand Jag1. We have employed a Notch decoy, N110-24, which specifically blocks Jag1 induction of Notch signaling. We determined that during physiologic ovulation, Jag1 inhibition suppresses luteal angiogenesis, including invasion of both endothelium and pericytes, but does not appear to alter endothelial-pericyte interactions. Conversely, after ovarian hyperstimulation (a condition occurring during fertility treatments), Jag1 inhibition causes vascular dilation, overt hemorrhage into the ovary, and disruption of pericyte organization. These results indicate that Jagged inhibition has different effects in different angiogenic conditions, and may be used to both positively and negatively regulate vessel formation. This work was supported by NHLBI award 1R01 HL112626 (JKK), CDMRP award BC170816 (JKK), and training grants T32DK07328 and 2T32EY013933 (NMK).

Program Abstract #619
Memory of prior temperature perception influences peroxide resistance in the nematode C. elegans
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Animals frequently encounter hydrogen peroxide in their environment. Hydrogen peroxide damages proteins and DNA; thus, animals must tightly regulate their peroxide defenses. Environmental perception regulates transcriptional programs that protect against peroxides, but direct links between individual sensory mechanisms and these protective responses are only beginning to be understood. We investigated how a single pair of thermosensory neurons coordinates peroxide defenses in the nematode Caenorhabditis elegans. We used the C. elegans Lifespan Machine to measure peroxide resistance with high temporal resolution. We found that prior exposure to warm temperature increased peroxide resistance; while prior exposure to cool temperature decreased peroxide resistance. To influence peroxide resistance, prior exposure to warm temperature requires the two AFD neurons, which together function as the primary temperature sensor of the worm. Consistently, loss of temperature-sensing cGMP signaling within the AFD neurons abolishes most of the effects of prior exposure to warm temperature on peroxide resistance. Furthermore, we found that to control peroxide resistance, cGMP signaling within AFD requires the DAF-16/FOXO and SKN-1/NRF transcription factors, which are central determinants of peroxide defense. This indicates that perception of prior temperatures can control subsequent peroxide resistance via protective transcription factors. Finally, the effects of prior temperature perception on
peroxide resistance persists for several days into adulthood dependent upon cGMP signaling within AFD. This suggests that cGMP signaling within AFD enables the maintenance of peroxide defenses in response to temperature changes. We propose that the AFD neurons form a temperature memory via cGMP signaling, and that the animals use this information to adjust peroxide defenses that require DAF-16/FOXO and SKN-1/NRF in target tissues. Funding: Northeastern University Tier 1 Award, Javier Apfeld

**Program Abstract #620**

**Shaping the zebrafish myotome by intertissue friction and active stress**

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Organ formation is an inherently biophysical process, requiring large-scale tissue deformations. Yet, understanding how complex organ shape emerges during development remains a major challenge. In particular, the process of organ shaping requires integrating scales from single cells up to whole tissues. During zebrafish embryogenesis, large muscle segments, called myotomes, acquire a characteristic chevron morphology, which is believed to aid swimming. Myotome shape can be altered by perturbing muscle cell differentiation or the interaction between myotomes and surrounding tissues during morphogenesis. To disentangle the mechanisms contributing to shape formation of the myotome, we combine single-cell resolution live imaging with quantitative image analysis and theoretical modeling. We find that, soon after segmentation from the presomitic mesoderm, the future myotome spreads across the underlying tissues. The mechanical coupling between the future myotome and the surrounding tissues appears to spatially vary, effectively resulting in spatially heterogeneous friction. Using a vertex model combined with experimental validation, we show that the interplay of tissue spreading and friction is sufficient to drive the initial phase of chevron shape formation. However, local anisotropic stresses, generated during muscle cell differentiation, are necessary to reach the acute angle of the chevron in wild-type embryos. Finally, tissue plasticity is required for formation and maintenance of the chevron shape, which is mediated by orientated cellular rearrangements. Our work sheds light on how a spatiotemporal sequence of local cellular events can have a nonlocal and irreversible mechanical impact at the tissue scale, leading to robust organ shaping. Funding from Singapore National Research Foundation Fellowship, HFSP Young Investigator Grant and Singapore Ministry of Education.

**Program Abstract #621**

**Regulation of Gonad Morphogenesis and Gametogenesis by the BTB Protein Ribbon**

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Cell adhesion and cell-cell signaling regulate the establishment and maintenance of organ structure and function. The *Drosophila* gonad has proven an excellent model for identifying genetic mechanisms underlying organogenesis. The gonad is formed by the coalescence of somatic gonadal cells and germ cells during embryogenesis. Subsequent development results in the establishment of the germline stem cell niche and stem cell populations that are maintained throughout the lifetime of the organism. In previous studies, the BTB transcription factor Ribbon (Rib) was identified as a gene required for embryonic gonad formation. Further study has revealed that Rib continues to be expressed during larval gonad development and in the adult ovaries and testes. These results suggest that Rib may regulate significant morphological changes that occur in the larval gonad and gametogenesis in the adult. We found that overexpression of *ribin* somatic cells throughout development causes significant defects in ovary and testis development. In females overexpressing *rib*, niche structures fail to form, resulting in ovaries with a blob-like appearance and a failure to produce eggs. In males overexpressing *rib*, niche structures form, but testes appear truncated and sperm do not progress through meiosis. To specifically examine the role of Rib in gametogenesis, *rib* overexpression was limited to adult tissues. When *rib* is overexpressed in somatic cells of the adult ovary, defects in oogenesis occur and a reduction in the number of follicle cells surrounding the egg chamber is observed. *rib* overexpression in somatic cells in the adult testes results in a failure of cells to transition to meiosis and abnormalities in somatic cell gene expression. Given that oogenesis arrests at a key transition regulated by the Notch signaling pathway, we are currently examining
the effect of fib overexpression on downstream Notch targets. Funded by Loyola University Chicago and a LUC Provost Fellowship.

Program Abstract #622
Molecular logic of the β-catenin-dependent ectodermal patterning in *Nematostella vectensis*

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The emergence of organisms with two body axes and bilateral symmetry was a major transition early in animal evolution, and most animals belong to Bilateria. Sea anemones are bilaterally symmetric non-bilaterians and members of Cnidaria, the bilaterian sister group. Like in Bilateria, their two body axes are patterned by β-catenin and BMP signaling, which raises the possibility of bilaterality being older than the cnidarian-bilaterian split. To assess this possibility, a comparison of the cnidarian and bilaterian axial patterning mechanisms is necessary. While bilaterian axial patterning is comparatively well-studied, cnidarian axial patterning mechanisms, especially the ones responsible for the β-catenin dependent oral-aboral (OA) axis, are not well-understood. Recently we showed that some β-catenin dependent genes in the sea anemone *Nematostella vectensis* respond to permissive “windows” of β-catenin signaling intensities, whereas others are upregulated until their expression levels saturate. Although clearly critical for the OA patterning, the mechanism underlying these differences in the dose-dependent response to β-catenin is unknown. Early *Nematostella* embryo is molecularly subdivided into an oral, midbody, and aboral domain. Here we show that four β-catenin-dependent transcription factors displaying saturating expression pattern the oral domain. These factors regulate each other as well as repress individual “window genes”, and their combined knockdown results in the loss of the oral domain identity. We demonstrate that a similar regulatory logic applies to patterning the whole OA axis, and identify a β-catenin dependent molecular determinant of the midbody identity. Given the similarity of the regulatory logic involved in the β-catenin-dependent OA patterning in the sea anemone and the posterior-anterior patterning in deuterostomes, our analyses point at the common evolutionary origin of these processes. This work was funded by the FWF grant P30404-B29 to G.G.

Program Abstract #623
Establishing a GFP Marker in Zebrafish to Study the Localization of Tinagl1

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Tinagl1 is a secreted protein found in the basement membrane under epithelial cells. The LeMosy lab previously showed that tinagl1 knockdowns resulted in abnormal spinal development and heart orientation during zebrafish development. These data, together with changes in length of motile cilia, suggested that tinagl1 is involved in cilia function during development. The mechanism of this interaction is unknown, and it is unclear whether tinagl1 is only in basement membranes at the basal side of cells, or if it also localizes to the apical side of cells where most cilia project. A deeper understanding of the localization of tinagl1 during development is a logical next step in understanding how this protein functions. Zebrafish provide an excellent model for studying this localization as they display strong phenotypic effects that can be easily imaged. The localization of tinagl1 will be tracked using a tinagl1-GFP fusion construct developed through PCR and insertion into a Tol2 transposon vector. This construct will be injected into early embryos together with transposase mRNA to create mosaic fish showing tinagl1-GFP in selected tissues. Successful germline integration of the tinagl1-GFP DNA will lead to the development of a transgenic line of zebrafish allowing imaging of tinagl1 localization during development. In the poster, plans and progress made prior to research lockdown will be described. This work is part of an honors thesis project, and is supported by a gift to EKL from the Charles Silbereisen Fund of the Vanguard Charitable Gifts Foundation.

Program Abstract #624
Zic genes play a role in convergent extension

Jocelyn Waggoner, Christa Merzdorf
Convergent extension is a cellular movement during embryonic development, in which cells intercalate along an axis to allow a change in tissue shape. This cellular movement is required for neurulation, which is closure of the neural tube to form the central nervous system. Neural tube defects are birth defects of the brain or spinal cord, when neural tube closure does not occur normally. It was found that knockout of members of the zic family of transcription factors in mice results in defects in neural tube closure, however it is not understood by which mechanism mutations in zic genes cause these defects. Based on findings from our lab and others, we hypothesized that zic genes are required for convergent extension. Neural plate tissue cannot be isolated to study whether lack of zic gene expression causes convergent extension to fail. In *Xenopus laevis*, dorsal blastopore tissue undergoing convergent extension during gastrulation also expresses the five zic genes. Dorsal blastopore explants from gastrula embryos allow analysis of convergent extension in an isolated tissue. A knockdown method (using morpholino oligonucleotides) for each of the five zic genes was combined with the ‘Keller explant’ method in order to study which of the very similar zic genes is required for convergent extension. Inhibition of any of the five zic genes allowed normal elongation (= extension) of the explants. With regard to convergence, inhibition of zic3 caused the highest constriction defects (around 70%), compared to inhibition of zic2 (around 50%). Inhibition of zic1 or zic4 resulted in constriction defects below 35%, and inhibition of zic5 showed no constriction defects. Thus, zic3 expression is required for convergence during gastrulation and probably during neurulation. Further research will investigate the mechanism by which zic3 controls convergence during gastrulation and neurulation. This work was supported by grants from the NSF and the NIH.

**Program Abstract #625**

**Title: Role of Ion Channels in Craniofacial Development**

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Mutations disrupting ion channels such as Kir2.1 and CaV1.2 are associated with craniofacial and digital defects. We have shown that a Kir2.1 homologue mediates BMP release and changes in intracellular calcium during fruit fly development. Reaching a positive membrane potential threshold in neurons causes an increase in intracellular calcium, driving vesicle fusion and neurotransmitter secretion. We hypothesize that ion channels mediate BMP release through regulating changes in intracellular calcium in mammalian cranial neural crest (CNC) cells to impact craniofacial development. We discovered that CNC cells from E9.5 mice and immortalized mouse embryonic palatal mesenchyme cells (iMEPMs) undergo changes in intracellular calcium. We see that compared to wild type cells, Kir2.1 KD cells have fewer calcium transients which are reduced in amplitude. In neurons, Kir2.1 helps restore a cell’s membrane potential so that it may produce another calcium transient. If the cell cannot be restored to resting potential, it will be unable to regulate intracellular calcium appropriately. Similarly, we see a reduced occurrence of these intracellular calcium pulses when we knockdown the CaV1.2 channel, which is responsible for the influx of calcium ions into the cell. These results suggest that Kir2.1 and CaV1.2 regulate changes in intracellular calcium levels in CNC cells and their derivatives. We are now testing the hypothesis that membrane potential regulates BMP release. We express BMP4 tagged with a pH sensitive GFP variant (SEP) to report release of BMP into the extracellular environment. We image these cells during depolarization to determine if depolarization induces increases in fluorescence in BMP4-SEP cells. Our preliminary results suggest that depolarization may induce BMP4 release. Because BMP4 is essential for craniofacial development, ion channel mediated release may explain why mutations in ion channels can lead to craniofacial defects. NIH-NIDCR-R01DE025311-01A1

**Program Abstract #626**

**A unique neural crest cell-autonomous function of Six1 in mandibular development**

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Han Liu, Chaochang Li, Jingyue Xu, Yu Lan, Rulang Jiang Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229, USA Six1 is a member of the SIX homeodomain family of transcription factors. Mutations in human SIX1 have been associated with Branchiootorenal and Branchiootic (BOR/BO) syndromes, characterized by
craniofacial, inner ear, and kidney developmental defects. During craniofacial development, Six1 is widely expressed in cells originated from ectoderm, mesoderm, endoderm, and the neural crest. Previous studies have shown that Six1 plays critical roles in cranial placode and maxillary development as well as in myogenesis, but whether Six1 is required in neural crest development is not known. Here we show that tissue-specific inactivation of Six1 in the premigratory neural crests or in post-migratory neural crest cell-derived mandibular mesenchyme in the mouse embryos caused mandibular incisor agenesis and distal mandible reduction. Molecular marker analysis showed that expression of Bmp4, Fgf3, Fgf10, Shh, and Pax9 was significantly down-regulated in the developing incisor tooth germs in Six1-deficient embryos. Analysis of ATAC-seq data from E10.5 mandibular mesenchyme identified Six1-binding motif among the most highly enriched motifs in putative cis-regulatory elements for genes encoding key signaling molecules or transcription factors regulating tooth development and osteoblast differentiation, respectively. Our results provide new insights into the mechanism of mandible and incisor development. This work is supported by NIH/NIDCR grant DE027046.

Program Abstract #627

Acetaminophen Induces Craniofacial Cartilage Defects and Widespread Developmental Abnormalities in Zebrafish

Derrick Glasco, Zhidong Wang, Seonwoo Kang, Daniel Pena

Bob Jones University, USA

Zebrafish is a well-established model organism for developmental toxicology studies. As part of a broader screening project, our lab has focused on the developmental effects of acetaminophen, the active ingredient in Tylenol. Previous studies have described the effects of acetaminophen on liver development and physiology; however, limited data exists on its potential broader interference with development. Using a high but non-lethal dose of acetaminophen, we probed for developmental defects across multiple systems. Acetaminophen-treated larvae had a smaller body size and gross morphological abnormalities. They exhibited delayed hatching, sensorimotor defects, and abnormal heart rates. Most interestingly, however, acetaminophen treatment caused variable but severe craniofacial cartilage defects, with Meckel’s cartilage and the ceratobranchial cartilages missing in virtually all embryos, and deformities in most other cartilage types. Craniofacial cartilages are derived from cranial neural crest cells; however, neural crest cell migration was largely intact, and cartilage precursors expressed the neural crest cell marker sox9a and eventually col2a1 (type II collagen), suggesting that the defects may be the result of a downstream process such as cartilage condensation. Not surprisingly, many craniofacial muscles which develop in close proximity to the missing cartilages were also absent in treated embryos. Since treated embryos also exhibited greater amounts of apoptosis in the craniofacial region, apoptosis is likely a contributing factor to these defects. Current experiments to elucidate the critical timing of acetaminophen exposure should give further insight into its mechanism of action. We are also investigating whether high exposure to acetaminophen during pregnancy can cause craniofacial defects in developing mice. These studies are funded by the Department of Biology at Bob Jones University.

Program Abstract #628

Morphometric analyses of adult and larval Frizzled 7a, 7b and Wnt5b mutants’ craniofacial elements

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Craniofacial birth defects are commonly caused by improper cartilage and bone formation. One signaling pathway of interest in cartilage and bone development is the Wnt/Planar Cell Polarity (PCP) pathway. Wnt ligands and Frizzled (Fzd) 7, the receptor in this pathway, play crucial roles in craniofacial chondrocyte shape. Here we focused on the role of the genes wnt5b, fzd7a and fzd7b through morphometric analyses of C terminal mutant adult and larval zebrafish using MorphoJ software. First, the heads of adult wnt5bta89, fzd7a3, and fzd7bhu395 zebrafish mutants were compared. Procrustes ANOVA of these data indicate the mutants significantly different from wild type were the fzd7a+/+; fzd7b+- and fzd7a+/+; fzd7b+/- mutants, in which fzd7a+/+; fzd7b+- had the greatest variation. Surprisingly, homozygous fzd7a; 7b mutants and fzd7a; fzd7b double mutants showed less divergence from wild type fish than heterozygous mutants. Next, images of wild type, fzd7a, and fzd7b mutants’ heads were examined at 6 dpf. Procrustes ANOVA analysis yielded no significant results and the canonical
variance test showed a notable difference between the \textit{fzd7a/-; fzd7b/-} mutants, and wild type and single \textit{7a} or \textit{7b} mutants. These results suggest that while mutating crucial elements of the PCP pathway can alter zebrafish cartilage and bone development, continued research should include a larger \textit{n} value and \textit{N} terminal \textit{frizzled 7a; 7b} mutants to draw generalized conclusions. We acknowledge the Ripon College Biology department, the Ripon College Knop Scholars Fund, and the Tri Beta Research Scholarship Foundation Fund for providing resources to facilitate this research.

**Program Abstract #629**

**Multifactorial genetic and environmental hedgehog pathway disruption sensitizes embryos to alcohol-induced defects**

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Prenatal alcohol exposure is likely the most common environmental cause of birth defects. These exposures cause a range of structural and neurological defects, including facial dysmorphologies, collectively known as fetal alcohol spectrum disorders (FASD). While prenatal alcohol exposure causes FASD, phenotypic outcomes vary widely. It is thought that multifactorial genetic and environmental interactions modify the effects of prenatal alcohol exposure. However, little is known on the nature of these modifiers. Disruption of the Hedgehog (Hh) signaling pathway has been suggested as a modifier of ethanol teratogenicity. In addition to regulating the morphogenesis of craniofacial tissues commonly disrupted in FASD, a core member of the Hh pathway, Smoothened, is susceptible to modulation by structurally-diverse chemicals. These include environmentally prevalent teratogens like piperonyl butoxide (PBO), a synergist found in thousands of pesticide formulations. Here, we characterize multifactorial genetic and environmental interactions in the pathogenesis of alcohol-induced craniofacial defects. We show that loss of a single-allele of \textit{shha} sensitized embryos to both alcohol- and PBO-induced facial defects. Co-exposure of PBO and a normally subteratogenic dose of alcohol synergized to cause more frequent and severe defects. The effects of this co-exposure were even more profound in the genetically susceptible \textit{shha} heterozygotes. Together, these findings shed light on the multifactorial basis of FASD outcomes. In addition to further implicating genetic disruption of the Hh pathway in alcohol teratogenicity, we found that co-exposure to environmental chemicals that perturb Hh signaling can be important variables in FASD and related craniofacial disorders. Funded by NIH T32AA007471 to JLE and NIH R01AA023426, R01DE020884, R35DE029086 and U01AA021651 to JKE.

**Program Abstract #630**

**Morphogenesis of mouse embryo extra-embryonic structures at gastrulation.**

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In human, early pregnancy loss after implantation is common. This particular window of development is hard to study, and little is known about the cause of early miscarriages, in particular the impact of defects in establishing the maternofoetal interface. After gastrulation, extra-embryonic (ExE) mesoderm cells give rise to organs responsible for foetal protection and nutrition as well as communication between mother and embryo, while embryonic (E) mesoderm cells form organs such as heart and muscles. We combined two-photon live imaging and transcriptomic analysis to gain insight into the early morphogenesis of ExE structures. We had previously observed that E and ExE mesoderm cells have distinct shape and migration phenotypes, possibly due to higher Intermediate Filaments (IF) content in the ExE region. Thanks to a Krt-8-eYFP knock-in mouse line, we captured the dynamics of IF formation in ExE mesoderm. Interestingly, we noticed the formation of stable, long and thick Krt-8 “cables”, mostly in the anterior part of the embryo, attached to the amnion and crossing the exocoelomic cavity towards the cavity wall or the chorion. High resolution imaging on fixed samples showed that those Krt-8 cables span several cells. Those IF-containing structures could be essential in cavity formation and stability, amnion elasticity, and allantois growth. In parallel, we studied mesoderm at the single cell level through single cell transcriptomes of ExE structures. We dissected the amnion, the allantois, and the chorion at E7.75. We established a model for the differentiation of populations through RNA velocity analysis, that highlighted naive and mature mesoderm populations. Single cell analysis also refined our candidates lists for markers and
regulation of each structure’s organization. To conclude, we characterized ExE early development through live imaging and single cell analysis. This work is supported by WELBIO

Program Abstract #631
Maternal Senescence Negatively Affects Distinct Embryonic Stages in *Drosophila melanogaster*
Halie Ostberg, Laura Boehm Vock, Margaret Bloch Qazi
*Gustavus Adolphus College, USA*
Increasing maternal age is commonly accompanied by decreased performance and fitness costs in offspring. In *Drosophila melanogaster*, increasing maternal age negatively affects oocyte size, embryonic viability, post hatching viability and offspring lifespan. Some of these maternal effects are multigenerational. Maternal senescence has a particularly large effect on embryonic viability. Elucidating at what developmental points embryogenesis is disrupted indicates potential causes underlying the maternal age effects. We examined the transgenerational effects of maternal age on embryonic development in two laboratory strains of *D. melanogaster* (Canton S and Oregon R). The developmental stage of embryos from eight groups, every combination of old and young mother, grandmother and great grandmother, was identified. It was also noted whether embryos appeared to be developing normally or abnormally at that stage. We modelled the survival of the embryos through developmental stages using binomial regression with random effects for replicates within each maternal age group. We then used a likelihood ratio test to test if survival trajectories were parallel and to identify developmental stages which had significantly higher rates of failure. This research was supported by Gustavus Adolphus College.

Program Abstract #632
Ion channel signaling in *Drosophila melanogaster* wing development
Laura George, Sarala Pradhan, Danae Mitchell, Megan Josey, Justin Casey, Matthew Belus, Karlie Fedder, Giri Dahal, Emily Bates
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Developmental research has elucidated multiple pathways in which cells communicate to control tissue patterning. Recent evidence has indicated that one of the ways cells communicate during development is via electrical signaling mediated by ion channels. However, the scope of ion channels that regulate morphogenesis and the mechanisms by which these ion channels influence development is not fully understood. Using the *Drosophila melanogaster* wing as a model to assess changes in developmental pathways, we individually disrupted 182 ion channels known to be expressed in the *Drosophila* wing disc. Of these channels, we identified 46 candidates that lead to significant defects in wing development when disrupted. Five of these ion channels, Best2, Sk, Stim, Orai and SERCA, regulate or respond to intracellular calcium, suggesting that intracellular calcium regulation is required for proper wing development. Using immunohistochemistry staining of downstream pathway components in the known canonical developmental signaling pathways, we found that knock down of Best2, a calcium activated chloride channel, disrupts BMP and Wnt signaling. Our results confirm that ion channels play a key role in development, and we identify a number of ion channel candidates for future study to further understand mechanisms by which ion channels influence development. We would like to thank the National Science Foundation, grant number NSF-IOS 1354282 and the National Institutes of Health, grant number R01DE025311 for the funds for this project.

Program Abstract #633
Whole embryo imaging demonstrates concerted morphogenesis of genital ridges and nephric ducts in the mouse
Corey Bunce, Jennifer McKey, Blanche Capel
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During organ development, molecular and cellular processes can be constrained by morphological context. The murine genital ridge, which develops into either a testis or an ovary, undergoes a unique and dynamic morphogenesis. While a mechanistic relationship between gonad morphology and sex determination has been proposed, the connection cannot be elucidated until several morphological events are untangled. Gonad
morphogenesis involves parallel processes of extension, shortening, thickening and turning. Limitations of conventional methods have prevented analyses from considering all processes simultaneously. We overcame these challenges by applying iDISCO tissue clearing coupled with immunofluorescence and light sheet microscopy to whole mouse embryos. We built a time course of genital ridge development that maintains morphology and embryonic context and analyzed the gonad with respect to each of its processes, including a considerable change in body position. Morphological processes of gonad formation have been reported to occur in an anterior-to-posterior direction. Our results recapitulate these findings while also revealing that the central region of the genital ridge undergoes more pronounced changes than the gonadal poles. These and other asymmetries provide a basis for hypotheses about the mechanisms and guiding forces underlying gonad morphological change. In particular, the growth and positional shift of the adjacent nephric ducts may explain the gonad's pattern of curvature or turning. Application of the technique to a sex-reversing mutant revealed defects in nephric duct development, supporting a connection between duct development and sex determination. These analyses highlight the importance of embryonic context and morphology to cell and tissue development. (CB supported by NIH R37HD039963)

Program Abstract #634
Shroom3 dependent morphogenesis of the optic cup
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Ocular development is dependent on epithelial folding events that include invagination of the optic cup and optic fissure formation and closure. Eye malformations such as colobomas and Peters anomaly are thought to be consequences of disrupted epithelial folding or fusion events but the molecular mechanisms directing these events and how they are disturbed to cause defects are not well understood. Shroom3 is a cytoskeletal protein required for the normal morphogenesis of several epithelial structures including the lens, neural tube, and gut and is expressed in the optic cup at the earliest stages of its development. To test whether Shroom3 is required for optic cup morphogenesis, homozygous mutant mouse embryos from a cre-expressing gene-trap line that disrupts the Shroom3 gene (Shroom3Gt/Gt) from stages E10.5-E17.5 were generated and analyzed. Immunofluorescent labeling of histological sections was then performed to identify specific alterations in optic vesicle morphology. Homozygous mutants display abnormal eye morphology such as incomplete closure of the optic fissure and an exaggerated ventral bend in the retina. To determine whether this phenotype is due to the absence of Shroom3 in the optic cup, a conditional Shroom3 allele was generated. The utility of the Shroom3flox allele was demonstrated by generating compound heterozygote embryos (Shroom3Gt/flox) and observing that they display neural tube defects that are identical to those observed in Shroom3Gt/Gt embryos. Using an optic vesicle-specific cre, conditional mutant mouse embryos (Shroom3flox/flox; mRf-cre) were found to have identical ocular phenotypes to the Shroom3Gt/Gt embryos demonstrating tissue specificity. These data strongly indicate the importance of Shroom3 during the morphogenesis of the optic cup and future experiments will be performed to determine its role in regulating cytoskeletal contractility, mitosis, or apoptosis during optic cup morphogenesis. Funding: R01EY026910

Program Abstract #635
Study of mouse embryo-endometrial interactions during implantation using 2D and 3D models
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The implantation process is an attachment and invasion of an embryo into the uterus wall, during which embryo interacts with its inner layer, endometrium, consisting of epithelium and stroma. After attachment to the epithelium, the embryo actively invades into the uterus wall. During invasion embryo interacts with the stromal cells directly. Decidualization is a differentiation of the stromal cells which is crucial for successful implantation. Despite growing body of evidence about the importance of embryo-stromal interaction the exact role of stromal cells in implantation is yet not fully understood. Not all embryos implant successfully. Implantation failure is the
cause of most spontaneous abortions in humans. Thus it is important to develop a model to study interactions of the embryo with primary endometrial stromal cells during the implantation. The aim of this work is to study embryo-endometrial interactions in mouse using 2D and 3D model substrate for implantation. First, we have developed protocols for isolating endometrial epithelial and stromal cells. Then, using 2D monolayer model we have identified that in vitro decidualized stromal cells have a lower proliferative potential than intact ones. Using time-lapse microscopy we showed stromal cells migrations near in vitro implanted embryo. Then we constructed a 3D model on the basis of collagen gel and primary cultures of mouse endometrial cells and demonstrated the attachment of mouse blastocysts to the substrate and subsequent development to the stage of the egg cylinder. The egg cylinder was characterized morphologically and by the localization of expression of markers specific for epiblast, extraembryonic ectoderm and primitive endoderm: Oct4 and Eomes, respectively. Thus, a new model was created to study mouse embryo implantation. The work was performed under the IDB RAS government basic research program ? 0108-2019-00042020 and using equipment of the Core Centrum of IDB RAS.

Program Abstract #636
Tamoxifen teratogenicity in the mouse
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Tamoxifen is an estrogen receptor (ER) ligand with widespread use in clinical and basic research settings. Beyond its application in treating ER-positive cancer, tamoxifen has been co-opted into a powerful approach for temporal-specific genetic alteration. The use of tamoxifen-inducible Cre-recombinase mouse models to examine genetic, molecular, and cellular mechanisms of development and disease is now pervasive in biomedical research. Understanding off-target effects of tamoxifen will inform its use in both clinical and basic research applications. Here, we show that prenatal tamoxifen exposure in the mouse causes structural birth defects. Administration of a single 200 mg/kg tamoxifen dose to pregnant C57BL/6J mice caused cleft palate and limb malformations in the fetuses, including posterior digit duplication, reduction, or fusion. These malformations were highly penetrant and consistent across independent chemical manufacturers. Tamoxifen exposure induced these same malformations in ERα−/− and ERβ−/− embryos. As opposed to 200mg/kg, a single dose of 50mg/kg tamoxifen at the same developmental stage did not result in overt structural malformations. Demonstrating that tamoxifen causes dose-dependent teratogenicity, these findings argue for more considerate application of tamoxifen in Cre-inducible systems and further investigation of tamoxifen’s ER-independent mechanisms of action. Funding sources: T35OD011078 and OVCGRE Fall Competition Award

Program Abstract #637
Shroom3 Dependent Formation of the Nephron
Ashmeet Hunjan, Joanna Cunanan, Ahsan Khan, Darren Bridgewater
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During kidney development, the formation of the nephron results from interactions between the nephron progenitor and ureteric epithelial cells. These interactions lead to the nephron progenitors to aggregate and migrate towards the ureteric epithelium. These cells will further aggregate and generate cellular projections that elongate towards the ureteric epithelium. This results in the nephron progenitor cells undergoing mesenchymal to epithelial transition and formation of a spherical structure composed of polarized epithelial cells termed the renal vesicle. While early nephron formation is dependent on changes in cell morphology to mitigate cell clustering and migration, the mechanisms by which this takes place remains unclear. Shroom3 is an actin-binding protein that changes cell morphology by modulating the actin cytoskeleton. We hypothesize that Shroom3 controls nephron progenitor cell clustering, migration, and cellular projection formation. To address this hypothesis we utilized Shroom3 deficient mice (termed Shroom3+/− and Shroom3−/−). Shroom3+/− and Shroom−/− exhibit abnormal nephron progenitor cell clustering and the presence of a gap between the progenitors and ureteric epithelium. The Shroom3−/− mice displayed a phenotype that was less severe compared to the null. NCAM immunofluorescence demonstrated a reduction of cellular projection formation in Shroom3−/− mice. These abnormalities resulted in disorganized renal vesicle formation and abnormal formation of glomeruli and nephron tubular segments. Together, these results demonstrate that Shroom3 is essential for nephron formation and could explain why
mutations in Shroom3 are strongly associated with kidney disease. Future experiments will be performed to determine the molecular pathways that Shroom3 utilizes to modulate cytoskeletal organization contractility during nephron progenitor morphogenesis. The funding was received from CIHR, Kidney Foundation of Canada, and McMaster University.

Program Abstract #638

Hypoxia-induced downregulation of Sema3a provides a template for endothelial strands for coronary artery stems and ostia

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During the formation of the coronary artery stems and ostia, endothelial strands from the endothelial progenitor pool surrounding the conotruncus penetrate into the aortic wall. Vascular endothelial growth factors (VEGFs) as well as CXCL12/CXCR4 signaling are thought to play a role in the formation of the coronary stem. However, the mechanisms regulating how endothelial strands exclusively invade into the aorta remain unknown. Immunohistochemistry showed that before the formation of endothelial strands, Sema3a was highly expressed in endothelial progenitors surrounding the great arteries. At the onset of/during invasion of endothelial strands into the aorta, Sema3a was downregulated and CXCR4 was upregulated in the endothelial strands. In situ hybridization showed that Cxcl12 was highly expressed in the aortic wall compared with in the pulmonary artery. Using avian embryonic hearts, we established two types of endothelial penetration assay, in which coronary endothelial strands preferentially invaded into the aorta in culture. Sema3a blocking peptide induced an excess number of endothelial strands penetrating into the pulmonary artery, whereas recombinant Sema3a inhibited the formation of endothelial strands. In cultured coronary endothelial progenitors, recombinant VEGF protein induced CXCR4-positive endothelial strands, which were capable of being attracted by CXCL12-impregnated beads. Monoazo rhodamine detected that hypoxia was predominant in aortic/subaortic region in ovo and hypoxic condition downregulated the expression of Sema3a in culture. Results suggested that hypoxia in the aortic region downregulates the expression of Sema3a, thereby enhancing VEGF activity to induce the formation of CXCR4-positive endothelial strands, which are subsequently attracted into the Cxcl12-positive aortic wall to connect the aortic lumen. This work was supported by JSPS Grant-in-Aid for Scientific Research 19K07251.

Program Abstract #639

The intersection of Notch and Hippo signaling pathways during hindbrain development

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The brain ventricular system consists of four cavities filled with cerebrospinal fluid and are surrounded by neuroepithelium. The anatomical development of the ventricles is well understood however we know little about the underlying molecular mechanisms. Both Notch and Hippo signaling pathways have been shown to be involved in neurogenesis and share function like regulation of differentiation, proliferation, and homeostasis of tissues. Our focus is to determine if the Notch and Hippo pathways interact at the level of hindbrain development during brain ventricle morphogenesis, using zebrafish as a model. Knocking out taz, a co-transcriptional regulator of the Hippo pathway causes defects in hindbrain ventricle morphogenesis, where the midline fails to fully separate. Taz mutants also display a downregulation of rfng expression at ventricle segments. Rfng is part of the Fringe gene family, and has been shown to be involved in regulating notch-ligand interactions during neurogenesis. These results suggest that Hippo signaling is involved in regulating Notch signaling at segment boundaries in the hindbrain during brain ventricle development. However, knockout of rfng by CRISPR-Cas9 does not appear to affect ventricle size or separation. These preliminary results indicate that rfng might not be essential for hindbrain ventricle development. Perhaps instead rfng acts along with another essential gene product or is a redundant gene and therefore there is compensation for its loss. Our results suggest an intersection of these two pathways, but the intricacies of the link are still unknown and more research will need to be done. Future studies will focus on all three Fringe family genes, rfng, lfng, and mfng to see whether their expression is reduced in taz mutants and whether they are involved in compensating for the loss of rfng. This expression analysis will help us to better understand the genetics of hindbrain ventricle morphogenesis. Funded by NSERC.
Program Abstract #640
Molecular Mechanisms of Cardiac Teratogenicity in Avian Maternal PKU
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Maternal PKU is a disease that affects embryos exposed to high levels of Phenylalanine (Phe) from mothers with PKU. This results in severe cardiac and cranial defects. It is not known which genes in the developing embryo are differently expressed in the presence of high Phe. Fertilized chicken eggs were treated with PHE through yolk injection and incubated until HH10-12-14 and then dissected. RNA was isolated from 3 thoracic samples of Control or Phe treated for each timepoint. RNA was shipped to Applied Biological Materials for enrichment for mRNA, library construction, and sequencing on the Illumina NextSeq500. Data was analyzed with the open source software Galaxy Suite. Resulting Differentially expressed genes were analyzed using DAVID for enriched signaling pathways. Two pathways, Retinoid (RA) and Focal Adhesion (FA), were significantly enriched. In review of the literature RA signaling is important in heart development and increases or decreases in levels of RA can cause significant developmental defects. Additionally, FA signaling effects cell behaviors including cell motility, proliferation, and survival. Further experiments, including Quantitative PCR (QPCR), in-situ hybridization, and immunohistochemistry are underway. This project was supported by funding from UCO CURE-STEM, College of Math and Science, RCSA, Office of High Impact Practices, Student Transformative Learning Record, and OK-INBRE.

Program Abstract #641
MicroRNA-mediated control of developmental lymphangiogenesis
Hyun Min Jung, Ciara Hu, Alexandra Fister, Andrew Davis, Daniel Castranova, Van Pham, Lisa Price, Brant Weinstein
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Although transcriptional programs directing lymphatic vessel formation have been described in recent years, post-transcriptional mechanisms contributing to molecular regulation of developmental lymphangiogenesis and lymphatic network assembly are not well understood. MicroRNAs have been shown to be important post-transcriptional regulators during development. We used high throughput small RNA sequencing to identify miR-204, a highly conserved microRNA dramatically enriched in lymphatic vs. blood endothelial cells in human and zebrafish. Suppressing miR-204 leads to loss of lymphatic vessels while endothelial overproduction of miR-204 accelerates lymphatic vessel formation, suggesting a critical positive role for this microRNA during developmental lymphangiogenesis. We also identify the NFATC1 transcription factor as a key miR-204 target in human and zebrafish, and show that NFATC1 suppression leads to lymphatic hyperplasia. The loss of lymphatics caused by miR-204 deficiency can be rescued by either endothelial autonomous expression of miR-204 or by suppression of NFATC1. Together, our results highlight a miR-204/NFATC1 molecular regulatory axis required for proper lymphatic development. This work was supported by the intramural program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health (ZIA-HD008808 and ZIA-HD001011, to BMW).

Program Abstract #642
Spironolactone affects the Cardiovascular System in Zebrafish (Danio rerio)
Hannah Petty, Erin Kosmowski, Christopher Lassiter
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Spironolactone is a commonly prescribed drug used to treat heart failure and high blood pressure by inhibiting the binding of aldosterone to receptors. The drug also acts as an androgen antagonist and can be used for excess hirsutism. Despite the lack of developmental defect studies, there are potential environmental concerns with spironolactone and how it may effect aquatic environments and species. We investigated zebrafish embryos after exposure to spironolactone. The cardiovascular system showed abnormalities such as decreased heart rate, a smaller atrium, and a smaller ventricle with concentrations as low as 1 µM. Smaller blood vessels were also observed. Further studies into the molecular pathways disrupted in these developmental processes could shed light on a link between endocrine disrupting compounds and developmental abnormalities. Funding sources: Roanoke College Office of Undergraduate Research and Biology Department.
Program Abstract #643
**Anatomy and Development of the Pectoral Fin Vascular Network in the Zebrafish**
Scott Paulissen, Daniel Castranova, Shlomo Krispin, Margaret Burns, Brant Weinstein
*NIH/NICHD, United States*
In this work, we use high-resolution imaging to describe the assembly of the vascular network supplying blood to the zebrafish pectoral fins, analogous structures to the forelimb of tetrapods. The superficial location of the pectoral fin and its developing vessels make it ideal for observing and studying a broad timespan of vascular development and vascular network assembly in living specimens. Our analysis of this process spans from initial sprout into the limb bud through appearance of an adult-like vascular network at approximately 3-4 weeks post fertilization. The blood vessels that feed the pectoral fins begin via a highly stereotyped process that starts with invasion of dorsal and ventral sprouts from the common cardinal vein into the fin bud. These sprouts grow around the endoskeletal disk of the pectoral fin and converge to become the primary arc of the pectoral fin vasculature. Completion of the flowpath and perfusion of the primary arc with blood, only occurs after a sprout from the base of the ventral arm of the primary arc migrates to and attaches to the dorsal aorta; this attachment follows a reproducible path in the trunk to supply an arterial feed near the base of the second trunk intersegmental vessel. After the initial embryonic flowpath through the primary arc is assembled, a number of other fascinating reorganization events occur to bring about an adult-like vascular structure. These include the growth and reorganization of the distal part of the primary arc to form complex vascular networks of arteries and veins aligned adjacent to and through the fin rays, as well as the formation of an entirely new proximal network of vessels feeding the future endoskeletal disk-derived musculature at the base of the fin. Together, our findings detail a complex yet highly choreographed series of steps involved in the development of complete, functional organ-and tissue-specific vascular networks. Funded by the NICHD Intramural Research Program.

Program Abstract #644
**Multi-scale cell communication underlies the regulation of limb size and body proportions in mouse embryos**
Alberto Rosello Diez, Jonathan Bensley, Thy Nguyen
*Monash University, AU*
Intra- and inter-organ coordination of organ growth during development is a key feature of metazoans. In previous work [1], we explored growth-regulating phenomena in mouse embryos by genetically reducing proliferation in ~50% of the cartilage chondrocytes that drive bone growth elongation. Strikingly, although we specifically targeted the left and not the right hindlimb, left-right symmetry was not affected, due to adaptive mechanisms: 1) compensatory proliferation by spared cells in the cartilage (i.e. cell-nonautonomous); 2) a ~10% systemic growth reduction affecting the whole body of the experimental embryos, allowing the targeted cartilage to keep up with the unaffected organs. The molecular and cellular mechanisms underlying these opposite local and systemic responses remain however obscure. We found that this effect is due in part to defective placental function, suggesting that the placenta responds to stress signals released by the cartilage. We also found that bilateral targeting of the limb cartilage leads to a milder systemic effect, suggesting that this response evolved to buffer asymmetries in the event of localized insults. Our current data supports a model in which the injury is conveyed to the placenta via multiple relay steps: first to the surrounding tissues and then to a central organ that integrates whole-body information such that buffering mechanisms (involving the placenta) are activated only if a left-right mismatch exists. We will discuss the molecular and cellular mechanisms underlying this multi-scale communication process. [1] Roselló-Díez A, Madisen L, Bastide S, Zeng H, Joyner AL. PLoS Biol. 2018. PMID: 29944650. Funding: ARD is supported by a Career Development Award from the Human Frontiers Science Program.

Program Abstract #645
**Using nuclear positioning to examine the mechanisms by which Hh signaling regulates hypothalamic neural stem cell proliferation.**
Anna Aristarkhova, Ira Male
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The hypothalamus is an evolutionarily conserved region of the vertebrate brain, controlling critical functions such
as homeostasis and reproduction. Adult neurogenesis has been linked to hypothalamic function, but how this neurogenesis is regulated is poorly understood. Our lab recently demonstrated a novel role for Sonic Hedgehog (Shh) as a positive regulator of neurogenesis in the zebrafish hypothalamus. Hh inhibition reduced both cell proliferation and cyclinD expression, suggesting Hh signaling might affect cell cycle progression. During embryogenesis, nuclei of neural precursors move between the ventricular and basal surfaces as they progress through mitosis in a process called interkinetic nuclear migration (IKNM), with nuclear position giving a rough estimation of cell cycle stage. Here we provide evidence that IKNM continues in the larval hypothalamus and nuclear positioning can be used to examine changes in cell-cycle progression within the neurogenic population. Using zebrafish transgenic lines that label the nuclei of Hh-responsive precursors, we examined nuclear position following cell cycle or Hh signaling inhibition. Blocking the cell cycle at G1/S resulted in a small but significant shift of nuclei toward the basal surface within the Hh-responsive population. In contrast, blocking the cell cycle at G2/M led to a repositioning of nuclei toward the ventricular surface. Inhibiting Hh signaling using cyclopamine or BMS-833923 led to a shift in nuclei toward the ventricular surface, consistent with Hh signaling affecting progression through the G2/M check-point. Understanding the mechanisms by which Hh signaling regulates hypothalamic neurogenesis could have major implications for understanding adult hypothalamic function and dysfunction. Funding Source: University of Amherst Commonwealth Honors College Research Fellowship.

Program Abstract #646
The transcription factor Ebf2 is a marker of somatosensory, motivation and reward system of the developing mouse brain.
A. Cecilia Barboza-Chávez, Lorena M. Cabrera-Alvarado, M. Blanca Delia Cepeda-Varela, Román Vidaltamayo  
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The functions of the nervous system depend on the correct connection between neurons and the formation of circuits between them. However, the molecular mechanisms involved in the formation of these circuits are not completely elucidated. In this work, we use the reporter tau-GFP (TGFP) under the control of the promoter of the Ebf2 gene in transgenic mice to trace the formation of neural circuits during mouse brain development. Ebf2 is a transcription factor involved in cell differentiation of the central nervous system, which is abundantly expressed in different areas of the murine brain. Our results show that the Ebf2-TGFP reporter is expressed in somatosensory system circuits connecting the trigeminal nuclei of the brainstem to the Ventral Posteromedial nucleus of the Thalamus, as well as in lamina II of the spinal cord and the Parabrachial nucleus. This distribution of the Ebf2-TGFP signals suggests that these circuits are involved in processing nociceptive signals. Moreover, we can also detect Ebf2-TGFP expression in circuits linking Septal Nuclei of the forebrain and the Bed Nucleus of the Stria Terminalis to the medial Habenula (mHb) via the stria medullaris. Some Ebf2-TGFP fibers link the mHb to the Interpeduncular Nucleus, where numerous Ebf2-TGFP axons that innervate the Dorsal Tegmental (DTg) nucleus originate. Some Ebf2-TGFP axons arising from the DTg and the ventral Periaqueductal Area join the medial forebrain bundle that traverse the hypothalamus and innervate the ventral pallidum region of the forebrain. The location and connectivity of these Ebf2-TGFP circuits suggest that they participate in the processing of motivation and reward signals in the brain. The expression of Ebf2-TGFP in these somatosensory and motivation-reward systems can be detected as early as E14.5 stage of mouse embryo development and is maintained in the adult mouse brain (P56).

Program Abstract #647
The role of axon guidance molecules in the early cerebellar circuit formation
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Many cerebellar neurological disorders are characterized by structural changes in neuronal connectivity. Recent experiments in our lab have shown that the foundation of the cerebellar connectivity is assembled by trigeminal afferent pioneer axons and cerebellar nuclei neurons; however, the cellular and molecular mechanisms underlying the early cerebellar connectivity establishment is not clear. This study aims to determine how axon guidance molecules regulate the complex cerebellar circuit formation that emerges from homogenous cell populations to provide insight into the cause of conditions such as autism spectrum disorder. The guidance
molecular alteration causes subtle structural changes in the early neural connectivity, in this study we have focused on the axon guidance molecule Sema5A and its receptor PlexinB3 during early cerebellar development using normal, mutant mice (Neurog1-/-) with the complete lack of the trigeminal ganglia, and nax mice in which Sema5A and its receptor PlexinB3 gene expression levels are upregulated. Our data show that a subset of the cerebellar nuclei neurons are strongly immunopositive for Sema5A in the rostral end of the cerebellar primordium, suggests a role in attracting primary projection of early pioneer axons to the cerebellar nuclei neurons. In addition, immunoreactivity with the putative axonal collateral branches may direct secondary projection towards the Purkinje cell plate. It is indicating a novel role for Sema5A and PlexinB3 receptor that may regulate trigeminal derived pioneer axons to target neurons during early cerebellar circuit formation. NSERC Program Abstract #648

Regulation of sexually dimorphic neuronal development and courtship behavior by the Dissatisfaction and Doublesex genes in Drosophila

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In Drosophila, the dissatisfaction (dsf) gene encodes a nuclear receptor that contributes to the development of male and female courtship behavior and fertility. Here, we identify a small subset of dsf-expressing interneurons in the abdominal nervous system of adult males and females that are required for most behaviors altered in dsf mutants. These neurons are sexually dimorphic in number and morphology. Males have about 3 bilateral pairs of local interneurons, whereas females have about 10 pairs of neurons that include anteriorly projecting neurons that are absent in males. These dimorphisms in neuron number and morphology are regulated by the sexual differentiation gene doublesex (dsx). In males, the loss of dsx causes neuron number to increase to an amount similar to what is seen in wildtype females and leads to the development of female-like projection patterns. Remarkably, the increase in neuron number observed in males lacking dsx is dependent upon dsf function. These results suggest a genetic pathway in which the male isoform of dsx represses dsf function in promoting neuron number thereby producing a sexual dimorphism in a set of neurons that contribute to courtship and fertility. This work offers insight into how a developmental gene patterns the circuits for an innate animal behavior.

Program Abstract #649

Smad4 Dependent Morphogenic Signals Play A Pivotal Role in Controlling Maturation and Axonal Targeting of Basal Vomeronasal Sensory Neurons to The Accessory Olfactory Bulb.

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The accessory olfactory system of mice provides a great model to understand the molecular mechanisms underlying the formation of functional neuronal circuits in mammals. The vomeronasal organ (VNO) of rodents is composed of two main types of vomeronasal sensory neurons (VSNs) that express distinct families of vomeronasal receptors and localize in different regions of the vomeronasal neuroepithelium (VNE). V2r-expressing neurons localize in the basal portion of the VNE and target posterior regions of the AOB (pAOB), while V1r-expressing neurons reside in the apical portion and project to anterior regions of the AOB. Morphogenic signals play pivotal roles in controlling genes that define neuronal identity and network formation. We found TGF-b and Bone Morphogenic (BMP) signals are actively transduced in the VNO of postnatal animals. In particular, we found that BMP signaling is restricted to the neurons in the basal regions of the VNO and to the cells in the marginal zones of the VNO, where neurogenesis occurs. Using different Smad4 conditional KO mouse models, we disrupted canonical TGF-b/BMP signaling in either maturing basal VSNs (bVSNs) or in all mature VSNs. We found that Smad4 functional ablation in immature bVSNs compromises dendritic knob formation, pheromone induced activation, correct glomeruli formation in the accessory olfactory bulb (AOB) and negatively affects cell survival. However, we observed that Smad4 loss-of-function in mature apical and basal VSNs only compromises the correct glomeruli formation of basal VSNs in the posterior AOB. Our results suggest that Smad4 mediated intracellular signaling specifically drives the functional maturation and connectivity of basal VSNs. This research was supported by NICHD’s awards 1R01HD097331-01 and 1R15HD09641101 and by NIDCD’s award 1R01DC017149-01A1.
Program Abstract #650

Postnatal cerebellar development is impaired by intrauterine growth restriction
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Intrauterine growth restriction (IUGR) caused by uteroplacental vascular insufficiency impairs the delivery of oxygen and nutrients to the fetus and results in subjects born small for gestation age (SGA). Clinical studies revealed that newborn SGA infants have smaller cerebella than normal-weight infants and have impaired postnatal sensory, behavioral, and cognitive development and a higher risk of cerebral palsy, but the underlying mechanisms remain poorly understood. During postnatal development, the numbers and distribution of granule cells in the cerebellum are dependent on cell proliferation, apoptosis, and migration. We used SGA pigs, which have a reduced cerebellar size at birth, as a relevant model for SGA infants to determine whether IUGR alters postnatal development of cerebellar granule cells. Immunohistochemistry was performed to quantify the numbers of proliferating and apoptotic cells, and the distribution of pH3+ and actCasp3+ cells in the external granule layer (EGL). The density of proliferating pH3+ granule cell precursors was moderately increased in SGA relative to NGA pigs, whereas the number of apoptotic cells was much more significantly increased in the EGL of SGA pigs. The thickness of the inner Tag1+ EGL was greater in SGA cerebella, suggesting an accumulation of pre-migratory granule cells in the inner EGL. Overall, our data suggest that cell proliferation in the EGL of SGA pigs is not dramatically affected. However, impaired migration of newly differentiated granule cells from the EGL to IGL was associated with increased apoptosis. An impaired granule cell migration from the EGL to the IGL persisted in SGA pigs even after twenty days of postnatal development. Long-lasting disruption of granule cell development is consistent with motor and behavioral deficits of infants born SGA.

Supported by the UTHSC Neuroscience Institute.

Program Abstract #651

A lysosomal gene regulatory network in microglia
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As scavengers of neuronal debris and effectors of immune pathways in the central nervous system, microglia drive both phagocytic and inflammatory responses in the brain during development, aging, and disease. Many microglial functions, such as pruning of synapses or phagocytosis of apoptotic debris, culminate in lysosomes for degradation and recycling. Of note, a strong correlation is observed between lysosomal dysregulation and aberrant microglial activity in neurodegenerative diseases. However, how lysosomal activities in microglia ameliorate or exacerbate the pathology in neurodegeneration remains unresolved. Central to lysosomal functions are Transcription factor EB (TFEB) and members of its protein family; TFEB and related transcription factors drive essentially all lysosomal processes, such as lysosomal biogenesis and autophagy. Despite the importance of lysosomal activity for microglial functions, the role of TFEB in microglia remains unexamined. Here we show that Tfeb, and the related transcription factor Tfe3, must be repressed for the normal development and function of microglia in zebrafish. We have previously shown that mutation in the lysosomal GTPase RagA (encoded by rraga), a known repressor of TFEB activity, results in aberrant microglial numbers and activity. Extending this observation, here we find that simultaneous loss of rraga and tfeb or tfe3 rescues a majority of defects observed in rraga mutants, indicating that the primary essential function of RagA may be to repress Tfeb and Tfe3. Furthermore, our data show that primitive macrophages in rraga mutants are abnormal, indicating that the microglial defects observed in rraga mutants are likely to extend to the peripheral immune system and precede the specification of microglia. Together, our studies demonstrate that RagA promotes survival and function of microglia by preventing aberrant expression of the critical lysosomal transcription factors Tfeb and Tfe3. Funding source: American Heart Association.

Program Abstract #652

Gpr161 regulates midbrain development via mediating cell proliferation
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The central nervous system is derived from the embryonic neural tube. Fundamental cellular processes such as cell proliferation, differentiation, and apoptosis, in neural progenitor cells are tightly regulated during early brain development. Sonic Hedgehog is secreted from notochord and designates neural progenitor cells into differentiated neurons depending on the morphogen gradients (Shh, Wnts, Bmps, and Fgfs). Gpr161 is known as a negative regulator of Shh signaling and its null and hypomorph mutant mice present with neural tube closure defects. Recently, the depletion of Gpr161 in neural stem cells and cerebellar granule progenitor cells in mice caused the Shh subtype-medulloblastoma. However, it is not well characterized the role of Gpr161 in the later stage of embryonic brain development. We utilized Gpr161 floxed mice mated to Wnt1-Cre that express Cre recombinases in the mesencephalon and neural crest derivatives to identify the role of Gpr161 in embryonic brain development. The gross phenotypes of Gpr161f/f;Wnt1-Cre/+ (Gpr161 CKO) mice included midbrain protrusion and craniofacial defects. We further identified the dysregulated molecular and cellular processes underlying the midbrain protrusion in Gpr161 CKO embryos. Using immunohistochemistry, we observed the cell proliferation marker was significantly increased in the dorsal ventricular zone of the mesencephalon in Gpr161 CKO embryos, indicating a hyperproliferation of midbrain progenitor cells. Gli1 RNA and protein levels were significantly increased in midbrain tissues of Gpr161 CKO embryos, indicating hyperactivation of Shh signaling. The RNA seq experiment further identified differentially regulated genes such as Fgf8, Fgf15, and Hes3, all of which are involved in the proliferation of midbrain progenitor cells. The data suggests that the midbrain protrusion in Gpr161 CKO embryos is secondary to the hyperproliferation caused by increased Fgf and Shh signaling. This study was supported by R01HD093758.

Program Abstract #653
Characterization and Regulation of Dopaminergic Cells in the Developing Zebrafish Hypothalamus
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The hypothalamus is an evolutionarily conserved region of the brain responsible for regulating essential processes such as circadian rhythms, reproduction, and feeding. Dopaminergic cells in the hypothalamus have varied functions, from regulating motor behavior to controlling neuroendocrine output, but the identities of distinct hypothalamic dopaminergic populations and how these populations are established during development is not well understood. This project investigated the morphology and distribution of dopaminergic cells in the zebrafish hypothalamus as well as the cell signaling mechanisms that regulate dopaminergic cell number during larval development. Prior studies showed that dopamine signaling is involved in the regulation of cell proliferation in the spinal cord, however, few studies have investigated the role of dopamine in hypothalamic cell proliferation. We used a drug treatment approach to investigate the role of cell-signaling pathways in controlling dopaminergic cell number, as well as the effect of dopamine signaling on the hypothalamus during larval development. Our findings suggest that dopaminergic cells in the hypothalamus are controlled by a combination of the Sonic Hedgehog, Wnt, and Notch signaling pathways. Increased dopamine levels in the hypothalamus led to an increase in Sonic Hedgehog-responsive neurogenic precursors, suggesting dopamine signaling may act indirectly through the Sonic Hedgehog signaling pathway to affect proliferation and/or differentiation within the hypothalamic stem cell niche. Funding Source: University of Amherst Commonwealth Honors College Research Fellowship.

Program Abstract #654
Initial structure-function analysis of the Xfeb gene, which is involved in neural crest development
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A network of transcription factors and signaling factors drive the induction of neural crest cells, which are essential for the formation of many tissues, including the peripheral nervous system, craniofacial structures, and melanocytes. The transcription factor Zic1 is essential for early neural development, including the establishment of neural crest cells. We identified the gene Xfeb as a direct target of Zic1 and overexpression of zic1 resulted in
ectopic expansion of Xfeb expression. In neurula embryos, Xfeb is expressed at the neural plate border posterior to the midbrain hindbrain boundary and in neural crest cells, which led us to hypothesize that it is involved in the development of the neural crest. Further experimentation in Xenopus embryos demonstrated that morpholino-based knock-down of Xfeb (using two separate morpholinos) resulted in a reduction in slug expression, a marker of premigratory neural crest cells. Conversely, overexpression of Xfeb resulted in an expansion of the slug expression domain. We also demonstrated that Xfeb acts downstream of both Zic1 and Pax3 during neural crest induction and seems to be part of the regulatory network that drives neural crest induction. Xfeb is necessary for the expansion of slug expression caused by overexpression of Zic1, while it also may be one of several downstream targets of Pax3. Xfeb consists of five tandem SCP (sperm-coating glycoprotein) domains, separated by short linkers. The first four of the five SCP domains contain the conserved residues required for serine protease activity. We developed deletion constructs of SCP domains in order to determine the necessity of these domains for slug expression. Loss of the first two SCP domains continued to allow Xfeb to expand slug expression, while removing domains 4 and 5 or domain 5 alone eliminated the ability of Xfeb to expand slug expression. Thus, it appears that the atypical 5th SCP domain is required for Xfeb activity. Funded by NSF.

Program Abstract #655
Finding dory – a role for cacna2d3 in sensory filtering
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Our nervous systems are constantly filtering irrelevant stimuli through fundamental and conserved processes, such as habituation learning and sensorimotor gating. These processes are critical to animal behavior and are disrupted in multiple human disorders of the nervous system, including ADHD, schizophrenia and autism spectrum disorders. Synaptic plasticity that underlies these processes has been described at the cellular level, yet the molecular-genetic regulators of this plasticity remain poorly understood, as do circuits that mediate sensory filtering. An unbiased, forward genetic screen identified the dory mutant line based on reduced habituation of the acoustic startle response (ASR) in mutant larvae. Whole-genome sequencing identified the calcium voltage-gated channel auxiliary subunit alpha-2/delta-3 (cacna2d3) as a candidate gene affected in dory mutants. cacna2d3 is known to regulate synaptic transmission but has no known role in sensorimotor gating. Behavioral characterization of zebrafish homozygous for two independent mutant alleles of cacna2d3 confirmed a critical role for this gene in habituation. These analyses also revealed reduced prepulse inhibition of the ASR in cacna2d3 mutants, indicating that cacna2d3 is required for sensorimotor gating. To identify the cell types in which cacna2d3 functions to mediate sensory filtering, we carried out an unbiased whole brain activity analysis using a mitogen activated protein kinase-mapping (MAP-Mapping) assay. This allowed us to examine how neuronal activity differs between wildtype and cacna2d3 mutant larvae during sensory filtering. Our data reveal a previously unrecognized yet critical role for cacna2d3 in sensory filtering and inform our understanding of the neural circuits that promote habituation and sensorimotor gating. This work was supported by the NSF GRFP and UW-Madison SciMed GRSF to N.J.S, and the UW-Madison Dept. of Integrative Biology

Program Abstract #656
Aberrations in rhombic lip development are a characteristic pathological feature of human Dandy-Walker malformation
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Dandy Walker malformation (DWM) is the most common human cerebellar malformation, affecting 1 in every 10000 live births. DWM is an imaging diagnosis that is characterized by three features: cerebellar vermis hypoplasia, an enlarged posterior fossa, and an enlarged fourth ventricle. Although recent advances in neuroimaging have improved diagnosis of DWM, virtually nothing is known about the cellular and histological defects that lead to DWM during brain development. One major reason is that little human specific data is available describing the histology of normal and abnormal human fetal cerebellar development. Currently, there is limited published fetal pathology of DWM and few comparative analyses available due to lack of molecular confirmations of diagnoses. We have carried out the first comprehensive prenatal histo-pathological analysis of human DWM. Our results indicate a significant reduction in foliar complexity of the developing human cerebellum. We also observe aberrations in the developmental trajectories of specific cell types like Purkinje cells, and progenitor zones like the rhombic lip and external granule layer (EGL). Significantly, proliferation and self-renewal of rhombic lip and posterior EGL progenitors is reduced, leading to hypoplasia that disproportionately affects the posterior lobe. Through our analysis of the human fetal DW cerebellum, we begin to directly address the developmental pathology of human DWM beyond that of the mouse models that share similar pathology. Our studies will fundamentally improve our view and understanding of the biology of the human cerebellar development and give us insights on the developmental pathogenesis of DWM. This work was supported by NIH-R01-NS080390 and R01-NS095733 (KJM) and the National Ataxia Foundation Young Investigator Research Grant (PH).

Program Abstract #657
Investigating mechanisms of the serine/threonine kinase TNIK in synapse patterning
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Synapse inhibition is a crucial process in the development of the nervous system, yet synapse dysregulation and an overall reduction in synapses is a pathogenic state characteristic of psychiatric diseases including schizophrenia and neurodegenerative diseases such as Alzheimer’s disease. The serine/threonine kinase TNIK, which is enriched at the synapse, is implicated in these disorders, yet the synaptic role of TNIK is not fully understood. We have shown, using C. elegans, that the TNIK ortholog mig-15 is critical in inhibiting the structural formation of synapses as mig-15 loss of function mutants exhibit an increase in synapse number. Conversely, over-expressing mig-15 results in a drastic decrease in synapse number, suggesting that mig-15/TNIK acts as a negative regulator of synapse number. However, the molecular mechanisms of mig-15/TNIK-mediated synapse regulation are unknown. To begin to investigate this, we conducted a forward screen to identify genes that function downstream of mig-15/TNIK. Here, we show ongoing work validating potential mig-15/TNIK genetic interactors and characterize their roles in synapse formation and patterning. This project is funded by the Canadian Institutes of Health Research (CIHR PJT-148667, K. Mizumoto).

Program Abstract #658
The development of mouse cerebral organoids in vivo
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Usually cerebral organoids are understood as 3D cultures consisting of the brain-specific cell types derived from embryonic or pluripotent stem cells. This approach is very important for studying brain development and disease modeling, but it has a number of drawbacks: a complex process of cell culturing, the absence of vascularization, absence of some cell types specific for the brain. We suppose that culturing of organoids in the ventricular cavities of the brain of the experimental animals in vivo could be the perspective alternative. It will be possible to use already committed cell types and culture media will be replaced by cerebrospinal fluid. The aim of this work was to obtain and study the cytoarchitecture of self-organized organoids in the ventricles of the adult mouse brain. For this purpose E14.5 GFP mouse neocortex was characterized by PCR and immunohistochemistry. Then suspension was prepared and stereotactically injected into the lateral ventricle of the adult mouse brain at the
concentration of 2x10^5/μl in Hanks solution. An immunohistochemical study of the organoids was performed at 5, 30 and 90 days after operation. We have shown that E14.5 neocortex consists predominantly of immature neurons and neural progenitors. We revealed that development of self-organized organoids takes 90 days. Mature NeuN+ neurons were located in the central part of the organoid. The periphery region looked like the first layer of the cortex with astrocytes GFAP+ forming the structure resembled "glia limitans" between organoid and ventricle cavity. Interestingly, there were no glial scar between organoid and recipient tissue. Moreover, the dense network of Synaptophysin+ processes were formed. It is importuned to notice that organoids were vascularized. Thus, the ventricles of the mouse brain can serve as an incubator for developing 3D organoids. This research was funded by the RSF project 19-74-00117.

**Program Abstract #659**

**The RNA binding protein Caper regulates tissues specific development of motor neurons**

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Motor neuron dysfunction results in a variety of debilitating diseases including Parkinson’s disease and amyotrophic lateral sclerosis (ALS). These individuals suffer from progressive loss of motoneurons and consequently loss of muscle control, which negatively impacts their quality of life and can ultimately be fatal. Recently, mutations in many different RNA binding proteins, including regulators of splicing, have been implicated in the development of motoneuron diseases. Alternative splicing is a significant contributor to co- and post-transcriptional gene regulation that increases the proteomic complexity of an organism. Interestingly, the nervous system has been demonstrated to have one of the highest frequencies of splicing events compared to any other tissue. Not surprisingly, the nervous system is particularly sensitive to dysfunction of core splicing machinery. Yet curiously, the vast majority of alternative splicing factors are ubiquitously expressed. Therefore, better understanding of the tissue-specific roles of alternative splicing would elucidate mechanisms that regulate neuron development, including motor neurons. In our research, we have demonstrated that the highly conserved RNA-binding protein and alternative splicing factor *caper* is necessary for proper development of motor neurons as indicated by aberrant morphology of neuromuscular junctions (NMJ) in *caper* deficient larvae. Furthermore, we have found that *caper* functions in motoneurons and glia during NMJ bouton morphogenesis but is dispensable in the muscle for bouton development. Notably, *caper* deficient larvae also demonstrate aberrant locomotor behavior, including compromised self-righting and decreased foraging behavior. Since *caper* is conserved across metazoa, this work may be broadly informative regarding the function of *caper* orthologs in neurogenesis across multiple species. This work was supported by the National Institutes of Health 1R15NS104976 to ECO and NINDS-NS080685 to MBT

**Program Abstract #660**

**Filling gaps in reconstruction of the ancestral traits of planktotrophic development in the annelid clade Opheliidae/Capitellidae/Echiura**

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Planktotrophic (p-trophic) larvae are hypothesized to be ancestral in annelids. Aiming to test this scenario, we study the development of the White Sea species Ophelia limacine from the Opheliidae/Capitellidae/Echiura (OCE) clade. First cleavages in Ophelia start as leotropic, equal, and synchronous with minor size differences in micromers and macromeres. Thoracophelia mucronate, Notomastus cf tenuis and two echiurids have strikingly similar pattern. Coeloblastulae such as in 7-20 hpf Ophelia are known in Notomastus and echiurids. Gastrulation by invagination (14-33 hpf in Ophelia) is typical for annelids without large yolky eggs. In early trochophore, 28-48 hpf, epithelization occurs, apical tuft appears. In trochophores, 1-4 dpf, wide episphere covers small hyposphere as in champignon a cap covers a stem, and forms a deep circular fold. Prototroch and metatroch run along the fold. Episphere forms a hood over a ciliated mouth. Esophagus makes a loop and leads to a stomach, which is separated by gastrointestinal valve from the intestine; anus dorsal. The first neurons along neurotroch. Ciliary episphere patch; long cilia of proto- and metatroch, 2-3 rows of short feeding cilia in the mentioned fold; some
non-motile preoral cilia; telotroch. Late trophophore, 16-20 dpf, elongates hyposphere, somatic and visceral muscles form, two roots of circumesophageal connectives extend. Similar larval shape, ciliation, and gut structure in trophophores of Armandia polyophthalma, T. mucronate, N. cf tenuis, Urechis caupo, Lissomyema mellita. Most p-trophic larvae of OCE clade have similar pattern of cleavage, gastrulation and trophophore. Future analysis of p- and l-trophic development among the annelids is one of the basic requirements to understand the evolution of development of annelids. The studies supported by the grant MK-1164.2020.4.

Program Abstract #661
Bradoriid (Arthropoda) Developmental Biology
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A hypothesized Antennapedia allele has been proposed as responsible for morphological changes in trilobites such as cephalic width changes and the development of macropleurae. Nigel Hughes presented an alternate proposal, linking the development of macropleural segments in trilobites to Abdominal-B Hox gene with expression being linked to the location of the genital opening as proximal trigger for macropleural development. Unfortunately for Hughes’ Abdominal-B scheme, if we equate macropleural development with appendage development (there exist good reasons to do so), Abdominal-B is known to antagonize the genital disc development of appendages. Bradoriids are an extinct Cambrian group of ostracod-like bivalved stem euarthropods. The remarkably deep/tall triangular shields of bradoriids belonging to Beyrichionidae and Hipponicharionidae have so far defied explanation. I propose here that shield depth in bradoriids was controlled by expression of an Antennapedia allele, with the locus of expression along the anterior-posterior axis of the animal determining whether its shields are preplete, amplete or postplete. This effect corresponds to the allele’s influence on the width of the trilobite cephalon. This research has been supported by the National Science Foundation.

Program Abstract #662
Novel protein from larval sponge cells is related to energy turnover enzyme conserved among invertebrates
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Phylum Porifera (sponges) occupy the basal position onto phylogenetic tree, therefore these primitive animals are an excellent object for studying the early stages of Metazoa evolution. Embryonic development in sponges mainly occur inside maternal body, in mesochyle, and finalize with releasing of free-swimming larva that attached to the bottom and transform into juvenile sponge, rhagon. The transition from a mobile larva to an attached filter-feeding organism is carried out due to metamorphosis, a process accompanied by a radical change in the plan of the body structure. Continuity between larval cells and adult tissues is still under discussion. Previously, polyclonal antibody was produced against major protein of the flagellated cells covering the larva of our sponge, Halisarca dujardini. Tracing of flagellated cells fate with this antibody demonstrate that flagellated cells redifferentiate to choanocytes, cells that produce water flow through sponge body and provide the feeding by flagella beating. In the present work, we identified the sequence of this major protein used for antibody production using tandem mass spectrometry and proteome searching. A search in open databases demonstrate presence of multiple orthologs of identified protein for sponges, cnidarians, platyhelmintes, ctenophores and echinoderms, but all of them have not been previously described. A polypeptide with a size of about 400 amino acid residues has two conservative domains: TIM-barrel, which has enzymatic activity against macroergic compounds, and canonical EF-hand, Ca-binding domain. In the genome of H. dujardini there are 4 close paralogs of the gene encoding this protein. mRNA of our protein is expressed in the surface flagellated cells of the larva. We suggest the participation of this protein in calcium-mediated regulation of energy metabolism, the activation of which precedes metamorphosis. The study was financially supported by the Russian Science Foundation (grant 17-14-01089).
Program Abstract #663
From where do beetles get their horn? The genetic underpinnings of novel trait development in dung beetles
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Naureen Fatima
West Virginia University, USA
The horns of dung beetle, Onthophagus taurus are used to address the fundamental biological question of novel
trait development. The horn is a secondary sexual trait and develops only in males (sexual dimorphism). At the
same time, it shows an extreme form of plasticity within males (polyphenism), which is influenced by nutrition
uptake. On the contrary, the primary sexual trait, genitalia, is not at all nutrition-dependent, and its size does not
vary under high or low nutritional conditions. Previous studies have shown a relationship between different
tissues in terms of gene expression patterns, and as of now, legs are considered to be the closest to horns.
However, not much is known about the relationship of horns with genitalia in the same context. We utilized
RNAsseq using four tissues and highlighted the gene expression pattern in polyphenic (head horns, where
nutritional conditions drastically affect its size), non-polyphenic (thoracic horns, where nutritional conditions have
moderate effects on size), and non-horn appendages (legs and genitalia, where nutritional conditions do not vary
their size) in the two beetle life stages, prepupa, and pupa. We will present our most current result and discuss
the significance of genitalia developmental genes in the beetle horn development. Funding source: WVU
Experiment Station Hatch Project from USDA NIFA (WVA00712)

Program Abstract #664
Molecular patterning during the development of Enchytraeus coronatus: insights into the evolution of direct
development in clitellate annelids
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Annelids are a numerous and widely distributed phylum of segmented worms, with great diversity in morphology
and ecology. A high degree of morphological diversity in this group of animals makes the annelids a useful system
for studying the mechanisms of animal body plan evolution. Like other spiralians the annelids display a highly
conserved pattern of early development known as “spiral cleavage” and often form a free-swimming larva.
Nevertheless, the clitellate annelids show a modified pattern of spiral cleavage and a “direct” development
without the ancestral planktonic larval stage. To study the evolution of development in the directly developing
clitellates, we first described cell lineages and generated fate map for the oligochaete Enchytraeus coronatus.
Next steps, we identified and cloned genes, which may have a conserved role in body plan formation, gut
development, germline and multipotent cell maintenance, various cell type specifications, and characterized their
expression pattern in the embryonic development of the studied oligochaete. Many of these genes, including Hox
and other homeobox genes, FoxA and Gata homologs, and Germline/Multipotency Program genes, are duplicated
and associated with a number of morphogenetic events in the embryo. Their co-options might be significant for
the evolution of direct development in Clitellata. This work was supported by the RFBR grant 19-04-01111-
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Program Abstract #665
Conserved transcriptional logics directing Nr2f1/2 expression in vertebrate atria
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NR2F orphan nuclear hormone transcription factors display conserved expression in atrial cardiomyocytes (ACs)
and regulation of vertebrate atrial development. Despite their centrality to vertebrate heart development, the
regulatory logic underlying Nr2f1/2 gene expression in ACs remain obscure. To begin to understand the
transcriptional regulation of Nr2f1/2 genes in ACs, we first cloned an ~1.8 kb region of the zebrafish nr2f1a
putative promoter and 5’UTR that is highly conserved among gnathostome Nr2f1 and Nr2f2 loci. However, this
region showed brain and low-level pan-cardiac expression, suggesting other conserved cis-regulatory modules
(CRMs) must direct AC-specific expression. Thus, we used Assay for Transposase Accessible Chromatin with high-
throughput sequencing (ATAC-seq) to compare open chromatin for the genomic environment of zebrafish nr2f1a
(730 kb) in isolated ACs to vertebrate Nr2f1 and -2 loci using VISTA plots and manual alignments. This approach identified a 280 bp enhancer 3' to nr2f1a, which we call 3'reg1, that is conserved in gnathostome Nr2f1 orthologs and able to direct expression in ACs of stable transgenic embryos. Integrating transcription factor binding site analysis and manual alignments allowed us to identify conserved putative binding sites within this CRM for Sox and Fox transcription factors. Deletion of the Fox/Sox sites dramatically reduced 3'reg1 expression in ACs. Despite also identifying nuclear hormone binding sites and the established responsiveness of Nr2f genes to retinoic acid (RA), pharmacological treatments and deletion analysis show 3'reg1 is not RA-dependent. Together, our data suggest that putative Fox/Sox sites within a conserved Nr2f1 3'-enhancer are necessary to promote expression within ACs, opening new avenues for our understanding of the gene regulatory networks driving vertebrate atrial development and the etiology of congenital heart defects in humans.

Program Abstract #666
Evaluation of the effects of TiO2 and Eu doped TiO2 nanocrystals in the development of Drosophila melanogaster
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There has been an increase regarding the use of nanoparticles in many fields, including the food industry, cosmetics, and medical. The titanium dioxide (TiO2) can be found in sunblock, chewing gum and paints. Because of this variety of uses it’s important that we understand its effects on living organisms. Here we evaluate the toxicity of the TiO2 and Europium doped TiO2 nanocrystals (NCs) during the development and lifespan of Drosophila melanogaster. Thirty-five first instar larvae were placed in 4mL of Drosophila standard culture medium (6 replicates) containing increasing concentrations of the TiO2 and TiO2Eu NCs (0.25 - 8.0 mg/mL). We dissected the gut of third instar larvae exposed to the NCs to perform the trypan blue exclusion test. The development of exposed animals was followed to determine the rates of larval mortality and pupation. The newly emerged flies were transferred to standard media to analyze the adult lifespan. The luminescence of the TiO2Eu NCs in adults was tracked. Animals that developed in the medium containing TiO2 NCs presented a larval stage delay in the two highest concentrations. The pupation rate was the lowest in the highest concentration and the larval mortality was higher which caused a lower number of emerged adult flies. The larvae exposed to TiO2Eu NCs presented a lower rate of pupation in the three highest concentrations and a greater larval mortality, leading to a low number of emerged flies. We also observed aberrant phenotypes in adults such as crooked wings and dark spots in the abdomen. We were able to track the TiO2Eu fluorescence in the fat body of newly emerged flies. The trypan blue assay showed larval gut damaged cells at higher concentrations. Although causing developmental delay and larval lethality we did not observe adult lifespan differences between exposed and control flies. Our results demonstrate that TiO2 and TiO2Eu NCs exposure can lead to larval lethality and developmental delay. Funding sources: none

Program Abstract #667
Toxicological effects of copper doped zinc oxide nanocrystals on the development of Drosophila melanogaster
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Nanocrystals have been increasingly incorporated into various branches of industry due to its properties to enhance the efficiency of many products. The zinc oxide nanocrystals are considered multifunctional because it has many properties of interest, such as broad UV absorption, high photostability, antifungal activity, biocompatibility, and biodegradability. Due to its properties, it has been included in the manufacture of sunscreens and as a food preservative. To increment these properties, nanocrystals can be doped with other elements. In the present work, the zinc oxide nanocrystals was doped with the transition metal copper, which has antibacterial activity. Drosophila melanogaster is a well-established model organism and due to its characteristics, it became the most viable option to be used in biocompatibility assays. Therefore, in the present study we
performed the initial evaluation of the exposure to copper-doped zinc oxide nanocrystals over the development of *Drosophila melanogaster*. The toxicological effect of the nanocrystals was evaluated by transferring Canton S L1 larvae (35/replicate, 6 replicates) to glass vials containing standard medium added of different concentrations of the nanocrystals (3.125-50 mM). The number of individuals that reached each stage of development was analyzed and the lethality at each stage was determined. Our analysis showed that the exposure to the nanocrystals caused a lower rate of daily pupation, a delay in larval development (4 days) and a high rate of larval lethality. The high rate of larval mortality was reflected in the lower number of animals that reached adulthood. Such results indicates that the copper doped zinc oxide nanocrystals are potentially toxic, since it has a negative impact in larval and pupal stages. Nowadays, we are evaluating the oxidative stress rate in different tissues during normal development which could explain the observed larval lethality and developmental delay. Funding sources: none.

Program Abstract #668
Importins regulating cytoplasmic histone dynamics in Drosophila
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Animal embryos typically store large amounts of maternal histones in the cytoplasm. Histone delivery to nuclei needs to be tightly regulated to support rapid cell cycles while preventing damage from histone overaccumulation. For the histone variant H2Av in Drosophila, this regulation is mediated by sequestering H2Av on lipid droplets (LDs) via the anchoring protein Jabba. From fertilization to the mid-blastula transition (MBT), H2Av constantly exchanges between LDs, supplying a small, steady free pool in the cytoplasm for nuclear import. But during the MBT, H2Av exchange halts abruptly, and nuclear import slows. Our goal is to identify how H2Av dynamics is developmentally controlled. Using proteomics and immunostaining, we find that Importin-α2 (Imp-α2) is dramatically enriched on LDs just as H2Av exchange ceases. Enrichment depends on Jabba, Jabba and Imp-α2 interact by luciferase complementation, and we have mapped a small motif in Jabba necessary for the interaction. Embryos from Imp-α2 mutant mothers do not develop, preventing a test of H2Av dynamics at the MBT. Using Fluorescence Recovery After Photobleaching, we find that in the wild type, H2Av already exchanges between LDs during oogenesis; in Imp-α2 mutant nurse cells, this exchange is abolished. By luciferase complementation, H2Av does not interact with Imp-α2, but with importin-9 (Ipo-9), consistent with mammalian studies where Ipo-9 binds to H2A/H2B dimers. We find that in Ipo-9 mutant nurse cells, H2Av exchange is virtually undetectable and that cytoplasmic H2Av levels are lower. Our working model is that Ipo-9 transports H2Av between LDs and protects it from degradation during its transit through the cytoplasm. Imp-α2, in turn, modulates Jabba’s ability to bind H2Av, and release of H2Av and Imp-α2 from Jabba are coupled. This model would explain why Imp-α2 is required for H2Av exchange and why its LD accumulation leads to static H2Av on LDs. Support: NIH grant R01GM102155.

Program Abstract #669
A region of SLBP distinct from the histone pre-mRNA binding and processing domains is essential for deposition of histone mRNA in the oocyte
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Metazoan histone mRNAs are the only eukaryotic mRNAs that are not polyadenylated. Instead they end in a 3’end Stemloop (SL). Processing of the histones pre-mRNAs is accomplished by an endonucleolytic cleavage after the SL. The stem loop binding protein (SLBP) binds to the SL, and SLBP is a key factor in all steps of the life cycle of histone mRNAs. We are studying the role of SLBP in Drosophila melanogaster in vivo. In Drosophila each histone gene contains a cryptic polyA site after the histone processing site, and when histone pre-mRNA processing is defective histone mRNAs are polyadenylated. Using FLY-CRISPRCas9 we obtained a 11 deletion (SLBP?11) null mutant and a 30nt deletion (SLBP?30) in the N-terminal domain (NTD) of SLBP. The 30nt deletion removed 10aa from the N-terminal domain of SLBP in a region of unknown function distinct from the processing domain. The SLBP?30 mutant was viable, but females were sterile. They laid eggs, but the eggs didn’t hatch, because they didn’t store histone mRNA in the egg. In Drosophila nurse cells produce large amounts of histone mRNA at the end of
oogenesis which is translated and stored in the egg to allow the development of the embryo until zygotic histone gene transcription turns on. The stored histone mRNA is produced in absence of DNA replication. In the ovary histone mRNA expression is normal in the rapidly replicating nurse cells throughout oocyte development but very little histone mRNA is expressed at the end of oogenesis after nurse cell replication is completed. Immunofluorescence data shows that the SLBP?30 protein is mainly localized in the cytoplasm at this stage, suggesting the deleted region is important for nuclear import of SLBP at the end of oogenesis. These results suggest that defective nuclear import of SLBP?30 may lead to a defect in HLBs and histone gene transcription. This work was supported by NIH grants R01GM58921 (W.F.M), R01 GM29832-41S1 (W.F.M. and J. P.-B). and R01GM100088 to G.G.

Program Abstract #670
TGF-beta expression during embryo development of sponge Halisarca dujardini
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The data on the organization and role of signaling cascades in development obtained on sponges (Porifera), the oldest group of multicellular organisms, can shed light to the early steps of body plan evolution (and axes in particular) in Metazoa. We have identified and analyzed in silico molecules-elements of the TGF-beta cascade, their domain structure, phylogenetic position and expression during development have been determined. The Halisarca dujardini (Demospongia) has 8 TGF-beta superfamily ligands, three of which belong to the TGF-beta family itself, while the rest are sponge-specific and not identical to bilateral animals in an evolutionary sense. The expression of these genes using in situ hybridization of nucleic acids is described. Four ligands have been shown to have dynamic patterns in the development of H. dujardini. One of them, HduTGFbE, is expressed throughout embryogenesis in the cells of the embryonic capsule, a unique structure that forms around the embryo from modified choanocytes. The other, HduTGFbD, is expressed diffusely in the cells of the embryo, starting from the mid-blastula and until releasing of the larva. The remaining ligands, HduTGFbA/G, are expressed in somatic tissues (choanocytes and mesochil cells), as well as from the period of late cleavage, in embryonic cells. Moreover, the pattern has a region of increased expression at one of the poles of the embryo. Whether these two regions coincide in the HduTGFbA and HduTGFbG transcripts, and whether this pattern is associated with the axis formed by the Wnt cascade, remains to be studied. Based on the data obtained, we assume that TGF-beta does not participate in the early specification of the axis of the embryo, but is involved in the late development. A comparative analysis of our data will allow us to identify the evolutionary pathways of the specification mechanisms of the body axes of Metazoa. The study was financially supported by the Russian Science Foundation (grant 17-14-01089).

Program Abstract #671
Development of tentacle apparatus in phoronids: from embryo to juvenile
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Ciliated tentacles are known in different groups of protostomes and deuterostomes. Such a distribution of tentacles within bilaterians and their presence in lower metazoans allow to suggest the existence of tentacle apparatus in the last common bilaterian ancestor (LCBA). Although recent palaeontological and molecular data evidence the simple organization of the LCBA, the ideas about complex LCBA that had tentacles are still discussed. In phoronids, early larva firstly acquires the specialized postoral ciliated band, which in advanced larva extends along the laterofrontal sides of tentacles. Tentacles together with the preoral ciliated band are used for collection of food particles from the water. Both the preoral and postoral ciliated bands beat from anterior to posterior. In metamorphosis, the fate of larval tentacles may be different. In some phoronid species, transformation of larval tentacles is expressed in destruction of the postoral ciliated band; in others, larval tentacles are completely consumed by the juvenile and the definitive tentacles form de novo or from small basal the anlages that arise at larval stage. In metamorphosis, nervous, muscular, and coelomic systems of tentacle apparatus undergo great changes. Experiments with the BrDU show that in competent larva and in juvenile, the most active proliferative
zones are located along the postroral ciliated band and at the tentacle basis. The transformation of larval tentacle apparatus in metamorphosis may correlate with the changes of life style from planktonic to benthic. It is likely that the transformation of larval tentacles in metamorphosis reflects some steps of the evolutionary history of phoronids and possibly all bilaterians, whose the last common ancestor initially used the tentacles apparatus to collect food on the substrate, and then this apparatus was transformed into the ciliated bands of bilateral pelagic larvae. This work is supported by Russian Science Foundation (18-14-00082).

Program Abstract #672

Validation of local feeding methods on the growth and fat color of juvenile mangrove crabs (*Scylla* spp)

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Mangrove crabs (*Scylla* spp) are high value commodities that are priced for their meat quality and texture, with the Philippines being second only to China in terms of production. The color of the fat affects the valuation of crabs in the market, with a bright orange color increasing their value by 10-15%. Interviews with fishermen across the island of Luzon indicates a feeding practice involving corn that is believed to cause the desired “yellowing” of the fat tissue. The augmentation of the usual fish meat diet with corn kernels increases the cost of pond maintenance by at least 5% in the hopes of getting better crab valuation. This practice also makes fishermen hesitant to implement algae-based diets that are rich with immunostimulants as these might result to the unwanted “greening” of the fats. To test this local practice and belief, the study compared the effects of standard fish meat diet, an algae-based diet, and a corn-augmented diet in the fattening and fat development of 48 juvenile mangrove crabs for 30 days. Growth rates of the crabs, based on carapace width and weight, were not significantly different (p = 0.03) at an average of 0.04 + 0.04 mm/day and 0.06 + 0.07 g/day. Dissection of the crabs showed a variety of colors within each diet, ranging from green to yellow. It is recommended that compositional analysis of tissues be done to check if nutritional content varied across feeding regimens. The use of lycopene-rich diets could also be investigated to help increase crab value by affecting fat color. This project is part of a national effort to validate local practices in the mangrove crab industry to assess the needs and opportunities to introduce enhanced technologies, and is funded by DOST-PCAARRD.

Program Abstract #673

Divergent skeletal function of sost/Sclerostin

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Sclerosteosis is an autosomal recessive disease that is characterized by an excess of bone growth caused by mutations in the gene SOST. This gene encodes a secreted glycoprotein, Sclerostin, that functions to inhibit bone growth in humans and mice. The specific and potent action of Sclerostin on bone growth is highlighted by the recent approval of Romosozumab, a neutralizing antibody that targets Sclerostin, for osteoporosis therapy. Recently, it has been shown that fish such as zebrafish and medaka express sost in bone. However, unlike in mammals, where SOST is abundantly expressed in osteocytes, sost expression in zebrafish occurs primarily in chordoblasts, osteoblasts, and weakly in osteocytes. Whether zebrafish sost/Sclerostin has a conserved function on bone growth is unknown. Here, we tested the hypothesis that Sclerostin inhibits bone growth in zebrafish. We generated adult zebrafish with loss-of-function mutations in sost. We analyzed 200 measures of vertebral bone morphology and mineralization using microCT-based phenomics. Compared to wildtype clutchmates, homozygous sost mutants exhibited reduced standard length and decreased vertebral bone mass and mineralization. The decreased bone measures in sost mutants were not solely attributable to developmental delay, as indicated by allometric analyses. No differences in bone measures were observed in heterozygous sost mutants. We conclude that sost/Sclerostin positively regulates body size, bone mass, and mineralization in zebrafish. This is in contrast to negative regulation on bone growth in mammals. Thus, our studies reveal unexpected flexibility in sost/Sclerostin in regulating bone growth in different species. This work was supported by the National Institutes of Health and the UW Department of Orthopaedics and Sports Medicine.
Program Abstract #674
The first report on structural and functional conservation of FGF signaling in regenerating annelid as exemplified by the polychaete worm *Alitta virens*
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Epimorphic regeneration is a complex process consisting of multiple stages, the most intriguing of which is blastema formation. Blastema initiation in vertebrates is known to depend on fibroblast growth factors (FGF) signaling. However, for invertebrates, which show outstanding regenerative abilities, there are only a few such works, interestingly none of which are dedicated to spiralian animals. Our research for the first time shows not only a variety of FGF ligands and receptors but provides valuable insight into their spatiotemporal expression and functions in this process in *Alitta virens* (Spiralia, Annelida). We found in *A. virens* two genes encoding FGF ligands and two genes of their receptors. Phylogenetically both ligands are placed into FGF8/17/18 subfamily and receptors’ sequences are associated with such of other Spiralian animals. In situ hybridization on both ligands and receptors demonstrate an early response to the amputation as soon as during the first day of regeneration. For the ligands, tissue specificity varies and the signal decreases as regeneration progresses over time. Noteworthy, their expression dynamics correlates with those found for receptors. The critical requirement of FGF signaling, especially on early stages, is also supported using inhibitors of this pathway followed by proliferation assay which demonstrates that blastemal cells are dependent on those molecules. Our results suggest it is FGF signaling, which enables regenerative response, while the ventral nerve cord and some mesodermal cells around the gut could be the place where the signal for it originates. This mechanism seems similar to vertebrates assuming such a response to the injury could be ancestral for bilaterians. This work was funded by the RSF grant 17-14-01089 and RFBR grant 20-34-70158.

Program Abstract #675
Morphogenesis of the mesodermal layer in *Alitta virens* (Annelida: Nereididae)
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Development of the mesodermal germ layer is a key evolutionary acquisition of bilaterian animals. The mode of morphogenesis by which mesodermal cells are distributed throughout an embryo largely determine the entire body plan. It is generally accepted that in annelids mesoderm morphogenesis has a teloblastic character. However, this trait substantially varies even in closely related species. In the absence of sufficient comparative data, the question on the evolutionary origin and mechanisms of the teloblasts activity remains unclear. Here we analysed 3D reconstructions of the confocal and semithin sections of embryos and juvenile *Alitta virens* worms. Our descriptions provide a valuable reference for comparison with *Platynereis dumerilii*, the intensively studied EvoDevo model. Till the differentiation of the first prototroch cilia, only the largest dorsocaudal cells, mesoteloblasts ML and MR, divide within *Alitta’s* entomesoderm. Consequently, the smaller daughter-cells bud off in different directions in a manner reminiscent of spiral cleavage. Bilateral symmetry and synchrony in the division of the left and right mesoteloblasts are not always visible, while the ML cell adjacent to the 4D macromere slightly lagged behind MR in mitosis progression. After the birth of eight small cells into the dorsal blastopore lip, the sizes of daughter-cells relative to the mesoteloblasts gradually increases. Thus, the 9ML/MR and 9ml/mr sisters are completely indistinguishable in size, but daughter-cells of the 6-8th generations are somewhat larger. At the following larval stages, cells become proliferative from anterior to posterior end of the mesodermal band. A few conspicuously larger cells were found at the caudal region of trochophores and adjacent to the ventral wall of the pygidial blood vessel in juveniles. Whether these cells originate directly from the mesoteloblasts remains to be determined. This work was funded by the RFBR grant 18-04-01335 and RSF grant 17-14-01089.

Program Abstract #676
The mesoderm formation in brachiopod *Coptothyris grayi*
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The data on mesoderm formation are used to resolve the relationships of large taxa and may be useful for clarification of brachiopod phylogeny and evolution. Development of the larval coelomic system are studied in embryos and larvae of rhynchonelliform brachiopod Coptothyris grayi by confocal laser scanning and transmission electron microscopy. In the gastrula, the epithelium of the archenteron forms two pairs of the lateral enterocoelic diverticula. The first pair forms the anterior coelomic pouches, i.e., the anterior mesoderm. The second pair forms the posterior coelomic pouches, i.e., the posterior mesoderm. In development, gastrula transforms into the two-lobed larva consisting of the apical and body lobes. In two-lobed larva, the anterior coelomic pouches are located in the apical lobe, whereas the posterior coelomic pouches are divided into two pairs and extend into the body lobe. After that, the larva becomes three-lobed, i.e., the body lobe is divided into the mantle and the pedicle lobes. Two pairs of setal bundles start to form in the mantle lobe. In three-lobed larvae, all coelomic pouches start to partially fuse. Therefore, the united coelomic anlage that is subdivided into three pairs of interconnecting chambers is formed. In the competent larvae, the posterior mesoderm forms the anlage of the perivisceral coelom that gives rise to branches extending into the mantle anlage. After metamorphosis, the anterior mesoderm forms the lophophoral coelom. Anterior and posterior sources of the mesoderm, which are known in annelids, phoronids, arthropods, and deuterostomes, are described in brachiopod development for the first time. Brachiopods, which is phylum among Lophotrochozoa, are characterized by the enterocoelic formation of mesoderm that is similar to that of the deuterostomes. Besides, the metameric structure of coelom is found in the brachiopod larvae. This work is supported by RFBR (19-04-00449) and RSF (18-14-00082).

Program Abstract #677
The BMP pathway is necessary for enteric progenitor colonization of the gut during zebrafish enteric nervous system development
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The vertebrate enteric nervous system (ENS) consists of a series of interconnected ganglia within the walls of the mature gut and is largely responsible for coordinating peristalsis, water balance, and regulation of hormonal secretions. During development, neural crest cells that contribute to the ENS migrate into the primitive foregut and migrate caudally along its length, during which time they are referred to as enteric neural crest cells (ENCCs). While ENCCs migrate as a group along the gut, they receive various extrinsic signals from the surrounding tissue and neighboring NCCs that promote their proliferation, migration, differentiation, and multipotency. These extrinsic signals include morphogens, such as GDNF, Sonic Hedgehog, and Wnts. Due to the combinatorial nature of the signaling mechanisms involved in the establishment of the ENS, it has been difficult to elucidate the major driving forces of many of the defects involved in aberrant ganglion formation in the gut. Recently we have identified through single-cell RNA sequencing analysis several BMP pathway members expressed in the enteric nervous system progenitor population from D. rerio. In-situ analysis of identified BMP ligands shows expression in the developing gut tube. Finally, we have also demonstrated that attenuation with a potent inhibitor of BMP signaling, K02288 small molecule inhibitor, reduces the number of enteric progenitors in the most distal portion of the gut in a temporally restricted manner. The identification of BMPs as important members of the gene regulatory network signaling pathways serves as a launch point to study BMP signaling during zebrafish ENS development. Funding provided by the Cancer Prevention and Research Institute of Texas.

Program Abstract #678
Runx2 Gene Evolution and Isoform Expression: Effects on transcriptional activity and the regulation of Mmp13 during jaw development
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Developmental control of jaw length is critical for species-specific adaptation and survival. The jaw skeleton is derived from neural crest mesenchyme, a progenitor population that regulates jaw length by exerting precise temporal and spatial control over osteogenic programs and associated transcription factors. One such transcription factor is Runx2, which is required for osteoblast differentiation and bone formation. Runx2 also regulates Matrix metalloproteinase 13 (Mmp13), which facilitates bone resorption. Runx2 contains two alternate
promoters (P1 and P2), tandem repeats of glutamine (Q) and alanine (A) with variable length in different species, a conserved DNA-binding domain, an exon that is alternatively spliced, as well as two possible C-termini, resulting in eight possible isoforms. To determine the extent to which Runx2 structure and isoform expression may underlie jaw evolution in birds, we compare white Pekin duck (Anas platyrhynchos), Japanese quail (Coturnix japonica), and chick (Gallus gallus), which vary in jaw length. We observe species-specific and dynamic regulation of Runx2 isoforms over developmental time when bone resorption is first detected and Mmp13 is induced. We also find that TGFβ1, a key regulator of Runx2, regulates differential expression of Runx2 isoforms with P1 showing no response and P2 isoforms being strongly induced. Overexpressing these isoforms reveals that some are transcriptionally activating, while others are repressive depending on the context of the RUNX2 target sequence especially regarding species-specific differences in the Mmp13 promoter. In addition, we determine that QA repeats affect the transcriptional activity of Runx2, with higher numbers of Q producing greater activity. Overall, we observe that Runx2 structure and isoforms regulate transcriptional activity and target genes in ways that are associated with jaw length. Support: NIH Grants R01DE025668, R01DE016402, and S10 OD021664 to RAS; and F32DE027283 to SSS.

Program Abstract #679
Axial Patterning of the Chondrichthyan Pharyngeal Skeleton and Origin of the Jaw
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The origin of the jaw is a long-standing problem in vertebrate evolution. Here, we used candidate and RNAseq/differential gene expression analyses to revisit a classical hypothesis of serial homology, which proposed that the upper and lower jaw are modified dorsal and ventral elements of the anterior-most gill arch, in a cartilaginous fish, the little skate (Leucoraja erinacea). We find that dorsoventral patterning mechanisms within the developing jaw in bony fishes are largely conserved with the gill arches in skate, including ventralising signalling pathways such as BMP and Endothelin, joint markers Bapx1 and Gdf5, pro-chondrogenic Barx1 and Goosecoid, and dorsalising transcription factor Pou3f3. We observe dorsal mesenchymal expression of Eya1/Six1 uniquely within the mandibular arch, and expression of the Notch signalling components Hey1 and Jag1 uniquely within the gill arches. We have also conducted a comparative transcriptomic analysis of pharyngeal arch progenitors in skate to test for novel markers of jaw identity and dorsoventral patterning mechanisms, candidate in situ validation is currently under way. Our findings reveal conserved patterning mechanisms in the mandibular, hyoid and gill arch in jawed vertebrates, consistent with serial homology, as well as unique transcriptional features that may underpin different jaw and gill arch morphologies. This work is funded by the Doctoral Training Partnership Programme of the Biotechnology and Biological Sciences Research Council (BBSRC DTP).

Program Abstract #680
Development of the pelvic fins in zebrafish
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Actinopterygian pelvic fins are a largely unexplored system that challenge our understanding of limb development. First, their position is remarkably labile along the body axis across different species. Furthermore, while tetrapod and chondrichthyan fore- and hindlimb buds appear near simultaneously, actinopterygian pelvic fins can develop weeks after the pectoral fin buds, such as in zebrafish. Both this positional, ontogenetic, and phylogenetic decoupling between the pectoral and pelvic fins raises the question- to what extent do they share developmental pathways in actinopterygians? The early formation of the zebrafish pectoral fin is reliant on a suite of signaling molecules: Tbx5a activates Fgf24, a convergence cue that instructs precursor fin field cells to condense into a fin bud. Fgf24 then induces an Fgf cascade (Fgf10, Fgf8) necessary for the sustained outgrowth of the fin bud. This Tbx-Fgf cascade for limb development is deployed in both fish and tetrapods, with the striking exception of Fgf24, which has been lost in tetrapods. We characterized this cascade in zebrafish pelvic fins by performing whole mount in situ hybridizations that stained for Tbx4, Fgf24, Fgf10a, Fgf8a, and Pitx1 expression in
stages up through pelvic fin bud formation. This work provides the basis for future studies to explore the specific function of these genes in driving pelvic fin development. T21HD055164; DNUFO.

Program Abstract #681
Turtle embryos exhibit a period of quiescence between two separate waves of trunk NCC migration
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The bones of the plastron, the turtle’s ventral shell, develop through intramembranous ossification, in the same manner as facial bones, suggesting they are also produced by neural crest cells (NCCs). Previous work has demonstrated the existence of a unique second migration of NCCs away from the neural tube of turtle (Trachemys scripta) embryos; these migrate ventrally, appear to be skeletogenic and may contribute to the bones of the plastron. The hypothesis being tested is that these late-emerging cells result from premigratory NCCs that are not depleted at the end of the first wave of migration, but instead remain dormant until the second migration commences. These premigratory NCCs may be unable to migrate from the neural tube during the period between migrations, due to intrinsic changes in their gene regulatory circuits or to the environmental conditions. The goal of this project is to determine whether the neural crest specifiers Sox9, Sox10, Pax3, Snail2, and FoxD3 are expressed in premigratory NCCs during this dormant period using immunofluorescence and whole mount in situ hybridization. Specifiers are transcription factors that control gene regulatory networks important for cell differentiation. Several of these genes have been shown to be involved in the epithelial-to-mesenchymal transition required for the emigration of NCCs, and the lack of expression of one or more of these specifiers could be preventing them from exiting the neural tube. Elucidating the molecular control of migration in this unique population of trunk NCCs would further our understanding of its role in the development of the turtle shell.
Funding sources: National Science Foundation, Millersville University

Program Abstract #682
Understanding the Role of Zinc in Drosophila Fertility
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Egg activation is required for oocytes to initiate embryogenesis. In mammals, a release of cellular zinc from the egg facilitates the completion of egg activation. It is unknown if similar processes occur in Drosophila, and whether zinc is required for normal fertility. In order to analyze the effects of dietary zinc deficiency, we assayed the fertility of flies raised in food containing zinc chelator TPEN. Our data showed that females raised in TPEN displayed impaired fertility while male fertility appeared unaffected. This effect is also dependent on TPEN dosage. Next, we tested whether disrupting the function of foi, the most highly expressed zinc transporter gene in Drosophila ovary, affects fertility. We attempted to knock down and knock out foi in the germline. We performed germline knockdown of foi using the GAL4-UAS system by crossing UAS-RNAi strain with germline GAL4 strains. However, RT-PCR showed an insignificant knockdown. For CRISPR/Cas9 knockout foi in the germline, we built a construct ubiquitously expressing gRNAs targeting foi and integrated it into the fly genome. After we establish the gRNA expressing strain, we will cross it to nos-Cas9 strain to achieve germline specific knockout of foi. I am grateful for support from the Society of Developmental Biology’s Choose Development! Program, which is funded by NSF grants (IOS-1239422; Broadening participation of underrepresented groups in Developmental Biology and REU DBI-1156528).

Program Abstract #683
Single cell mRNA-seq reveals two potential germ cell lineages in early sea urchin development
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Specifying the germline is an important aspect of embryonic development as it enables the continuity of the species. The sea urchin S. purpuratus follows an inherited method of germline formation in which localized maternal signals are inherited by a subset of cells, the putative primordial germ cells (PGCs). It has been shown that these cells promptly behave differently compared to somatic cells, and even the sister cells of this lineage,
the large micromeres. The PGCs become quiescent at blastula stage, which includes repression of the cell cycle, transcription, RNA degradation, translation, and mitochondrial activity. Remarkably, when the precursor of these cells – the micromeres from the 16 cell stage – are removed from the embryo, the animal somehow compensates and restores germ line function, yielding fertile adults. We used single cell RNA seq technology to investigate the specification of these rare PGCs, their maintenance, and regulation of quiescence. Our single cell sequencing analysis of eight sea urchin developmental stages reveals cell types derived from the three germ layers and the germline. Nanos expression, directed by a distinct mechanism than that present in the PGCs, has been shown in the cells adjacent to the PGCs, at gastrula stage. Two populations of Nanos-expressing cells are detected in our analysis allowing for comparison of the germline and this secondary source of Nanos expression. We are using these datasets to parse out 22 cell lineages of the embryo identified by this approach and to focus on the key transitions that the germ line cells may utilize in normal specification and maintenance. Funding: National Institutes of Health 1R01GM132222 9R01GM125071 1P20GM119943

Program Abstract #684
The function of engrailed and invected in sequentially segmenting insects.
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The key to successful development in all metazoans revolves around the establishment and maintenance of lineage compartments. Two homeobox containing transcription factors, engrailed and invected, play an essential role in establishing and maintaining lineage boundaries during different stages of Drosophila melanogaster development. These two genes are presumed to share the same function in other arthropods, due to their strongly conserved expression pattern in the posterior compartment of segments in all arthropods studied to date. However, analysis of engrailed and invected in other insects suggest they are playing a more complicated role in organizing segmental pattern. We report here the use of RNA interference combined with lineage tracing to explore the consequence of loss of engrailed and invected function, both individually and together, in the red flour beetle Tribolium castaneum. Loss of function of both paralogues has not been explored outside of D. melanogaster. Our results support previous reports documenting a role for engrailed or invected in limb development, maintenance of segment boundaries (observed as segment fusions), and an unexpected loss of abdominal segments. Our complete data set uncovers additional severe phenotypes, likely resulting from unstable segment compartment boundaries, and a loss of cell adhesion that has yet to be reported elsewhere. This work was supported by an NSF grant (1817873) to Lisa Nagy and Terri Williams.

Program Abstract #685
Stem cells sense the identity of missing tissues to launch targeted regeneration in planaria
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In order to regenerate tissues successfully, stem cells must first detect injuries and then produce missing cell types through largely unknown mechanisms. Planarian flatworms are an ideal model organism to study how injury induces tissue repair due to their extensive stem cell population that fuels regeneration of any organ. To investigate this question we compared stem cell responses to different injuries by amputating a single organ, the pharynx, or removing tissues from many other organs by decapitation. We find that planarian stem cells adopt distinct behaviors depending on what tissue is missing: loss of non-pharyngeal tissues increases numbers of non-pharynx progenitors, while removal of the pharynx specifically triggers proliferation and expansion of pharynx progenitors. By pharmacologically inhibiting either proliferation or activation of the MAP kinase ERK, we identify a narrow window of time during which proliferation, followed by ERK signaling, produces pharynx progenitors necessary for regeneration. Further, unlike pharynx regeneration, eye regeneration does not depend on proliferation or ERK activation. These results indicate that stem cells tailor their proliferation and expansion to match the regenerative needs of the animal. Our findings imply that damaged organs communicate with stem cells to instruct their behavior, providing a mechanism for flexible cellular output necessary for regeneration. Funding: Startup funds-Cornell College of Veterinary Medicine.
Program Abstract #686

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

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Nematostella vectensis polyps are highly regenerative and can rebuild any missing or damaged parts, and even regenerate their entire body. We have been investigating the role of Wnt expression and functionality during Nematostella regeneration and find that regeneration of oral and polyp structures by an amputated physa fragment is accompanied by expression of all 14 wnts in the Nematostella genome. A few of these genes are activated within several hours of amputation, followed by sequential expression of the remaining wnt gene complement as the body regenerates. In contrast to the physa, wnt genes are not activated at the aboral-facing cut site on the upper polyp piece, indicating that wnt expression is polarized to the oral-facing wound end. Wounding caused by amputation triggers a rapid MAPK (Mek-Erk) signaling response at the wound sites of the physa and upper polyp pieces, and this activity is required for wnt gene activation in the physa fragment. Inhibition of MAPK or Wnt signaling inhibits head regeneration by the physa. Following wnt activation, a burst of cell proliferation occurs at the future oral end of the physa, and this requires Wnt signaling. Regeneration of aboral structures is not accompanied by elevated cell proliferation and is insensitive to Wnt inhibition. We conclude that Wnt signaling is required for regeneration of Nematostella head structures, and moreover that hydrozoan and anthozoan cnidarians share an evolutionarily conserved, Wnt-dependent mechanism for oral regeneration. The underlying basis for orally-polarized wnt activation during cnidarian polyp head regeneration remains to be deciphered. Funded by NYSTEM and a Departmental supplement.

Program Abstract #687

Macrophages directly contribute collagen to scar formation during heart regeneration

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Canonical roles for macrophages in mediating the fibrotic response after a heart attack (myocardial infarction) include turnover of the extracellular matrix and activation of cardiac fibroblasts to initiate collagen deposition. Here we reveal that macrophages can directly contribute collagen to the forming scar through studying the functional kinetics of fibrosis during zebrafish heart regeneration. Unbiased transcriptomics revealed an upregulation of collagen isoforms in zebrafish macrophages following injury. Adoptive transfer of macrophages from collagen-tagged transgenic zebrafish donors enhanced scar formation and induced fibrosis in the heart, via cell autonomous production of collagen. The majority of tagged collagen was deposited proximal to the injury, within the overlying epicardial region, suggesting a possible distinction between macrophage-collagen deposition and that predominantly laid-down by activated myofibroblasts. Macrophage-specific targeting of collagen 4a binding protein and cognate collagen 4a1 followed by adoptive transfer led to significantly reduced pericardiac scarring in cryoinjured hosts. These findings contrast with the current model of scarring whereby collagen deposition is exclusively attributed to myofibroblasts, and implicate macrophages as direct contributors to fibrosis during heart repair. This work was funded by the British Heart Foundation.

Program Abstract #688

Mechanisms for Brain Repair after Hypoxic Injury

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Hypoxia is one of the leading causes of brain injuries, including learning deficiencies, loss of motor coordination, memory deficit, and visual impairment. Many of these symptoms improve over time, suggesting the presence of mechanisms in the human brain to recover from hypoxic injuries; however, the mechanisms involved in hypoxic injury and recovery are not well understood. To study hypoxic brain injury, we have developed adult zebrafish as a model organism. The zebrafish offers several advantages for use in this research. These include tolerance to low oxygen level, the ability to visualize and manipulate specific neuronal populations in the brain, and the potential to identify the regenerative mechanisms after hypoxic injury. Our preliminary studies showed that adult zebrafish
exhibited abnormal swimming behavior after hypoxic treatments in an air-proof water chamber. Histological analyses revealed a correlation between the extent of hypoxic treatment and damage. The data suggest that zebrafish can be used to understand the biological processes associated with hypoxia-induced brain injury and repair. It has been reported that inhibition of alpha-tubulin acetyltransferase 1 (ATAT1) expression causes neurological defects in zebrafish. Our preliminary data showed that fish lacking acetylated tubulin (atat1-/-) are more sensitive to hypoxic insult suggesting a protective role for modified microtubules in response to the loss of oxygen. Current experiments are focused on determining molecular and cellular changes associated with the hypoxic insult to understand how the brain recovers from a hypoxic injury. Fund provided by Department of Cellular Biology and University of Georgia.

Program Abstract #689
The change in the thickness of regenerating rat sciatic nerve fibers after administration of mesenchymal stem cells

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Currently, mesenchymal stem cells (MSCs) derived from bone marrow, adipose tissue and other sources are often used in experimental studies on the stimulation of damaged nerve regeneration. The aim of this work was to study the effect of single transplantation of MSCs on the regenerating fibers of a rat damaged sciatic nerve. In the present work, the sciatic rat nerve was damaged by ligature, and Wistar-Kyoto rat bone marrow MSCs were transplanted (50000 cells in 5 μl of medium) into nerve. The animals of the control group, which had a ligature, 5 μl of culture medium was injected subperineurally. Bromodeoxyuridine-labeled MSCs were identified in the recipient's nerve for a week and were found not only in the endonervium, but also in the epi- and perineurium. Apparently, a part of the transplanted MSCs migrated to the outer shells of the peripheral nerve after breaking the barrier by ligation. A study of the regenerating nerve fibers in the distal segment of crushed nerves was performed using immunohistochemical detection of peripherin. Peripherin is a type III intermediate filament protein of molecular weight 57 kD, which is involved in the process of nerve fibers elongation in damaged nerve. Detection of this protein is often used to study the structures of the peripheral nervous system. Two months after the operation, peripherin-immunopositive nerve fibers were counted and measured on transverse sections of the distal segment of the recipient's nerve. Morphometric analysis of regenerating fibers performed using ImageJ software (NIH, USA) showed that the average thickness of nerve fibers in animals of the experimental group was increased. A study of the nerve fibers thickness distributions of the damaged nerve distal segment showed that in animals treated with MSCs, the percentage of larger diameter fibers is higher than in animals of the control group. This study was implemented within the frames of the state assignment.

Program Abstract #690
Death and regeneration of sensory hair cells of the developing inner ear.

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Death of mechanosensory hair cells of the inner ear results in hearing loss and balance disorders. Unlike mammals that lack the capacity to regenerate hair cells, teleost fishes and frogs are capable of regenerating sensory inner ear hair cells that have been lost following acoustic or ototoxic trauma. We have visualized regeneration of inner ear hair cells as they regenerate in zebrafish. Unlike methods where all cells of the inner ear epithelium are exposed to a drug or a deafening sound, we are able to specifically target individual cells or parts of cells and leave surrounding cells undamaged using a two-photon laser. Ablation of hair cells in these embryos has allowed us to trace the fate of nearby cells, supporting cells, which differentiate into hair cells by either direct transdifferentiation or asymmetric cell division. Our ultimate goal is to determine which signals play a role in regenerating sensory hair cells. To this end, we use embryological manipulations in Xenopus to test the regenerative potential along specific axes and the timing of axes determination in the otic placode as it gives rise to the inner ear. We explore the role of Wnt signaling with gain and loss of function experiments.
Program Abstract #691
The function of Okapi, a novel FERM domain protein, in regulating the number of follicle stem cells in Drosophila oogenesis
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FERM domain proteins modulate cell adhesion and cell signalling pathways. Here, we show that a novel FERM domain protein, which we named Okapi (Oka) has an important function in regulating follicle stem cells (FSCs) in Drosophila oogenesis. FSCs give rise to several somatic cell lineages that are important for egg development, including follicle cells that envelope germline cysts and interfollicular stalk (IFS) cells that connect follicles. We found that loss of oka resulted in a strong increase in the number of FSCs and IFS cells, and additional defects in oogenesis. As a similar phenotype had been described for hippo (hpo) mutants, we asked whether Oka might be a positive regulator of Hpo signalling, which is known to limit the nuclear translocation and activity of the transcriptional co-activator Yorkie (Yki). We observed that in oka mutants nuclear Yki was present in a greater portion of FSCs than in wild-type ovaries. Further, we found that a reduction of Yki partially and completely rescued the number of FSCs and IFS cells, respectively, in oka mutant ovaries. As hpo mutant FSCs had been observed to displace wild-type FSCs in mosaic ovaries, we tested the behaviour of oka mutant FSC clones. Interestingly, in the presence of wild-type FSCs, oka mutant FSCs were outcompeted, lost over time, and seemed to undergo differentiation. Together, our results indicate that Oka regulates the number of FSCs and IFS cells, potentially by regulating Hpo signaling, and is required to make FSCs competitive through a yet unknown mechanism. This work has been supported by the Department of Cell and Systems Biology at the University of Toronto.

Program Abstract #692
The tumor suppressors merlin and expanded integrate signaling inputs to regulate stem cell division in the Drosophila testis stem cell niche
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The ability of adult organisms to maintain tissue homeostasis is mediated by small groups of adult stem cells that support the tissues they reside in. Adult stem cells are regulated by signals from their microenvironment, the stem cell niche. One of the best-characterized model niches is that of the Drosophila testis, which contains two stem cell populations that cluster around a group of nondividing somatic cells called the hub. The germline stem cells (GSCs) produce cells that differentiate into sperm, while the somatic cyst stem cells (CySCs) produce cyst cells, which guide germline cell differentiation. Recently, contact inhibition, mediated by the tumor suppressor and human disease gene Merlin/NF2, was proposed as a mechanism that regulates proliferation of CySCs and their coordination with GSCs. In other Drosophila tissues, Merlin acts redundantly with the related tumor suppressor protein, Expanded. We investigated the role of these genes, and found that inhibition of either merlin, expanded, or both genes together in the cyst lineage resulted in an increase in the number of CySCs, a corresponding decrease in the number of GSCs (due to CySCs outcompeting them in the niche), and inappropriate division of cyst lineage cells outside the niche. More severe phenotypes were observed in the double mutant, suggesting partial redundancy of these genes in this tissue. Surprisingly, expression of a phosphomimetic constitutively active allele of merlin showed a similar phenotype to the loss-of-function testes. We found that the merlin loss-of-function phenotype could be attributed to increased dpERK accumulation, whereas the gain-of-function phenotype could be attributed to a reduction in signaling through the differentiation-promoting Tor pathway. Our findings support a model in which the function of merlin and expanded is to balance and integrate multiple signaling inputs within the cyst stem cells. This work was supported by NIH R15 GM102828 to J.L.

Program Abstract #693
Feeding-dependent changes in gene expression during the quiescence to proliferation transition of Follicle Stem Cells in the Drosophila ovary
Daniel Zinshteyn1, Eric Lee1, Zhen Fu2, Alana O'Reilly1
1FCCC Program in Molecular Therapeutics, United States; 2 Department of Entomology, Texas A&M, United States
Feeding-dependent changes in gene expression during the quiescence to proliferation transition of Follicle Stem Cells in the Drosophila ovary
Daniel Zinshteyn, Eric Lee, Zhen Fu, Alana O'Reilly
1FCCC Program in Molecular Therapeutics, United States; 2 Department of Entomology, Texas A&M, United States
Stem cells cycle between periods of quiescence and proliferation, a process that is thought to promote healthy tissue aging. Proliferative stem cells compete more effectively for niche occupancy in some tissues, with drift toward clonality suppressed by synchronization of stem cell pools via periods of quiescence. The quiescence to proliferation transition is controlled, in part, by diet, such that periods of starvation or nutrient restriction promote quiescence, with rapid transition to proliferation upon feeding. *Drosophila* ovarian Follicle Stem Cells (FSCs), an epithelial stem cell population, arrest division after entering a nutrient-restricted state and initiate proliferation rapidly after feeding. Some of the molecular mechanisms that regulate FSCs are known, but much of the biology behind FSC pliability is still a mystery. Here, we utilized TU-tagging, a cell-specific method for biosynthetically labeling RNA transcripts, to map the gene expression changes that occur during the first 24 hours of the FSC quiescence to proliferation transition. By identifying transcripts enriched in targeted cells, we are able to describe the adult FSC transcriptome. We find that these projection-forming cells are enriched for transcripts typically associated with neuronal function. We also identified 281 FSC-enriched genes whose expression is feeding-dependent, many of which are also associated with neuronal projections and response to bacterial infection. Our findings shed light on the complex interaction of diet and life history in a single dynamic cell population. This research was supported by the NIH [HD065800 (AOR), CA06927 (FCCC)].

Program Abstract #694
Neurodevelopmental disorder risk gene DYRK1A is required for ciliogenesis and brain size in *Xenopus* embryos
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DYRK1A (dual specificity tyrosine-(Y)-phosphorylation-regulated kinase 1 A) is a high confidence autism risk gene that encodes a conserved kinase. In addition to autism, patients with putative loss of function variants in DYRK1A exhibit microcephaly, intellectual disability, developmental delay, and congenital anomalies of the kidney and urinary tract. DYRK1A is also located within the critical region for Down syndrome; therefore, understanding the role of DYRK1A in brain development is crucial for understanding the pathobiology of multiple developmental disorders. To characterize the function of this gene, we used the diploid frog, *Xenopus tropicalis*. We discover that Dyrk1a is expressed in ciliated tissues, localizes to ciliary axonemes and basal bodies, and is required for ciliogenesis. We also demonstrate that Dyrk1a localizes to mitotic spindles and that its inhibition leads to decreased forebrain size, abnormal cell cycle progression, and cell death during brain development. These findings provide hypotheses about potential mechanisms of pathobiology and underscore the utility of *X. tropicalis* as a model system for understanding neurodevelopmental disorders. Funding: NIMH 1R21MH112158-01 and 1U01MH115747-01A1; NIGMS R35GM127069.

Program Abstract #695
Associations of the ANKK1/DRD2 gene polymorphism with morphofunctional and psychological characteristics in Russian and Transnistrian females
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The DRD2 gene is one of the most popular neurotransmitter system genes among researchers, which is often considered in connection with the human mental health. Recently, the polymorphism of this gene has also been studied in association with physique. The purpose of this study was to investigate the morphological features of the physique and psychological characteristics of females in Russia and Transnistria in connection with rs1800497 polymorphism of the ANKK1/DRD2 gene. A total of 307 female volunteers aged 16 to 29 years were examined and genotyped. Over 20 different morphological and functional indicators were measured including bioimpedance body composition analysis, while the level of stress tolerance was analyzed using the Fetiskin test for perceptual stress tolerance followed by performing a multi-stage statistical analysis of anthropogenetic data with the
software Statistica 10.0. Participants were informed about the aims of this study and provided informed consent. The research of the alleles' distribution in the examined youth sample showed the predominance of the C allele over the T allele (0.774 and 0.226, respectively) which is consistent with data from other researchers obtained from samples of Russian residents. Significant differences (p<0.05) were revealed for obesity-related morphological traits: females in the subgroup ?? genotype carriers have increased body fat mass, higher Body mass index values with reduced metabolic rate and low phase angle values (indirect indicator of the level of physical activity). Homozygotes CC also demonstrated a higher heart rate and reduced stress resistance according to the Fetiskin test. The revealed statistically significant associations of the ANKK1/DRD2 polymorphism with morphofunctional indicators can be used in applied aspects, for example, in predictive personified medicine. Funding: the reported study was funded by RFBR according to the research project ? 18-09-00290.

Program Abstract #696
Behavioral analysis of zebrafish with mutation of the HFC1A gene
David Paz
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Methylmalonic acidemia homocysteinemia, cblX type (cblX) is a multiple congenital anomaly disorder characterized by metabolic deficits, defects in brain development, craniofacial abnormalities, and intractable epilepsy. Between 15-25 percent of multiple congenital anomalies are associated with genetic mutations and mutations in the HCF1 gene cause cblX syndrome. The HCFC1 gene encodes a transcriptional cofactor that regulates the expression of 5000 different downstream target genes. Previous studies suggest that mutation or decreased Hcfc1 expression disrupts neural precursor proliferation and survival; however, the functional consequences of these cellular deficits are unknown. Mutation of Hcfc1 in mice is embryonically lethal and mice undergo in utero gestation, limiting analysis of developmental brain function via traditional behavioral assays. Recent studies have shown that zebrafish larvae are an emerging system to study developmental behavioral phenotypes. In this study, we present high throughput behavioral analysis of the Co64 allele. The Co64 allele, is missense mutation produced via CRISPR/Cas9 methodology and is homozygous viable. Behavioral analysis of homozygous carriers of the Co64 allele revealed a hypomotilic phenotype relative to wildtype siblings upon light stimulus. Interestingly, these behavioral deficits are associated with increased neural precursors as indicated by flow cytometry. These data represent a novel hcfc1a homozygous viable allele with defects in brain development and locomotion. Financial support for this project was provided by NINDS award 1K01NS099153-01A1.

Program Abstract #697
Low folate concentration exacerbates mismatch repair deficiency in neural tube defects
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Genomic variation can influence an individual’s risk of disease. Neural tube defects (NTDs) are a complex polygenic disorder and resulted from the failure of neural tube closure (NTC), yet its genetic feature remains largely unknown. We aim to characterize sequence variants in NTD cases in specific genes that control NTC. We sequenced 280 NTC genes in 355 unrelated NTDs individuals and 225 ethnicity-matched controls. A significant increase in rare variants was identified in NTD cases. After annotated rare variants in eleven histone modifications occupied regions, we found a greater number of potentially deleterious rare variants in occupancy regions of H3K36me3, which can recruit mismatch repair (MMR) machinery. The folate level of brain tissues was then assessed in the subgroups of subjects enrolling 110 NTD cases and 120 controls, lower folate concentrations were identified in 69% of cases versus 33% of controls. In vitro assays in mouse E9 neuroectoderm cells showed that folic acid insufficiency decreased the level of MMR protein Msh6, attenuated the association of Msh6 to H3K36me3, and altered binding of H3K36me3 and Msh6 to a set of genes required for NTC. Comparison to MMR deficiency or sufficiency featured genome sequencing database, rare variants in NTD cases showed a pattern with a signature of MMR deficiency mutations, moreover, microsatellite instability, another hallmark of MMR deficiency, was significantly severer in 105 NTD cases compared to 69 matched controls. Our work suggests a mechanistic link between folate insufficiency and MMR deficiency corresponding to increase rare variants in genes required for NTC. The work was supported by the National Natural Science Foundation of China (No.
Program Abstract #698
Analyzing Pelvic Ganglia and Bladder Neural Deficits in Mouse Models of Spina Bifida
Anoop Chandrashekar, Karen Deal, E. Michelle Southard-Smith
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Neural crest (NC) progenitors form the pelvic and accessory ganglia that innervate the lower urinary tract (LUT) and are essential for normal bladder contractility and urinary continence. Pediatric patients suffering from spina bifida (SB) often exhibit bladder dysfunction accompanied by reduced bladder innervation and smooth muscle deficits. Pax3, a gene mutated in SB, is expressed in both NC progenitors and somites that form smooth muscle. We utilized the *Pax3* Splotch-delayed (*Pax3Sp-d*) mouse model of SB and quantified neuronal differentiation in the pelvic ganglia (PG) and bladder innervation density of *Pax3Sp-d/Sp-d* mutants. Additionally, we implemented NC-specific deletion of *Pax3* in the context of the *Pax3Sp-d* allele to assess whether Pax3 function in NC progenitors is required for normal bladder innervation. Fetal *Pax3Sp-d/Sp-d* mutants displayed deficient PG neurogenesis, absence of PG neuron subtypes, and reduced density of bladder wall innervation. Mice with NC-specific ablation of *Pax3* survived postnatally and exhibited reduced bladder wall innervation and interganglionic connectivity. Our results indicate that *Pax3* function within NC derivatives specifically is required for normal bladder innervation and neuron subtype differentiation in pelvic autonomic ganglia. Funding: NIH, R01-DK078158

Program Abstract #699
In vivo analysis of REEP5 as a regulator of cardiac function in zebrafish
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Dysfunction of the sarcoplasmic reticulum (SR) plays an important role in the development and progression of many heart diseases. However, how its structure is organized and maintained remains largely unknown, particularly in cells, like cardiomyocytes, with a highly differentiated SR network. In this study, we show that REEP5, a cardiac enriched and conserved membrane protein, plays an important role in regulating SR organization and the cellular stress response of cardiac myocytes in heart failure. REEP5 levels increase in human idiopathic heart disease and mouse hypertrophic cardiomyopathy. Depletion of REEP5 in isolated mouse cardiac myocytes causes SR membrane destabilization and vacuolization, resulting in disrupted Ca2+ cycling and decreased myocyte contractility. Using zebrafish, we show that *in vivo* depletion of Reep5, by antisense morpholino and CRISPR-mediated loss-of-function, disrupts SR morphology, causing vacuolization, reduced contractility, defective cardiac looping, general cardiac dysfunction, and eventual death. We also found that *reep5*-/- fish have sensitized cardiac dysfunction when treated with verapamil, a Ca2+-channel blocker, though they did not phenocopy the morphant and F0 crispant fish. In these mutants, loss of Reep5 led to increased expression of Rtn4 and decreased expression of Atl3 and Ckap4, which are proteins involved in SR membrane shaping, indicating a possible adaptation mechanism. Together, our data suggest a key and conserved role for REEP5 in SR organization and heart function. Our work also provides a framework for investigating a pathway whose dysregulation may lead to heart failure. Future studies aim to investigate how Reep5 and other pathway members interact to coordinate SR structure and function, their role in heart failure, and their potential as therapeutic targets. Funding: Ted Rogers Centre for Heart Research Innovation Fund and the Heart and Stroke Foundation.

Program Abstract #700
Postembryonic screen for mutations affecting spine development in zebrafish
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One of the most common disorders of the spine in human is adolescent idiopathic scoliosis (AIS), which is characterized by the onset of an abnormal lateral curvature of the spine of <10° around adolescence, in otherwise healthy children. The genetic basis of AIS is largely unknown and the mechanisms required for postembryonic maturation and homeostasis of the spine remain poorly understood. Here we report the results from a small-scale forward genetic screen for adult-viable recessive and dominant mutant zebrafish, displaying overt morphological abnormalities of the adult spine. Germline mutations induced with N-ethyl-N-nitrosourea (ENU) were screened for dominant phenotypes in 1,229 F1 animals, and bred to homozygosity in F3 families, from these, 314 haploid genomes were screened for recessive phenotypes. We found 39 adult-viable (3 dominant and 36 recessive) mutations each leading to a defect in the morphogenesis of the spine with at least 16 independent mutant loci. The largest phenotypic group displayed larval onset axial curvatures, leading to whole-body scoliosis without vertebral dysplasia in adult fish. Using sequencing and meiotic mapping we revealed the molecular identity of several genes. We identified new mutations in the skolios/kinesin family member 6 (kif6) gene, causing neurodevelopmental and ependymal cilia defects in mouse and zebrafish. We also report several recessive alleles of the scospondin and a disintegrin and metalloproteinase with thrombospondin motifs 9 (adamts9) genes, as regulators of spine morphogenesis. Our results provide evidence of monogenic traits that are critical for normal spine development in zebrafish, that may help to establish new candidate risk loci for spine disorders in humans.  

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Program Abstract #701

Investigating the Role of the Multimerin-2 gene in the Wound-Healing Processes of Xenopus laevis Embryogenesis

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Xenopus laevis embryos have a profound ability to recover from severe physical perturbations at early stages of development. To analyze the molecular basis of plasticity we performed an RNA-Seq experiment following rotations of mid-gastrula stage presumptive neural tissue. One of the genes that was differentially expressed and upregulated in the screen was the Multimerin-2 (MMRN2) gene. We hypothesized that this gene may play a role in plasticity and wound healing; the objective of this project is to better understand the role of this gene in both normal Xenopus laevis embryogenesis and in wound healing. To do so we employ both expression analysis and functional assays. Normal MMRN2 expression in embryos is observed through chromogenic in situ hybridization (ISH), utilizing constructed DIG-labeled RNA probes. Embryos of specifically chosen stages are then physically perturbed, and ISH is conducted at various time points to observe the effect of wounding on gene expression. The project has thus far completed in situ hybridization to determine normal MMRN2 expression. Gene expression appears subtly in late stage embryos, and is restricted to the embryo’s head region, appearing strongest around the ear and eye vesicles, with lower levels of expression in the anterior notochord. No significant expression was observed in embryos prior to late tailbud/early hatching stages. Because of its low level of expression, additional approaches to assay gene expression in situ will be conducted. This research will contribute in the efforts to better understanding wound healing mechanisms in Xenopus laevis and in humans. Given the enormous clinical and personal burden of chronic wounds, more research based on novel genes, like MMRN2, is essential to creating new types of treatments for afflicted patients. This project is funded by NSF Grant 1257895 and NIH Grants 1R15NS067566-01, 1R15HD077624-01, and 1R15HD096415-01 provided to Dr. Margaret Saha.

Program Abstract #702

Hydrocephalus likely caused by defects in ADAMTS20-mediated assembly of Sco-spondin in mouse B3glct mutants

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Classical Peters plus syndrome (MIM #261540 PTRPLS) phenotypes include defects in eye development,
prominent forehead, hypertelorism, short stature and brachydactyly, with isolated cases of fetal hydrocephalus. PTRPLS is caused by recessive mutations in the B3-glucosyltransferase (B3GLCT) gene. B3GLCT adds glucose to O-linked fucose on properly folded thrombospondin type 1 repeats (TSRs) forming a glucose-fucose disaccharide. The disaccharide is important for stabilization of the TSR fold. In mice, loss of B3GLCT causes similar craniofacial and skeletal abnormalities with white-spotting and hydrocephalus occurring at high frequency. Reduced function of the secreted metalloprotease ADAMTS20, with 12 predicted B3GLCT-modified TSRs, was proposed to contribute significantly to white spotting and hydrocephalus in mouse B3glct mutants. In this study, we demonstrated that cerebral spinal fluid (CSF) flow was reduced in B3glct mutants. We provide evidence that defects in CSF flow likely stemmed from altered Sco-spondin (SSPO) processing and assembly into Reissner’s fiber in the central aqueduct, rather than a defect in polarization of the lateral ventricle ependymal cells. SSPO has 16 TSRs predicted to be modified by B3GLCT. Moreover, we demonstrated that ADAMTS20, like SSPO, was expressed in the subcommissural organ. Secretion and assembly of SSPO was similarly altered in Adamts20 belted mutants, suggesting that ADAMTS20 was required for assembly of Reissner’s fiber. Taken together, these studies provide evidence that hydrocephalus in B3glct mutant likely stems from defects in secretion/function of ADAMTS20 and SSPO causing abnormal assembly of Reissner’s fiber and impaired CSF flow in the central aqueduct. Supported by NIH (R01HD090156 and R01HD096030 to R.S.H. and B.C.H.) and 2019 Hydrocephalus Association Innovator award (BCH).
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