ABSTRACTS

Program Abstract #1

Dynamics and Shaping of a BMP Morphogen Gradient

Joe Zinski[2], Francesca Tuazon[2], Wei Duo[1], Yan Huang[1], David Umulis[1], Mary Mullins[2]

[1]Department of Agricultural & Biological Engineering, Purdue University, USA; [2]Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

The vertebrate embryonic dorsoventral (DV) axis is patterned by a bone morphogenetic protein (BMP) activity gradient during blastula and gastrula stages. This BMP morphogen gradient is shaped by BMP antagonists emanating from dorsal regions that block signaling dorsally and lead to a gradient of signaling with highest levels ventrally. Quantitation of this gradient, defining its range and the dynamics of its formation, as well as its modulation during gastrulation has not been investigated. We quantified in every cell of the embryo in a temporal developmental series the nuclear intensities of phosphorylated-Smad5 (P-Smad) protein, the BMP signal transducing protein. We use automated algorithms to identify the thousands of individual nuclei present at each embryonic time point, and to measure their corresponding P-Smad intensities. The quantitative dynamics of the gradient in blastula and gastrula stages will be presented, cell-to-cell variability of BMP signaling levels, as well as the distinct roles of BMP antagonists and their modulators in shaping this gradient.

Program Abstract #2

DDR regulates collective cell migration and antagonizes VEGFR to maintain TVC leader/trailer polarity during *Ciona* development.

Yelena Bernadskaya, Stephanie Gline, Lionel Christiaen

New York University, USA

Collective cell migration is required for diverse processes such as gastrulation, angiogenesis and neural crest migration. We use the migration of bilateral pairs of cardiopharyngeal precursors of the chordate *Ciona intestinalis* as the simplest possible model to study polarized collective cell movement. The cardiopharyngeal precursors (trunk ventral cells, TVCs) arise from the asymmetric divisions of the B7.5 blastomeres and migrate with stereotyped leader/trailer polarity from the embryo tail to the ventral trunk. We developed quantitative methods to assay TVC movement, morphology, and tissue interactions in 4D data sets and use these techniques to generate a detailed profile of TVC migration. Using these methods we identify two Receptor Tyrosine Kinases (RTKs), DDR and VEGFR, as regulators of TVC migration and polarity maintenance. Expression of dominant negative (dn) VEGFR or DDR in the B7.5 lineage causes the TVCs to deviate from normal migration parameters, alters their morphology, and disrupts their contact with surrounding tissues. Specifically, expression of dnDDR disrupts TVC leader/trailer polarity and causes the cells to move in a tumbling motion while reducing the TVC contact with the epidermis and increasing interactions with the endoderm. The dnDDR2 TVC phenotype depends on the tissue integrity of the endoderm and epidermis, suggesting integration of external mechanical attributes of surrounding tissues and RTK signaling during the maintenance of TVC leader/trailer polarity and migration. dnDDR and dnVEGFR produce opposite effects on TVC phenotypes and coexpression of both can restore the wild type TVC morphology, suggesting antagonistic relationships between RTKs maintain leader/trailer polarity. We therefore propose a model in which DDR/VEGFR antagonism maintains TVC leader/trailer states and integrates biomechanical cues to confer directionality to TVC migration. This work is funded by NIH F32 GM108369-02 to Y.B. and NIH/NIGMS R01GM096032 to L.C.

Program Abstract #3

Multicolor clonal labeling reveals biased apoptosis in fetal male germ cell development

Daniel Nguyen, Diana Laird

University of California, San Francisco, USA

Embryonic development is a critical period when tissue compartments are established by the expansion and maturation of precursor cells. In the germline, primordial germ cells proliferate, migrate, and colonize the gonad whereupon they continue differentiating into germ cells (GCs) and then gamete-producing cells. Interestingly, many GCs are eliminated in mammals by scheduled waves of apoptosis during the fetal period. The basis for this apoptosis and its effects on the composition of the germline are poorly understood. We investigated the spatial distribution of GC apoptosis in the mouse testis during the fetal wave (e12.5-e15.5) using wholemount imaging and showed mathematically that it occurs nonrandomly in highly localized clusters. We found that this clustering was environment-independent and did not require cytoplasmic sharing in male GC cysts. To determine if apoptotic clusters resulted from cell-intrinsic defects shared among

clonally related cells, we undertook random multicolor labeling with a GC-specific drug-inducible Cre and the *Rainbow* and *Confetti* reporter alleles. After inducing labeling at the conclusion of GC migration (e10.5), we observed at e13.5 that apoptotic GC clusters frequently shared the same color and were derived from the same clonal ancestor. We did not observe propagation of apoptosis between clones in clusters at clonal borders. We further investigate the functional role of clonal elimination in apoptosis-deficient *Bax*-mice to identify aberrant GC development when apoptotic clones are retained. Multicolor labeling also facilitates a comparative perspective on clonal development and how clonal differences alter GC composition; variance in clone size suggests that clonal development is individualistic and uncoordinated across the GC compartment. Our results suggest that GC clones in the fetal testis have different developmental fates and that apoptosis may act as a quality control mechanism in targeting specific clones for elimination.

Program Abstract #4

Labors of the Embryo: Balancing Work and Dissipation during Frog Morphogenesis.

Lance Davidson, Deepthi Vijayraghavan, Joseph Shawky
University of Pittsburgh, USA

Recent studies of morphogenesis have revealed the importance of mechanics in guiding morphogenetic movements. These efforts have identified how patterned forces are generated and highlight the role of mechanical constraints. For instance, mediolateral cell intercalation, transient rosette formation and resolution, and apical constriction all produce local tensional forces that are converted into extensional movements (e.g. during convergent extension) or into out of plane tissue folding (e.g. during ventral furrow formation or neurulation). Morphogenetic movements driven by these complex patterns of force are constrained by an equally dynamic mechanical microenvironment in the embryo. For instance, studies by our group and others have found order-of-magnitude changes in tissue stiffness and viscosity over time coincident with early morphogenetic movements. As we turned to understand how force production and mechanical constraints are integrated we have begun to reinvestigate the physical principles of work and the energetics of morphogenetic movements. By measuring the strain energy stored and dissipated during dorsal convergence and extension we can understand how long embryos "remember" mechanical events and how soon events are forgotten. Limits on the storage and recovery of mechanical energy have important consequences on the range of force transmission and restricts the role of mechanical signaling during embryogenesis.

Program Abstract #5

How, when and where in pattern formation: Spying on embryonic development one molecule at a time

Hernan Garcia
UC Berkeley, USA

An abiding mystery in biology is how a single cell develops into a multicellular organism. Despite great advances in identifying the molecular players of developmental programs, the quantitative prediction of gene expression patterns from knowledge of DNA regulatory sequence has proven elusive. Technological limitations have kept us in the dark about the dynamics of these regulatory decisions, a necessary first step towards the predictive understanding of developmental response. In this talk I present new technologies and theoretical methods to access and predict developmental decisions in living fruit fly embryos at the single nucleus level. Using this approach we can measure where, when and how fast nuclei express a gene in response to an input morphogen and bridge these dynamics to the resulting macroscopic domains of gene expression that arise throughout the embryo and that lead to the specification of future body parts. In contradiction with the standard picture of gene regulation, we discovered that transcription factors can regulate gene activity in two decoupled ways. First, they determine a random subset of nuclei that is able to participate in the regulatory game. For those genes that are randomly turned on, transcription factors also determine the rate at which the gene product is produced. Both of these modes of regulation are necessary in order to create sharp boundaries in the embryo. This work provides a framework to predictively understand and control developmental response by identifying the different regulatory strategies employed by the fly in the generation of patterns of gene expression.

Program Abstract #6

Decoding Embryonic Developmental Pathways Using 4D-High Content Imaging of *C. elegans* embryos

Karen Oegema[1], Stacy Ochoa[1], Renat Khaliullin[1], Lior Galanti[2], Zhiling Zhao[1], Shaohe Wang[1], Ronald Biggs[1], Adina Gerson-Gurwitz[1], Arshad Desai[1], Kristin Gunsalus[2], Rebecca Green[1]
[1]Ludwig Institute for Cancer Research, USA; [2]New York University, USA

An important challenge is to systematically define the genetic pathways that function during embryogenesis to coordinate the complex events driving morphogenesis and formation of multi-cellular tissues. To this end, we developed a 4D-high-

content imaging based approach to functionally classify the ~2600 genes essential for embryonic development in the model metazoan, *C. elegans*. In this screen, we imaged embryogenesis (~10hrs) in two engineered marker strains that report on: (1) the specification and positioning of cells in the three germ layers and (2) epidermal and neuronal morphogenesis. Imaging was performed on a high throughput spinning disk confocal imaging system that enables collection of high-resolution developmental data for 80-100 embryos in a single experiment. Using 500 genes as a test set, we imaged embryos after RNAi of individual targets and manually scored the timelapse datasets for embryonic developmental defects; this effort recovered expected phenotypes for previously identified developmental genes, validating our experimental approach, and also revealed as yet unreported phenotypes for many uncharacterized genes. We have used this 500-gene dataset to develop custom algorithms that automate scoring of phenotypic features. The phenotypic profiles generated by this analysis are facilitating functional groupings and network analysis. To date, we have collected 4D developmental data on ~900 genes. When complete, this project will provide a systems-level view of embryonic development in a complex multicellular organism. Since ~750 of the 2600 targets in our screen are uncharacterized genes that are conserved in humans, we anticipate that our screen will be relevant to understanding the genetic basis for human development and may also shed light on poorly understood congenital defects in humans, such as neural tube, craniofacial, and ventral body wall closure abnormalities. This work was funded by the Ludwig Institute for Cancer Research.

Program Abstract #7

Differentiation of human embryonic stem cells in micropatterned colonies recapitulates early embryonic spatial patterning

Eric Siggia, Fred Etoc, Christoph Kirst, Iain Martyn, Anna Yoney, Ali Brivanlou
Rockefeller University, USA

Stem cells are commonly grown on surfaces and when induced to differentiate show disorganized arrangements of fates. The simple process of spatial confinement and BMP4 induction leads to a reproducible arrangement of extraembryonic and germ layer fates as a function of colony radius that mimics the proximal distal axis in the mammalian embryo. Fate is defined by distance from the colony boundary which can be hundreds of microns away. The stem cell colonies also form a radially localized primitive streak and exhibit gastrulation like movements. We have characterized the molecular events that control our radial patterns, which include receptor occlusion in apical-basal polarized colonies, and the production and diffusion of secreted inhibitors and secondary morphogens. The patterns induced by Wnt stimulation resemble those induced by BMP4, less the outermost extraembryonic ring. However the Wnt patterns are sharper and reveal new pathways controlling fate boundaries. This quantitative assay shows in a context very different from the embryo how the canonical signaling pathways, generate spatial patterns over large scales, but it also reveals cell biological aspects of signaling that are difficult to study in mammalian embryos.

Program Abstract #8

Imprinted gene expression at the Dlk1-Gtl2 cluster is controlled by both maternal and paternal Gtl2 germline IG-DMRs in a tissue-specific fashion.

Katherine Alexander, Maria Garcia-Garcia
Cornell University, USA

Genomic imprinting depends on allele-specific DNA methylation at imprinting control regions known as germline differentially methylated regions (DMRs). However, the mechanisms by which these germline DMRs influence allele-specific expression are not fully understood. By re-examining mouse mutants containing deletions of the *Gtl2* germline *IG-DMR*, we uncovered previously unappreciated requirements for the maternal and paternal *IG-DMRs* in different embryonic tissues. Consistent with previous findings, we found that the maternal/unmethylated *Gtl2 IG-DMR* is required in early embryos for expression of *Gtl2* in cis. However, we also found that the paternal/methylated *IG-DMR*, previously thought to be dispensable for imprinted gene expression, is required at E14.5 in the yolk sac for cis repression of *Gtl2* and in the lung for expression of *Dlk1*. By using chromatin immunoprecipitation and qRT-PCR, we show that the paternal *IG-DMR* has features of active enhancer elements in the embryonic lung and liver, including the presence of enhancer-specific histone modifications and bidirectional transcription of nuclear non-coding RNAs. Interestingly, we found that early in embryogenesis, the transcriptional repressor TRIM28 is required to prevent enhancer activity at the paternal *IG-DMR*. Together, our experiments reveal a previously unrecognized requirement for the paternal *IG-DMR* to regulate imprinting, and provide evidence that this imprinting control region functions through different mechanisms at different embryonic tissues and developmental stages.

Program Abstract #9

Super-resolution imaging of DNA folding in vivo implicates chromatin bridges as a mechanism of repression

Alistair Boettiger[1], Ajaz Wani[2], Bogdan Bintu[1], Nicole Francis[3], Xiaowei Zhuang[1]

[1]Harvard University, USA; [2]University of Kashmir, India; [3]Institut de recherches cliniqes de Montre, Canada

Polycomb Group (PcG) proteins play a critical role in the maintenance of gene repression throughout development. PcG complexes affect chromatin conformation at multiple scales, but the nature of the chromatin structures they form and underlying mechanisms are incompletely understood. We used stochastic optical reconstruction microscopy (STORM) to analyze both the distribution of Polycomb Repressive Complex 1 (PRC1) in the nucleus, and the organization of large genomic regions bound by PRC1 in *Drosophila* embryonic cells. We discovered that PRC1 components are organized into hundreds of previously unresolved sub-micron clusters (30-500nm) in the nucleus, in addition to a handful of more prominent ~1 micron clusters previously characterized as PcG bodies. This observation challenges the interpretation of previous association studies, as nuclear elements (e.g. certain gene loci) not associated with microscale PcG bodies may still be associated with smaller nanoscale bodies. Mutation of a polymerization interface in the conserved Sterile Alpha Motif of the PRC1 component, Polyhomeotic (Ph), disrupts clustering of all PRC1 components we tested. Interestingly, this mutation does not substantially alter the DNA binding patterns of Ph as assayed by ChIP, but affects higher-order chromatin structure on the scale of 10s to 100s of kilobases as assayed by 4C. To visualize these changes, we labeled PcG domains of different sizes (10 - 400 kb) using Oligopaints and imaged them with STORM. Compared with similar domains that do not bind PcG proteins, the PcG bound regions are more locally intermixed, more densely coiled, and show a stronger avoidance of transcriptionally active chromatin. Knockdown of Ph abrogated these spatial features and resulted in leaky gene expression. Taken together, our data suggest that PcG protein bound chromatin forms a dense, spatially isolated structure that is refractory to transcription and is driven by protein-protein bridges mediate by Ph.

Program Abstract #10

Concentration Dependent Activity of the Bicoid Transcriptional Activator

Colleen Hannon[1,2], Shelby Blythe[1,2], Eric Wieschaus[1,2]

[1]Princeton University, USA; [2]Howard Hughes Medical Institute, USA

In order for embryonic development to proceed correctly and reproducibly, the expression of genes in individual cells must be coordinated with precision. In *Drosophila*, graded expression of the maternal transcription factor Bicoid (Bcd) provides positional information to pattern the anterior-posterior (AP) axis of the developing embryo. Bcd is known to bind hundreds of sites in the genome, activating target genes at different positions along the AP axis. To measure Bcd binding states at specific concentrations along its gradient, we have developed a series of transgenic lines that express defined uniform concentrations of Bcd. Using chromatin immunoprecipitation for Bcd followed by high throughput sequencing in these transgenic embryos, we group Bcd-bound target regions into several "affinity" classes based on their *in vivo* occupancy by Bcd at different concentrations. We find that the occupancy of a given target sequence for Bcd is dependent not just on the biochemical affinity of its Bcd binding sites, but its genomic context. Further, we find that some low affinity target regions are dependent on Bcd for maintaining an open chromatin state. This suggests a model in which Bcd is able to influence chromatin structure to gain access to low affinity targets at high concentrations. In contrast, high affinity targets are more accessible either through their native chromatin structure or as a result of other chromatin modifying factors.

Program Abstract #11

Quantifying the mechanisms controlling context specificity and robustness of developmental enhancer dynamics at single-cell resolution

Amanda Zacharias, Teddy D. Lavon, Elicia Preston, Shaili D. Patel, John Isaac Murray

University of Pennsylvania, USA

Signaling pathways achieve specificity in target gene activation through the dependence on additional inputs, commonly referred to as "context". To evaluate whether quantitative differences in Wnt pathway activity could also provide context, we measured the nuclear localization of the Wnt pathway effectors, TCF and β-catenin, in all cells throughout development in *C. elegans* embryos. We found that the response to Wnt compounds over successive exposures: nuclear localization of TCF and β-catenin is significantly increased in Wnt-signaled cells whose parents had also received a Wnt signal. This trans-generational "memory" of Wnt signaling influences target gene regulation suggesting that the level of signaling pathway activity can act an additional form of context. To understand how context information is encoded in Wnt target enhancers, we are investigating the dependence of enhancer activity on the organization and affinity of binding sites for TCF and other co-regulatory, cell-specific "context" transcription factors. We identified 20 targets of the Wnt

signaling pathway in *C. elegans* embryonic development that likely depend on distinct context factors. Using bioinformatics, we identified 88 putative Wnt target enhancers for these genes. We used time-lapse confocal imaging and lineage reconstruction to characterize reporter expression driven by many of these enhancers across developmental time and space with single-cell resolution. We identified a number of enhancers that drive part, but not all, of the full gene expression pattern. For several genes, multiple enhancers drive partially overlapping patterns, suggesting that expression in a particular sub-lineage may be distributed across multiple enhancers to provide robustness, thus serving as partial "shadow" enhancers. Our results provide a foundation to study the encoding of context in enhancer function and robustness quantitatively with dynamic cellular resolution.

Program Abstract #12

A positional Toll receptor code directs polarized forces during convergent extension in *Drosophila*

Adam Pare, Athea Vichas, Christopher Fincher, Zachary Mirman, Dene Farrell, Avantika Mainieri, Rodrigo Fernandez-Gonzalez, Jennifer Zallen

HHMI and Developmental Biology Program, Sloan Kettering Institute, New York, NY, USA

A major challenge in developmental biology is to understand how tissue-scale changes in organism structure arise from events that occur on a cellular and molecular level. My lab uses multidisciplinary approaches from cell and developmental biology, physics, engineering, and computer science to study how tissue architecture is dynamically established and remodeled throughout development. The embryonic body axis elongates dramatically from head to tail through the rapid and coordinated movements of hundreds of cells, in a process that is conserved throughout metazoans. We identified the force-generating machinery that produces the polarized cell movements that drive axis elongation in *Drosophila*. In addition, we discovered that the organization and dynamics of this machinery are controlled by a global system of spatial information provided by an ancient family of Toll-related receptors that are widely used by the innate immune system for pathogen recognition. These spatial cues direct cell-cell interactions that produce local cell rearrangements and the collective formation and resolution of multicellular rosette structures that promote efficient elongation. We showed that rosettes form through a mechanically regulated process in which an initial asymmetry in actomyosin contractility is propagated by the force-sensitive activation of myosin in neighboring cells, amplifying the effects of chemical spatial cues. These studies elucidate general principles that link cellular asymmetries and local mechanical forces to global tissue reorganization.

Program Abstract #13

Differential Growth Triggers Mechanical Feedback that Elevates Hippo Signaling

Yuanwang Pan[1], Idse Heemskerk[2], Consuelo Ibar[1], Boris Shraiman[2], Kenneth Irvine[1]

[1]Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway NJ 08854, USA; [2]Kavli Institute of Theoretical Physics, Santa Barbara, CA 93101, USA

Mechanical forces have emerged to be an important regulator of tissue growth. For example, high cytoskeletal tension enhances tissue growth while low cytoskeletal tension decreases tissue growth. On the other hand, growth has also been suggested to affect tissue mechanics: heterogeneous growth could lead to mechanical stress that feedbacks into cells to maintain growth homeostasis. However, whether and how such a mechanical feedback mechanism functions in vivo are not clear. Here we test the mechanical feedback hypothesis by inducing differential growth in *Drosophila* wing disc epithelia through distinct approaches. We show that differential growth triggers a mechanical response that lowers cytoskeletal tension along apical cell junctions within faster-growing cells. This reduced tension modulates a biomechanical Hippo pathway, decreasing recruitment of Ajuba LIM protein and the Hippo pathway kinase Warts to junctions, thus reducing the activity of the growth-promoting transcription factor Yorkie. This provides the experimental support and a molecular mechanism for lowering growth rates within faster-growing cells by mechanical feedback. Additionally, bypassing mechanical feedback induces tissue distortions and inhomogeneous growth. Thus our research further identifies the roles of mechanical feedback in maintaining tissue shape and controlling patterned growth rates during development.

This research was supported by NIH grant R01 GM078620 and the Howard Hughes Medical Institute (KDI), and NSF PHY-1220616 and GBMF #2919 (BIS).

Program Abstract #14

The role of oriented cell division in prostate progenitor cell homeostasis

Maxwell Shafer[1], Mathieu Tremblay[1], Marine Lacomme[2], Carine Monat[2], Michel Cayouette[2], Maxime Bouchard[1]

[1]Goodman Cancer Research Centre, Department of Biochemistry, McGill University, Canada; [2]Institute de Researches

Cliniques de Montreal, Universitie de Montreal, Canada
The formation of multiple lineages and the stratification of cell types during epithelial morphogenesis is controlled by both extrinsic and intrinsic cell fate determination signals. The epithelial lineages and stratified architecture of the prostate are generated during development by coordinated symmetric and asymmetric divisions in bi-potent basal stem cells. These divisions control the relative amounts of the stem-like basal, and differentiated luminal cells. Using conditional gene inactivation in the mouse, we have observed that the transcription factor Gata3 controls this process by regulating the expression and localization of the protein kinase aPKC, a member of the apical Par complex. Deregulation of aPKC by loss of Gata3 leads to spindle orientation randomization in basal stem cells, and an increase in the formation of 'double-positive' progenitor cells. These defects ultimately lead to aberrant prostate branching morphogenesis and epithelial hyperplasia. aPKC controls spindle orientation by directly interacting with the spindle complex protein LGN, which links the spindle with the apical or lateral cortical membranes during asymmetric or symmetric divisions, respectively. In many different contexts, inhibition of LGN by apically localized aPKC is thought to block asymmetric stem cell divisions, whereas interaction of LGN with INSC is hypothesized to promote asymmetric divisions. In contrast to its necessity for asymmetric cell divisions in the epidermis, we recently found that LGN is required for symmetric divisions in prostate stem cells. Loss of LGN leads to epithelial hyperplasia and increases in progenitor cells. Further analysis of LGN, and other polarity genes in the developing prostates will shed light on the mechanisms of epithelial lineage determination and stratification, and highlight the critical role of spindle orientation within stem cells.

Program Abstract #15
Control of Organ Size by the Hippo Pathway
Kieran Harvey
Peter MacCallum Cancer Centre, Melbourne, Australia
The Hippo pathway is a complex signalling network that controls developmental tissue growth and is frequently deregulated in different human cancers. Discovered by us an others in 2002, the Hippo pathway is now the subject of intense investigation, but despite this the mechanism of signal transduction within this pathway is incompletely understood. We have used large-scale screens to address these knowledge deficiencies. Using genetic screens, we identified Tao-1 as a kinase that regulates activity of the Hippo kinase, and Hipk as a kinase that promotes activity of the Yorkie transcription co-activator. Using proteomic screens, we identified a new branch of the Hippo pathway, from transmembrane receptor to the nucleus, that operates downstream of the Dachsous cadherin. We also discovered the GTPase regulatory proteins Pix and Git as proteins that activate the Hippo kinase. These studies have provided important insights into the signalling logic that operates in the Hippo pathway in the context of tissue growth.

Program Abstract #16
Amyloid-like aggregation of Xvelo drives Balbiani body formation
Elvan Boke[1], Martine Ruer[2], Martin Wuhr[1], Margaret Coughlin[1], Steve P. Gygi[1], David Drechsel[2], Simon Alberti[2], Anthony A. Hyman[2], Timothy J. Mitchison[1]
[1]Harvard Medical School, USA; [2]Max Planck Institute of Molecular Cell Biology and Genetics, Germany
A key characteristic of female germ cells, oocytes, is that their complement of mitochondria and RNA is kept intact for decades before fertilization. However, we have little idea how these are protected for such long periods of time. One prominent feature of dormant oocytes is the Balbiani body, which is conserved from fish to humans.These enigmatic structures, which can be thought of as a non-membranous compartment, or super-organelle, are prominent in the cytoplasm of early oocytes, and are packed with mitochondria, ER, Golgi and RNA. Very little is known about their organization and structure. We have studied the organization of the Balbiani body in *Xenopus*, the large size of which makes it amenable to biochemistry. We show by quantitative mass spectrometry that the most enriched protein in the Balbiani body of *Xenopus* is Xvelo, a homolog of the germ line protein Bucky ball in zebrafish. Xvelo has a prion-like domain in its N terminus, which is sufficient and necessary to target to the Balbiani body. We show that Xvelo forms a mitochondria-embedding, amyloid-like matrix pervading the entire volume of the Balbiani body. Moreover, recombinant Xvelo forms micron-sized networks *in vitro* that can cluster mitochondria on its own in a cell free system, thus reconstituting aspects of a Balbiani body *in vitro*. We propose that the Balbiani body forms by amyloid-like aggregation of Xvelo. Because the prion-like domain of Xvelo is conserved in different germ plasm-related proteins in other species, Balbiani body formation by amyloid-like aggregation could be a conserved feature in evolution for maintaining the immortal character of germ cells.

Program Abstract #17

Divergent roles of noncanonical Wnt signaling in PGCs and their somatic microenvironment

Andrea Cantu[1], Amanda Butler[2], Kameswari Potharaju[1], Mary Lou King[2], Diana Laird[1]

[1]University of California, San Francisco, USA; [2]University of Miami School of Medicine, USA

The autonomous versus microenvironmental regulation of cellular decisions remains a central question for cells that move during development such as primordial germ cells (PGCs). Despite the conservation of PGC migration across species, the mechanisms governing motility, maturation, survival, and proliferation of these cells in mammals remain largely unknown. We previously found that the rate of mouse PGC proliferation varies by location rather than by embryonic age, suggesting microenvironmental regulation of the cell cycle. We further determined that proliferation of PGCs is restricted by Wnt5a-Ror2 signaling specifically in the hindgut. Given the broad expression of Ror2 receptor in PGCs as well as neighboring somatic cells, here we studied its function in each. Using immunofluorescence, we established that Ror2 is highly expressed in the mouse hindgut epithelium and dorsal mesentery, lowly expressed in the gonadal ridges, and present on migratory PGCs. Conditional knockout of *Ror2* in early PGCs recapitulated the defects in migration observed in full Ror2 mutants; however, instead of the ultimate reduction in PGC number observed when Ror2 function is ubiquitously disrupted, PGC-specific deletion produced a sustained and significant increase in the total germ cell population throughout the migratory period. Interestingly, several established PGC survival signals were misregulated in Ror2 mutant somatic cells. In parallel studies in *Xenopus laevis*, x*Ror2* knockdown by morpholino caused a significant increase in PGC number in tailbud stage embryos, whereas x*Ror2* knockdown directed to the germline significantly reduced PGC number; PGC migration was disrupted in both knockdowns. These data from mouse and *Xenopus* suggest that Ror2 signaling plays divergent roles in developing PGCs: regulating germ cell survival and cell cycle via the soma and autonomously mediating migration.

Funding: NSF GRFP Fellowship (AVC), NIH 1DP2OD007420 (DJL), NIH GM102397 and HD072340 (MLK)

Program Abstract #18

Slow multicellular Ca2+ waves patterned by dynamic morphogen gradients coordinate collective mesenchymal cell migration and are manipulable by novel optogenetic tools

Ang Li[1], Jung-Hwa Cho[2], Chun-Chih Tseng[1], Lian He[3], Peng Tan[3], Chao-Yuan Yeh[1], Brian Reid[4], Yuwei Li[5], Min Zhao[4], Yubin Zhou[3], Robert Chow[2], Cheng-Ming Chuong[1]

[1]Dept of Pathology, University of Southern California, USA; [2]Dept of Physiology & Biophysics, University of Southern California, USA; [3]Institute of Biosciences and Technology, Texas A&M University Health Science Center, USA; [4]Dept of Dermatology, UC Davis School of Medicine, USA; [5]Dept of Molecular & Computational Biology, University of Southern California, USA

The factors organizing stem cells into distinct organ topologies include biochemical molecules, physical forces and bioelectric signals. Embryonic chicken feathers can robustly develop the proper shape and polarity due to highly-controlled mesenchymal cell migration as shown by 4D imaging and cell tracking data. Through vibrating probe measurement we detected an electric circuit formed between anterior and posterior part of developing feathers. RNA-seq and in situ hybridization data indicate this circuit is potentially constituted by voltage gated Ca2+ channels (VGCCs), Ca2+ release activated Ca2+ channels (CRACs), gap junctions and Ca2+ activated K+ channels. Combining constant flow circulation system, confocal microscopy and retrovirus-based ratiometric Ca2+ sensors, we mapped real-time Ca2+ response in feathers and dissociated cells and detected a small population of feather mesenchymal cells with active VGCCs communicating with others using gap junctions. Interestingly, 4D Ca2+ imaging revealed presence of slow oscillatory multicellular Ca2+ waves during feather elongation. Blocking VGCC or gap junction activities significantly altered mesenchymal cell movement patterns, leading to altered feather morphology and polarity. Additionally, the spatial configuration of the Ca2+ waves correlate with that of Shh responding cells as shown by a Gli responding GFP and Patched1 in situ hybridization. Further functional assays revealed a role of Shh signaling in increasing spontaneous Ca2+ fluctuations and Wnt signaling in inducing gap junction expression. Perturbing either pathway would alter cell movement patterns and feather morphology. Finally, using photoactivatable CRAC we artificially created collective Ca2+ oscillations and induced feather elongation in ectopic directions. We thank supports from the National Institute of Arthritis and Musculoskeletal and Skin Diseases AR47364, AR60306, AR 42177, NIH BRP 5R01EY022931, Doerr Stem Cell Challenging grant.

Program Abstract #19

Imaging how transcription factors bind DNA to control cell fate in the living mouse embryo

Melanie White[1], Juan Angiolini[2], Yanina Alvarez[2], Gurpreet Kaur[1], Ziqing Zhao[1], Stephanie Bissiere[1], Valeria Levi[2],

Nicolas Plachta[1]
[1]*Agency for Science, Technology and Research, Singapore;* [2]*Universidad de Buenos Aires, Argentina*
Transcription factor (TF) binding to DNA is fundamental for gene regulation. However, it remains unknown how the dynamics of TF–DNA interactions change during cell fate determination *in vivo*. We have established new imaging techniques to quantify TF–DNA binding in single cells of developing mouse embryos. We show how the TFs Oct4 and Sox2 re-partition between specific and non-specific DNA sites as cells decide their fate *in vivo*. Furthermore, we demonstrate that Sox2 DNA binding varies between blastomeres and is regulated by histone methylation. Live-cell tracking demonstrates that those blastomeres with more long-lived binding contribute more pluripotent progeny, and reducing H3R26 methylation decreases long-lived binding, Sox2 target expression, and pluripotent cell numbers. Therefore, Sox2-DNA binding predicts mammalian cell fate as early as the four-cell stage. More generally, our work reveals the dynamic repartitioning of TFs between DNA sites driven by physiological epigenetic changes.

Program Abstract #20
Comparative transcriptomics in the sea anemone *Nematostella vectensis* suggests regeneration is a partial redployment of the embryonic gene regulatory network
Jacob Warner, Eric Röttinger
Institute for research on cancer and aging of Nice, FR
Regeneration, as it restores missing tissue, is thought to employ processes originally used during embryonic development. If so, how does injury trigger those processes? Furthermore, are the ensuing genetic interactions similar to those of embryonic development? This project explores the mechanistic basis of regeneration by comparing the gene regulatory networks (GRN) governing regeneration and development. To achieve this, we use the developmental model system *Nematostella vectensis*. This sea anemone can regrow half its body when bisected. In order to examine the global similarities and differences in transcription during regeneration and embryogenesis we used RNA-seq to measure gene expression spanning 16 time-points of regeneration and compared this to existing embryonic datasets. We found that, compared to embryogenesis, regeneration is a transcriptionally modest process, involving approximately 10% of the total number of genes that are activated during embryonic development. By performing co-expression analysis we identified discrete gene modules that are activated during embryogenesis and regeneration. Several of these modules are conserved during the two processes suggesting a partial re-deployment of the embryonic gene network during regeneration. To test if the regulatory interactions of these GRNs driving these processes are conserved we performed knockdown experiments to compare the targets of wnt signaling in embryogenesis and regeneration. Here we found that a subset of the wnt targets are conserved in regeneration compared to embryogenesis again suggesting a partial re-deployment of the embryonic network.

Program Abstract #21
Early development of neural lineages in the *Drosophila* central brain: primary neurons derive from neuroblasts in the embryo to form neural circuits of the larval brain
Jennifer Lovick, Amelia Younossi-Hartenstein, Satkartar Khalsa, Volker Hartenstein
UCLA, USA
The central nervous system of the fruit fly *Drosophila melanogaster* is a complex assemblage of neural circuits composed of clusters of neurons, or lineages, which share similar anatomical and functional characteristics as well as a common developmental origin. In the central brain, approximately 100 neural progenitors, neuroblasts, make lineages during two proliferative waves. The first, embryonic wave, produces primary neurons that form the functional larval brain; a second wave, which lasts from mid-larval stages to early pupa, creates adult-specific, secondary neurons that differentiate during metamorphosis and integrate with surviving embryonic-born neurons. Thus, neuroblasts, which are first specified in the embryo, are responsible for generating all neurons of the brain. In previous studies, we documented the development of secondary lineages from larva to adult, establishing a comprehensive neuroanatomical map of adult-specific neurons. Using global markers which allow us to visualize primary and secondary neurons of lineages within the same brain, we generated an atlas of primary lineages at the early larval stage. Lineages are morphologically identifiable by their characteristic axonal projections (primary lineage tracts, PATs). We are using the atlas of PATs to (1) reconstruct single primary neurons at high resolution within an early larval brain EM stack using CATMAID in an effort to understand how individual neurons, organized into lineages which share common wiring properties, form circuits; and (2) link the morphologically defined lineages to their parent neuroblasts in the early embryo, effectively determining the origin and genetic specification for each central brain lineage. I here present an overview of our two-pronged approach, by which we

follow the PAT atlas backwards in time into the late embryo, and use sets of genetic markers to determine the early embryonic neuroblasts of origin of individual lineages.

Program Abstract #22

Developmental nonlinearities and the mechanistic basis for phenotypic robustness

Rebecca Green[1], Jennifer Fish[2], Nathan Young[3], Francis Smith[1], Katie Dolan[2], Benjamin Roberts[2], Charles Roseman[5], Trevor Williams[4], Ralph Marcucio[3], Benedikt Hallgrimsson[1]

[1]University of Calgary, CA; [2]University of Massachusetts - Lowell, USA; [3]University of California San Fransisco, USA; [4]University of Colorado Denver, USA; [5]University of Illinois - Urbana, USA

Robustness to perturbation is a fundamental feature of complex life. In complex organisms, the ability to tolerate potentially disruptive genetic variation is necessary for evolution to occur; however little is known about the generation of robustness. One possible mechanism is non-linear correlations between genotype and phenotype. Here, we use a large alleleic series of loss of *Fgf8* to show a strong non-linear relationship between *Fgf8* gene dosage and phenotypic shape. We show low *Fgf8* dosage generates alterations in mean phenotype and phenotypic variance. Phenotypic variance is not due to increased variance at the RNA level; only changes in the mean expression levels of *Fgf8* and its target genes are observed. This non-linear phenomena may form a general explanation for stability in development. Our results imply that contrary to the received view, robustness may not mask cryptic variation that is selectable when exposed by mutations. Further, major mutations don't necessarily produce increased variation because of systemic perturbation. Finally, this explanation implies that robustness can evolve by shaping nonlinearities among determinants of phenotypic variation.

*first and second author contributed equally; Funding sources: NIDCR R01DE019638 to RM and BH, NIDCR R01 DE019843 to TW, NIDCR F31 DE022214 to RG, NSERC 238992-12 to BH, ACHRI fellowship to RG

Program Abstract #23

Embryonic origin and ontogeny of a pluripotent adult stem cell compartment

Erin Davies[1], Alejandro Sánchez Alvarado[1,2]

[1]Stowers Institute for Medical Research, USA; [2]Howard Hughes Medical Institute, USA

Neoblasts, adult somatic stem cells responsible for producing all cell types required for tissue maintenance and regeneration in freshwater planaria, exhibit levels of plasticity and pluripotency akin to embryonic stem cells. We investigated the developmental origin of neoblasts during *Schmidtea mediterranea* embryogenesis and report that neoblasts arise from a persistent, cycling, *piwi-1*+ cell compartment as organogenesis commences. *piwi-1* expressing cells are detected throughout development, and notably cell cycle activity is restricted to this compartment. Many genes with neoblast enriched expression in adult animals are expressed throughout embryogenesis and are co-expressed in *piwi-1*+ blastomeres. However, dynamic shifts in gene expression within the *piwi-1*+ compartment are observed at the onset of organogenesis: early embryo enriched genes are downregulated as key regulators of lineage commitment and differentiation are upregulated. Remarkably, *piwi-1*+ cells from embryos undergoing organogenesis behave similarly to adult neoblasts in cell transplantation assays: they engraft, proliferate, produce differentiated progeny, and are capable of rescuing lethally irradiated adult hosts. In contrast, *piwi-1*+ cells from early embryos do not persist in a heterotopic environment. Taken together, these data suggest that the *piwi-1*+ compartment is required for the construction of all major organ systems during embryogenesis. Here, in a triploblastic animal not thought to undergo gastrulation, heterogeneous expression of key developmental regulators within a pluripotent, cycling cell compartment generates the panoply of lineage-dedicated progenitors required for organogenesis. Moreover, progenitor subpopulations established during embryogenesis persist into adulthood, facilitating redeployment of developmental pathways to maintain and replace missing tissue.

Program Abstract #24

A Meiotic-Vegetal Center Couples Oocyte Polarization with Meiosis at A Nexus of Oocyte Differentiation

Yaniv Elkouby, Allison Jamieson-Lucy, Mary C Mullins

University of Pennsylvania Perelman School of Medicine, USA

A universal feature of early oocyte differentiation is formation of the Balbiani body (Bb), a large granule of specific mRNAs, proteins, and organelles. The zebrafish Bb establishes oocyte animal-vegetal (AV) polarity by specifying the oocyte vegetal pole. The Bb has been observed for two centuries, but how it forms and is asymmetrically positioned was unknown. Using quantitative image analysis, we traced oocyte symmetry breaking to a nuclear asymmetry at the onset of meiosis called the chromosomal bouquet. The bouquet is a universal feature of meiosis where all telomeres cluster to one pole on the nuclear envelope (NE). Telomere movements and clustering on the NE facilitate chromosomal pairing and

meiotic recombination. We show that Bb precursors first localize with the centrosome to the cytoplasm apposing the bouquet telomere cluster. They then aggregate around the centrosome in a specialized nuclear cleft that we identified, assembling the early Bb. We found that the bouquet nuclear events and the cytoplasmic Bb precursor localization are mechanistically coordinated by microtubule. Thus the AV axis of the oocyte is aligned to the nuclear axis of the bouquet. This symmetry breaking lays upstream to Bucky ball, the only known regulator of Bb formation. We link two universal features of oogenesis, the Bb and the chromosomal bouquet, to oocyte polarization, and propose that the centrosome forms a cellular organizer that we term the meiotic–vegetal center (MVC), coupling meiosis and oocyte patterning. We next revealed that oocytes are organized in cysts with synchronized MVCs. We detected bouquet specific, cilia-like structures that may mechanically regulate cyst MVCs. Moreover, the MVC localizes near cytoplasmic bridges that connect sister cyst oocytes, suggesting its positioning by a previous mitotic division plane. These results link polarity to cyst organization. We uncovered key, functionally coordinated meiotic and cellular polarity events of the early oocyte.

Program Abstract #25

Cilia-mediated Hedgehog signaling controls form and function in the mammalian larynx

University of texas, at Austin, USA

Jacqueline M. Tabler[1]*, Maggie M. Mitchell[1]*, Rebecca Fitch[1], Christopher Carter[1], Karen J. Liu[2], Steven Vokes[1], Roian Egnor[3], and John Wallingford[1]*

Vocal communication is fundamental to social interactions, yet while the neurobiology of speech and the developmental biology of hearing are thoroughly characterized, we know strikingly little about development of the organs of vocalization, the larynx and vocal folds. This gap in our knowledge is significant because laryngeal defects in human patients with ciliopathies and Hedgehog-related birth defects dramatically impair the voice and thus degrade quality of life. Here, we present a genetic fate map of the mammalian larynx and vocal folds and we demonstrate a key role for cilia and HH signaling in mouse laryngeal development. Mice with mutations in the ciliogenesis and planar polarity effector gene *Fuz*, display severely disrupted laryngeal morphogenesis, while *Gli3* mutant mice display less severe phenotypes. In both cases, laryngeal defects involve an invasion of excess neural crest-derived mesenchyme. In *Gli3* mutants, the crest-derived vocal ligaments are expanded at the expense of reduced mesoderm-derived vocal muscles. Strikingly, this phenotype is dose-dependent and recordings of viable heterozygous *Gli3* mutant mice reveal significantly altered vocalization acoustics. Together these data provide a foundation for further studies of the molecular genetics of vocal organ development and establish the mouse as a tractable model for studies of human laryngeal birth defects.

Funding: HHMI Young Investigator Award, NIH F32DEO23272. **Authors contributed equally*

Program Abstract #26

Biological Experiments in Space – Open Science and NASA's GeneLab Project

Sigrid Reinsch[1], Oana Marcu[1,2]

[1]NASA-Ames Research Center, USA; [2]SETI Institute, USA

This tutorial will describe the vision and progress of the GeneLab project: an open science initiative to generate and make publicly accessible omics data generated through biological research in space by: (1) developing a unique public bioinformatics database that includes space bioscience relevant "omics" data (genomics, transcriptomics, proteomics, and metabolomics) and experimental metadata; (2) partnering with NASA-funded flight experiments to expedite omics data input to the GeneLab database; and (3) developing community driven reference flight experiments. Data sets are available for unrestricted download and analysis from experiments using numerous organisms including microbes, yeast, *C. elegans, Drosophila, Arabidopsis*, rodents and human cell lines. In 2015 GeneLab partnered with two experiments that examined the proteomic and transcriptomic responses of *Arabidopsis* to spaceflight, and to obtain multiple organ tissues from Rodent Research-1 (RR-1), the maiden flight to test the recently developed rodent habitat. Partnerships under development for 2016 and 2017 include various microbial, plant, invertebrate and rodent experiments. GeneLab's data system currently allows search and download capabilities. Future capabilities will include data federation, computational tools and a collaboration framework. Analysis of GeneLab data will contribute fundamental knowledge of functional and regulatory networks responsive to the spaceflight environment and foster new hypothesis-driven research for future spaceflight studies spanning basic science to translational research. GeneLab is funded through NASA's Space Life and Physical Sciences Research and Applications Division (SLPSRA) and the International Space Station Research Integration Office (ISSRIO).

Program Abstract #27

Retinoid metabolism affects gonadal sex determination in mice

Peter Koopman[1], Chun-Wei Feng[1], Jessica Ineson[1], Kim Miles[1], Vincent Harley[3], Andrew Sinclair[2,4], Josephine Bowles[1]
[1]The University of Queensland, Australia; [2]Hudson Institute of Medical Research, Australia; [3]Murdoch Children's Research Institute, Australia; [4]The University of Melbourne, Australia

Mammalian sex determination depends on an interplay of signals that promote either testicular or ovarian development from the bipotential genital ridges, with many components of these regulatory networks remaining to be identified. Retinoic acid (RA) is a morphogen that is present in fetal ovaries, where it is known to stimulate germ cell meiosis during fetal development, but is removed from fetal testes by the action of the p450 enzyme CYP26B1. Here, we show that endogenous RA also affects primary sex determination and gonad differentiation. We demonstrate, using mouse genetic experiments and cell and organ culture studies, that RA signaling promotes ovarian development and antagonises testis development. In XY *Cyp26b1*-null embryos, which have elevated endogenous RA levels, the testis pathway was partially diverted to the ovarian development pathway, resulting in ovotestis development. As a result, steroidogenesis was impaired, and the reproductive tract was feminized. Our results reveal that RA must be fully removed from the developing gonadal tissue by CYP26B1 if normal male sex determination and testis development are to proceed. The data also implicate the retinoid metabolism pathway as a possible target for environmental endocrine disruption during fetal sex development, and suggest that disturbances in RA metabolism may underlie gonadal dysgenesis syndromes in humans.

Program Abstract #28

Conditional targeting of key developmental signal, Shh, in the adult mouse IVD causes Premature Intervertebral Disc Degeneration

Sarthak Mohanty, Elfie De Jesus, Chitra Dahia
Hospital for Special Surgery, USA

Disc degeneration and associated back pain are very common, affecting almost 1/7 individuals. The current treatments are palliative, mainly due to poor understanding of the molecular mechanisms of disc growth and differentiation, and of the changes associated with of its degeneration. Our approach has been to identify signaling pathways involved in the normal postnatal growth and differentiation of the disc, and to see if the abrogation of these signals causes premature disc degeneration, using the mouse as a model. Our previous studies showed that Shh is synthesized postnatally by the nucleus pulposus (NP) cells, derived from the embryonic notochord, and is a key regulator of postnatal disc growth and differentiation. Shh expression decreases with age. Here we test the hypothesis that Shh signaling continues to be essential for maintenance of the IVD during adult life, by generating a conditional mouse model using a NP Cre driver line to conditionally target Shh in the adult (9 month old) mouse disc following tamoxifen treatment. Three months later there were dramatic changes in the L5/L6 and L6/S1 discs of Shh targeted mice compared to the controls, including the loss of NP cells, disc height, disc width, and loss of structure in the surrounding annulus fibrosus compared to controls. In these discs, the NP space became filled with cells that did not express NP cell markers, with an appearance similar to those of 2 year old mice, and to human degenerated discs, with the appearance of chondrocyte-like cells. The results suggest that Shh signaling is also critical for maintenance of the lower lumbar and sacral mouse discs (the discs most affected during aging in humans), and its loss is associated with degeneration of the intervertebral disc. In contrast, more cranial discs examined (L3/L4) seemed unaffected. The reasons for these different effects are not yet clear.

Program Abstract #29

Hedgehog signaling is required to form the cartilage callus during large-scale bone regeneration

Stephanie Kuwahara, Nikita Tripuraneni, J. Gage Crump, Francesca V. Mariani
University of Southern California, USA

Bone repair typically involves the formation of a cartilage template, however how this cartilage forms and its importance in repair is not clear. By using a model for large-scale bone repair in the mouse rib, we have found that the repair callus consists of an unusual type of cartilage that is quite different from cartilage seen during bone development. This cartilage has bone-like properties both in the expression of bone-associated genes and in cell morphology and appears to regenerate full thickness bone more effectively than cells that undergo direct ossification. Although the formation of the callus is known to involve contributions from the periosteum, little is known about how periosteal cells generate cartilage only in response to injury. Using transgenic lineage tracing we have identified a rare periosteal subpopulation, marked by the expression of *Sox9*, that is responsive to injury and builds the repair callus. In addition we present data indicating that Hh signaling is required in Sox9+ cells for the formation of the cartilage callus and may have a distinct role in repair versus development. Thus large-scale regeneration of the mouse rib uses a cartilage differentiation mechanism distinct from

development, with the bone-forming capability of chondrocytes in the callus possibly due to their origin from the periosteum.

Program Abstract #30

The *C. elegans* spermatheca as a model system for calcium signaling in a contractile and compliant tube.

Jeff Bouffard, Anand R. Asthagiri, Erin J. Cram
Northeastern University, USA

Biological tubes are found in organisms across the animal kingdom, often functioning as conduits for material transport. Robust regulation of the contractility and compliance of these tubes ensures transport proceeds with proper pace and directionality. The spermatheca, a 24-cell tissue in the *C. elegans* reproductive system, provides an ideal model for detailed studies of such tubes. During ovulation, the spermatheca is stretched significantly by oocyte entry, remains distended while the eggshell forms, and finally initiates coordinated contraction of the cells, expelling the egg. With much of the calcium and contractile machinery known, and parallels with many smooth muscle systems, the spermatheca presents a ready model system for examining contractility and calcium signaling in biological tubes. Mechanisms at work in this tissue potentially represent fundamental biological responses applicable to other contractile and compliant tubes. Using the genetically encoded calcium sensor GCaMP, we monitored calcium activity in the spermatheca during ovulation events by acquiring movies of live, intact animals with a widefield fluorescence microscope. To analyze our imaging data we developed semi-automated image and signal processing and analysis pipelines in Fiji and Matlab. These pipelines extract metrics from the GCaMP datasets, such as maximum signal, time from start of oocyte entry to half maximum signal, and percent of timeseries over half maximum signal. These metrics enable quantitative observations, for example the loss of the worm Filamin *fln-1* results in a decreased maximum signal and increased percent of timeseries over half maximum compared to wildtype. These results demonstrate that our processing and analysis pipelines provide a platform to develop a deep quantitative understanding of this model contractile tube.

An NSF EAPSI fellowship to JB, a TIER1 award from NEU to ARA and EJC, and NIH grants 1R01GM085077 and 1R01GM110268 to EJC funded this work.

Program Abstract #31

Cavefish Evolution as a Natural Model for Metabolic Diseases

Jaya Krishnan[1], Ariel Aspiras[2], Cliff Tabin[2], Nicolas Rohner[1]
[1]Stowers Institute for Medical Research, USA; [2]Harvard Medical School, USA

Understanding the genetic basis of adaptation has broad implications not only for a basic understanding of evolution, but also for human pathologies given that many human diseases are a consequence of mis-adaptation to modern societies. The emerging model system *Astyanax mexicanus* has become an important fish species to address adaptation to extreme environments due to its unique ecology and the availability of genetic tools and genomic resources. There are distinct yet still interfertile populations of this species living in both river and cave environments. While the surface fish live in a rich ecological niche with typical fish physiology, the cave populations have adapted to survive in conditions of extreme starvation through much of the year punctuated by brief floods that bring excessive nutrients. To cope with these conditions they have evolved impressive metabolic adaptations such as hyperphagia (increased appetite), elevated body fat levels, insulin resistance and altered feeding behaviors. Despite these drastic metabolic changes cavefish live long and healthy lives, probing the question whether they have acquired mechanisms allowing them to cope with extreme nutritional levels. Here, we have focused on the fatty livers these fish develop, reminiscent of human Non-Alcoholic Fatty Liver Disease. Using RNA-Seq data of fed and starved cavefish livers we confirmed the impressive starvation resistance of these fish on a molecular level and identified protective pathways specifically altered in cavefish to circumvent negative health effects. We are currently using genome editing to functionally test identified candidate genes in zebrafish, cavefish, and mouse models. Such a comparative approach will allow us to study in detail the molecular mechanisms underlying the adaptation of cavefish to the extreme and nutrient poor environments, thereby providing potential new insights into human health.

Program Abstract #32

Transcriptomic analysis reveals novel regulators of germ cell development in the parasite *Schistosoma mansoni*

Harini Iyer[1], Tracy Chong[1], Caitlin B. Dingwall[1], James J. Collins III[2], Phillip A. Newmark[1]
[1]Howard Hughes Medical Institute and University of Illinois at Urbana-Champaign, USA; [2]UT Southwestern Medical Center, USA

Schistosomiasis is a major neglected tropical disease caused by members of the genus *Schistosoma.* The pathogenicity of

schistosomiasis is due to the host's immune response to the hundreds of eggs the parasites lay each day in the host vasculature. Thus, schistosomiasis is a disease in which the parasite's tremendous reproductive output, in combination with the host immune machinery, results in considerable morbidity. Significant progress has been made in the understanding of immunobiology of schistosomiasis. However, the reproductive biology of schistosomes remains poorly understood. To better understand the reproductive biology of these parasites, we used RNA-seq to enrich for mRNAs associated with the male germline of *Schistosoma mansoni*. We find 400 transcripts (>1.5X, $p \leq 0.05$) putatively enriched in *S. mansoni* male germ cells. Among the top candidates are *nanos* and *boule,* genes known to have conserved germ cell functions, validating the efficacy of our approach. In situ hybridization reveals that a majority of the differentially expressed transcripts are expressed in *S. mansoni* testes, and interestingly, also in the ovaries. We have functionally validated many of these genes by RNAi, and find that a number of both conserved and schistosome-unique genes result in a range of male germ cell defects in the parasite in vitro. Furthermore, our studies reveal that many parasite germ cell-enriched genes have homologs in the free-living flatworm *Schmidtea mediterranea.* In situ hybridization and RNAi of the *S. mediterranea* homologs show that these genes are expressed and function in the planarian male germ cells, indicating molecular conservation between free-living and parasitic flatworms. Our studies establish the free-living planarian as an excellent model for the study of parasite reproductive biology and may have implications in preventing and treating schistosomiasis.

Program Abstract #33

Repeal, Replace, and Redeploy: Neofunctionalization of a conserved gene regulatory network during *Aedes aegypti* development

Kushal Suryamohan[1,6], Casey Hanson[2], Emily Andrews[3], Saurabh Sinha[2], Molly Duman-Scheel[3,4], Marc Halfon[1,5,6]
[1]University at Buffalo-SUNY, USA; [2]University of Illinois Urbana-Champaign, USA; [3]Indiana University School of Medicine-South Bend, USA; [4]University of Notre Dame, USA; [5]Roswell Park Cancer Institute, USA; [6]NY State Center of Excellence in Bioinformatics & Life Sciences, USA

Gene regulatory networks (GRNs) provide the mechanistic basis for metazoan development. We are studying GRN evolution and developmental system drift during development of the central nervous systems of the fruitfly *Drosophila melanogaster* and the dengue fever and Zika virus vector mosquito *Aedes aegypti*. Despite substantially similar nervous system morphology, the two species show significant divergence in a set of genes coexpressed in the *Drosophila* ventral midline, including the master midline regulator *single minded (sim)* and downstream genes including *short gastrulation (sog), Star,* and *NetrinA (NetA)*. In contrast to *Drosophila*, we find that midline expression of these genes is absent or severely diminished in *A. aegypti*. Instead, they are co-expressed in the lateral nervous system. This suggests that in *A. aegypti* this "midline GRN" has been redeployed in a new location while lost from its previous site of activity. In order to characterize the relevant GRNs, we employed SCRMshaw—a computational method we previously developed for *cis*-regulatory module discovery—to identify 6 novel regulatory sequences from *A. aegypti* and 7 from *D. melanogaster.* Analysis of these enhancers in transgenic *Drosophila* suggests that most of the altered gene expression observed in *A. aegypti* results from *trans*-dependent redeployment of the GRN, potentially stemming from *cis*-mediated changes in the expression of *sim* in combination with other as-yet unidentified regulators. Our results illustrate a novel "repeal, replace, and redeploy" mode of GRN evolution in which a conserved GRN undergoes neofunctionalization at a new site while its original function is co-opted by a different GRN. Remarkably in this instance, the dramatic shift in gene expression does not result in gross morphological changes, but rather only in subtle differences in development and function of the late embryonic nervous system.

Funding: NIH grants R01 GM85233 and R01 AI081795; USDA grant 2012-67013-19361.

Program Abstract #34

Understanding the immune response to injury in a regenerating mammal (*Acomys spp.*)

Thomas Gawriluk[1], Jennifer Simkin[1], Shishir Biswas[1], Stephen Kiama[3], Vanessa Ezenwa[2], Ashley Seifert[1]
[1]University of Kentucky, USA; [2]University of Georgia, USA; [3]University of Nairobi, Kenya

Discovering molecular pathways and cellular mechanisms that induce epimorphic regeneration in mammals using classic regeneration models has been largely unsuccessful. We recently reported that the African spiny mouse (*Acomys cahirinius*) undergoes epimorphic regeneration in response to a 4 mm circular ear wound. In contrast, *Mus musculus* and sympatric African rodents heal by scarring. This discrepancy is associated with aborted cell-cycle progression and reduced axonal outgrowth. The ability to compare regeneration and scarring in closely related vertebrates (diverged 35.6 million years ago) is unprecedented in regenerative biology. Using this paradigm, we tested the long-standing hypothesis that a bias towards an innate immune response over an adaptive response is associated with epimorphic regeneration in

response to injury. We compared the humoral killing ability of serum and the local *and* systemic cytokine profile between two regenerating species (*A. cahirinus* and *A. percivali*) and two scarring species (*M. musculus* and *Myomyscus brockmani*). Our data show that *Acomys spp.* have stronger humoral killing capacity in response to injury, compared to non-regenerating species supporting a shift towards an innate immune response in regenerating species. Interestingly, our cytokine data strongly suggest that regeneration and scarring induce different pro-inflammatory responses. Scarring is associated with a classic IL-6/IL-8 response, while regeneration is associated with a IL-1/IL-4/IFNγ response. This indicates that regeneration is concurrent with an immune-cell-mediated response. To test this, we characterized cellular immunity (mast, neutrophils, macrophages, and T-cells) present in the healing tissue during regeneration and scarring. These studies provide groundwork to understanding whether or not mammalian regeneration is mediated through the immune response with future studies aimed at functionally depleting or adding specific cell-types and ligands into animals.

Program Abstract #35

Uncovering the RNA-protein interaction network that control vertebrate embryogenesis

Daniel Cifuentes[1,2], Romain Christiano[3], Charles Vejnar[2], Stephanie Lau[2], Carter Takacs[2], Valeria Yartseva[2], Alfredo Castello[4], Matthias Hentze[5], Tobias Walther[3], Antonio Giraldez[2]

[1]Boston University School of Medicine, USA; [2]Yale University, USA; [3]Harvard University, USA; [4]Cambridge University, UK; [5]EMBL, Germany

Clearance of maternal mRNAs is a fundamental step during embryogenesis. To understand this post-transcriptional regulatory mechanism we have investigated I) what are the RNA-binding proteins (RBPs) that mediate mRNA clearance during embryogenesis? II) What are their mRNA targets? III) What are the sequence elements that mediate maternal mRNA regulation? Using Interactome Capture in zebrafish embryos, we have detected 165 proteins that directly bind to RNA during early embryogenesis. Interestingly, most of these proteins are maternally provided, rising the question of how exquisitely timed post-transcriptional regulation is achieved if the RNA-binding proteins are present since oocyte maturation and before maternal mRNA clearance. To address this point we analyzed the binding dynamics to mRNA of these RBPs during embryogenesis. We found that the binding to RNA of 45 of these proteins is developmentally regulated. iCLIP analysis for 22 RNA-binding proteins allowed us to uncover which groups of proteins bind to each functional class of mRNAs (destabilized targets, microRNA targets, highly translated mRNAs) and regulate their fate in a cooperative manner. Finally, we uncovered the embryonic role of Ddx6, a RNA-helicase recently involved in microRNA-mediated translational repression. Interestingly, analysis of the in vivo iCLIP of Ddx6 shows that it binds mainly at the 3'UTRs and Ddx6 zebrafish mutant embryos show impaired microRNA activity at the level of protein translation while microRNA-mediated mRNA decay is intact. In summary, the concerted identification of RBP binding dynamics and their mRNA targets by Interactome Capture and iCLIP provides an invaluable tool to decipher the post-transcriptional regulatory network that shapes gene expression during early vertebrate embryogenesis and establish the framework to understand post-transcriptional regulation in other biological transitions.

Funding: NICHD-NIH K99 grant to D.C.

Program Abstract #36

MicroRNAs: what are they doing in germline cells?

Alexandra Dallaire[1,2], Irfan Bukhari[3,4], Martin Simard[1,2]

[1]St-Patrick Research Group in Basic Oncology, CHU de Quebec - Laval University Cancer Research Centre, CA; [2]Laval University Cancer Research Center, Quebec City, CA; [3]Cancer Research Center, Massachusetts General Hospital, Boston, USA; [4]Harvard Medical School, Boston, USA

Germ cells provide maternal mRNAs that are stored in the oocyte until their translation at a specific time of development. In this context, germline mRNA stability and translation can be decoupled from each other and the control of gene expression mainly depends on post-transcriptional regulators. Among them, microRNAs regulate gene expression by associating with Argonaute proteins to form the microRNA-induced silencing complex (miRISC). In the germline, microRNAs coexist with a high proportion of potentially targeted transcripts. However, the mode of post-transcriptional regulation used by germline microRNAs is currently unknown. Using *C. elegans* as a model organism, we sought to determine how microRNAs affect their targets in germ cells. We observed that germline microRNAs surprisingly stabilize their targets while repressing their translation. This is in striking contrast with somatic microRNA-mediated repression, which mainly results in mRNA destabilization. We hypothesize that microRNAs function through a different mechanism in germ cells and somatic cells. In order to gather new insights on microRNA function, we purified and compared protein complexes associated to germline or somatic microRNAs. Mass spectrometry analyses revealed new miRISC interactors,

among which some are specific to germ cells. Using *in vivo* GFP reporters which are either specific to germline or somatic microRNA activity, we identified the factors differentially involved in somatic or germline microRNA-mediated target regulation. Our results show that germline microRNAs can block translation without initiating mRNA degradation. This study highlights an unexpected function for microRNAs in animal germ cells and suggests their contribution in the stability of maternal mRNAs transmitted to the embryo.

Program Abstract #37

Ciliary transcription factors and microRNAs form a module to collectively regulate multi-ciliogenesis in development and disease

Peter Walentek[1], Ian Quigley[2], Rui Song[1], Dingyuan Sun[1], Umeet Sajjan[1], Lin He[1], Christopher Kintner[2], Richard M. Harland[1]

[1]University of California Berkeley, Molecular and Cell Biology Department, USA; [2]Salk Institute for Biological Studies, Molecular Neurobiology Laboratory, USA

Vertebrates produce several ciliated cell types during development and in adulthood, using a set of conserved ciliary transcription factors (TFs). RFX family TFs are required for all types of cilia (primary and motile), while Foxj1 confers cilia motility, and the Multicilin/E2f/Dp1 complex regulates ciliation in multiciliated cells (MCCs). In addition, ciliated cell type-specific microRNAs (miRs), such as the *miR-34/449* family in MCCs, regulate the production of functional cilia. Here we provide evidence that MCC-specific TFs directly regulate the expression of *miR-34/449s*, which in turn repress multiple targets in MCCs. A key target in MCCs is the centriolar protein *cp110*, because its levels need to be precisely titrated to promote cilia formation: Only optimal Cp110 levels allow for ciliogenesis, while loss of Cp110 prevents ciliary adhesion complex formation and excess levels inhibit cilia formation by distal end capping. These optimal Cp110 levels are achieved through (1) transcriptional activation of *cp110* by ciliary TFs, (2) co-activation of *miR-34/449s* by ciliary TFs, and (3) subsequent post-transcriptional repression of *cp110*. This MCC-specific transcriptional/post-transcriptional regulatory module exemplifies a potentially broader mechanism in development and disease: On the one hand, *cp110* is likely regulated through ciliary TFs in other ciliated cells where it is also subject to miRNA repression e.g. by *miR-129*. On the other hand, ciliary TFs and *miR-34/449s* are misexpressed in certain cancer types, e.g. in gastric cancer, where they are associated with ciliated metaplasia and influence proliferation and malignancy. Taken together, ciliary TFs and miRs seem to form regulatory modules to facilitate ciliogenesis in development and disease.

Funding: PW (DFG, Wa3365/1-1; NIH, K99HL127275), RMH (GM42341), CK (GM076507)

Program Abstract #38

microRNA-31 regulates skeletogenesis in the sea urchin embryo

Nadezda Stepicheva, Jia Song

University of Delaware, USA

The microRNAs are small non-coding RNAs that repress the translation and reduce the stability of target mRNAs in animal cells. microRNA-31 (miR-31) has been found to play a role in cancer, bone formation, and lymphatic development. However, the function of miR-31 in embryogenesis is not well described. We examined the role of miR-31 in early development, using the sea urchin embryo as a model. We found that miR-31 is expressed in all stages of development and its knockdown (KD) resulted in defects in the patterning and function of the primary mesenchyme cells (PMCs). PMCs form the embryonic skeleton that facilitates larval swimming and feeding. Using bioinformatics and luciferase reporter constructs, we identified miR-31 to repress directly *Pmar1*, *Alx1*, *Snail* and *VegfR7* within the gene regulatory network (GRN) of PMCs. Further, blocking the miR-31-mediated repression of *Alx1* and/or *VegfR7* genes in the developing embryo resulted in defects in PMC patterning and skeletogenesis. The majority of the mislocalized PMCs in miR-31 KD embryos did not express *VegfR10*, indicating that miR-31 regulates the ability of PMCs to respond to positioning cues. In addition, miR-31 indirectly suppresses expression of *Vegf3* in the ectoderm. These results indicate that miR-31 coordinately suppresses genes within the GRN of PMCs and in the ectoderm to impact PMC patterning and skeletogenesis. To understand the function of miR-31 at a systems level, we will use miR-31 pull down assays to identify its direct targets. This study will reveal how miR-31 cross-regulates GRNs and signaling pathways to ensure proper development. Since miR-31, GRNs, and signaling pathways are highly conserved in animals, this study will enhance the understanding of fundamental mechanisms used by a developing embryo to build its precise organization.

Funding sources: NSF CAREER (IOS 1553338) to JLS

Program Abstract #39
Tale of tails
Yoshiko Takahashi
Kyoto University, JP
The tail is an important part of the boy that most characterizes the vertebrate. The tail also allows a variety of diversities because in many cases tails are specified to particular purposes of habitation (e. g. long vs short tails, decorative appealing for reproductive tactics). The tail, forming posteriorly to the hind limb/anus level, consists of two major components: the ectoderm (nervous system) and mesoderm (muscles and bones). For such formation, the tail bud, a mass of mesenchymal cells, plays critical roles. In particular, the tail bud cells that participate in the neural tube formation undergo EMT and MET, and this process is called secondary neurulation (SN), markedly different from the well-known neural plate folding seen in the anterior body. Using chickens, we have recently identified the presumptive SN region located posteriorly to Hensen's node in stage 8 chicken embryo (equivalent to ~E8 mouse embryo). Importantly, this region does not contribute to the mesodermal components. Thus, neural tube-forming cells and mesodermal cells are segregated early in the tail-forming region, in which Sox genes play important roles. Furthermore, SN-forming precursors in the tail bud appear to behave as stem cell-like cells. We will discuss the roles of SN in the tail formation and its physiological functions in both embryology and evo-devo.

Program Abstract #40
Collective cell movement in looping morphogenesis
Erina Kuranaga[1,2]
[1]Laboratory for Histogenetic Dynamics, RIKEN CDB, Japan; [2]Laboratory of Histogenetic Dynamics, Graduate School of Life Sciences, Tohoku University, Japan
Although collective migration of cohesive cell groups in vivo is particularly prevalent during embryogenesis and drives complex formations of tissues and organs, we still lack a mechanistic understanding of the underlying concepts. During morphogenesis of *Drosophila* male terminalia, genitalia perform 360 degrees clockwise rotation, which finally induces the dextral spermiduct looping. Our imaging analysis indicated that the unidirectional movement of epithelial cells in A8 tergite that surrounded male genitalia was observed during the rotation. We found that genitalia was able to rotate even in an ex vivo condition, suggesting that this rotation would take place singly without relying on the external force such as attached hindgut or internal tissue deformation. Cells in A8 simultaneously kept adhesion while cells frequently intercalated each other, and we confirmed the contribution of Myosin II and DE-Cadherin, that are responsible for cell intercalation, to the genitalia rotation. Epithelial cells in A8 tergite adopt a left-right asymmetric cell shape within their plane and A8 cells recapitulated contraction and relaxation of their apical plane known as pulsed contraction. To verify whether these cellular behaviors observed within cell plane are enough to induce collective cellular movement, we have performed mathematical considerations and numerical simulations based on a widely accepted model to describe collective dynamics of epithelial cells, called a "vertex model". Through the use of live-imaging technique and mathematical modeling, we are trying to gain better insight into the mechanisms of collective cellular movement during epithelial morphogenesis.

Program Abstract #41
PDGF signaling directs cardiomyocyte movement toward the midline during heart tube assembly
Joshua Bloomekatz[1], Reena Singh[2], Ariel Dunn[1], Megan Vaughan[1], Chin-San Loo[1], Richard Harvey[2], Deborah Yelon[1]
[1]Division of Biological Sciences, University of California, San Diego, USA; [2]Development and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Australia
Communication between neighboring tissues plays a central role in the guidance of cell movement during organ morphogenesis. During heart tube assembly, for example, interactions with the adjacent endoderm are crucial for the movement of bilateral populations of cardiomyocytes toward the midline, a process referred to as cardiac fusion. However, the molecular underpinnings of the relationship between the myocardium and the endoderm remain unclear. Here, we show a new role for platelet-derived growth factor (PDGF) signaling in mediating tissue communication during heart tube assembly. We find that mutation of the zebrafish *pdgfra* gene, encoding PDGF receptor alpha, causes cardia bifida, a failure of cardiac fusion. This phenotype indicates a previously unappreciated function for PDGF signaling during early heart morphogenesis, and this function appears to be conserved, since we find that mouse *Pdgfra* mutants exhibit cardiac fusion defects. Cell tracking experiments in zebrafish *pdgfra* mutants demonstrate that their cardia bifida is the consequence of misdirected cell movements, indicating a specific role of PDGF signaling in steering cardiomyocytes toward the midline. Intriguingly, the PDGF ligand *pdgfaa* is expressed within a portion of the endoderm

that is positioned just medial to the *pdgfra*-expressing lateral plate mesoderm, which contains myocardial precursors. Overexpression of *pdgfaa* throughout the embryo interferes with cardiac fusion, suggesting an instructive influence of PDGF signaling on guiding cardiomyocyte movements. Together, these data uncover a novel mechanism through which endodermal-myocardial communication directs the stereotyped patterns of cell movement that initiate cardiac morphogenesis.

Program Abstract #42

Parallel regulation of convergence and extension by Planar Cell Polarity and notochord boundary signaling

Margot Williams[1], Atsushi Sawada[1], Chunyue Yin[2], Terin Budine[1], Lila Solnica-Krezel[1]

[1]Washington University School of Medicine, USA; [2]Cincinnati Children's Hospital, USA

The remarkable complexity of all animals can be traced back to gastrulation, when germ layers are induced, patterned, and shaped into a rudimentary body plan. During gastrulation, the developing embryo exhibits striking elongation along the anterior-posterior (AP) axis. This axial elongation occurs via highly conserved convergence & extension (C&E) movements, which are driven by polarized cell behaviors including mediolateral (ML) intercalation and directed migration. Although molecular regulation of ML cell polarity underlying C&E is largely attributed to planar cell polarity (PCP) signaling, there is evidence that PCP-independent polarity cues may also be involved. We have identified the chromatin factor Ugly duckling (Udu)/Gon4l as a novel regulator of C&E in zebrafish. Maternal zygotic (MZ) *udu* mutant embryos display a short AP axis, abnormal notochord boundaries, and reduced ML polarity and intercalation of axial mesoderm cells. PCP signaling appears to be unaffected in MZ*udu* mutants, suggesting that *udu* regulates ML cell polarity in parallel to PCP. Indeed, axial mesoderm cells in *kny/glypican4* -/- PCP mutant embryos acquire ML polarity only when adjacent to the notochord boundary, indicating that this boundary provides a PCP-independent cell polarity cue. Importantly, this ability of the boundary to induce ML polarity is lost in *kny;udu* double mutants. Although MZ*udu* embryos exhibit severe mesoderm patterning defects, restoration of Udu/Gon4l in the paraxial mesoderm does not rescue cell polarity in the axial mesoderm. Instead, Udu/Gon4l appears to have a tissue autonomous role in cell polarity within the axial mesoderm, possibly by regulating tension at the notochord boundary. This boundary-associated polarity cue acts in parallel to PCP, and our studies genetically dissect these overlapping signals that regulate ML cell polarity underlying C&E gastrulation movements.

Funding sources: W.M. Keck Fellowship, NIGMS F32 GM113396, NIGMS RO1 GM55101, R35GM118179

Program Abstract #43

Mechano-Devo: Proprioception during development with mechanical signals

Olivier Hamant

Univ. Lyon, ENS de Lyon, INRA, CNRS, France

There is accumulating evidence that single cells in culture can sense mechanical cues to control their division, their growth and their fate. In a multicellular context, this implies that a given cell can use growth-derived stress and shape-derived stress to control its behavior. In this scenario, the presence of mechanical forces in tissues may thus be a way for the cell to know its position and control its differentiation accordingly, during development. To illustrate this idea, I will show how the microtubule response to mechanical stress in Arabidopsis channels cell and organ growth. Beyond the cytoskeleton, mechanical cues are also controlling gene expression. Our recent data suggest that the perception of mechanical stress acts in parallel to major hormones to regulate some of the master regulators in the plant stem cell niche. Altogether, this provides a picture in which mechanical forces add robustness to morphogenesis, by channeling the dynamics of cell effectors and molecular pathways.

Program Abstract #44

The ZP domain protein DYF-7 promotes dendrite extension by preventing rupture of a neuron-glia epithelium

Isabel Low[1], Claire Williams[1], Ian McLachlan[1], Irina Kolotuev[2], Maxwell Heiman[1]

[1]Harvard Medical School, Boston Children's Hospital, USA; [2]University of Lausanne, Switzerland

Epithelia are the interface between an organism and its environment. The outward-facing, or apical, surface of an epithelium is coated with a specialized apical extracellular matrix (aECM) that is distinct from the more well-studied basement membrane ECM. Almost all aECM contains factors bearing a zona pellucida (ZP) domain, suggesting this protein family plays some universal role in epithelial function; yet, no such unifying role for ZP domain proteins has been identified. Previously, we showed that the *C. elegans* ZP domain protein DYF-7 promotes sensory dendrite extension by anchoring nascent dendrite endings at the embryonic nose while the neuron cell bodies migrate away. We wondered if this activity represents a novel role for ZP domain proteins in the nervous system, or if it could shed light on a role shared with

other ZP domain proteins. We show that the sensory dendrites and their ensheathing glia constitute an epithelium with an outward-facing apical surface bounded by tight junctions, and that DYF-7 localizes to this apical surface. In the absence of DYF-7, the dendrites fail to remain anchored at the nose because this epithelium ruptures at glia:glia junctions, likely due to the mechanical pulling force produced during cell migration. Ultrastructural analysis in embryos reveals highly oriented bundles of extracellular filaments coating the apical surface of the neuron-glial epithelium, and these filaments co-localize with DYF-7 and are absent in *dyf-7* mutants. Furthermore, DYF-7 forms similar bundled filaments when expressed in vitro. Therefore, we propose that DYF-7 assembles extracellular filaments that coat the apical surface of an epithelium and prevent it from rupturing under mechanical stress. We speculate that such filaments confer elasticity to the apical surface of an epithelium, and may represent an ancestral and unifying role for ZP domain proteins. *Funding: NIH/NIGMS.*

Program Abstract #45

Mechanical coupling coordinates the co-elongation of notochord and presomitic mesoderm

Fengzhu Xiong[1,2,3], Wenzhe Ma[4], L Mahadevan[3], Olivier Pourquié[1,2]

[1]Department of Pathology, Brigham Women's Hospital, USA; [2]Department of Genetics, Harvard Medical School, USA; [3]School of Engineering and Applied Sciences, Department of Physics, and Department of Organismic and Evolutionary Biology, Harvard University, USA; [4]Department of Systems Biology, Harvard Medical School, USA

Mesodermal structures notochord (NC) and presomitic mesoderm (PSM) elongate together during gastrulation, producing stereotypic pairs of somites sandwiching a continuous NC. This configuration is essential for somite and neural tube patterning. However, PSM and NC elongate with distinct tissue and cellular dynamics. For example, PSM volumes grow drastically with proliferation and addition of new cells from the caudal progenitor zone while NC volume grows little despite continued lengthening.NC cells remain closely clustered while PSM cells exhibit significant local migration and mixing. These differences raise the question if/how PSM and NC elongation are coupled to each other. Here we report that, in avian embryos, while removal of either posterior PSM or NC significantly halts elongation, NC narrowing requires PSM but not vice versa. By implanting soft gels and following their deformation, we found that PSM compresses the NC lateral-medially. The elongating NC in turn pushes on the progenitor zone, whose caudal movement promotes addition of new PSM cells. These highly motile cells may cause the posterior PSM expansion that exerts compression on the NC. Together this mechanical coupling induced by cellular dynamics forms a positive feedback loop, which is sufficient to produce coordinated, steady, and self-sustained elongation in an agent-based computational model. Furthermore, the model recapitulates various perturbations and predicts regulation of elongation speed in response to mechanical and geometrical changes. Our results thus showcase a level of poorly explored mechanical coupling that drive coordinated morphogenesis. We are currently investigating the cellular basis of tissue force generation in the PSM following several possibilities: 1. Expansion driven by motile PSM cells; 2. Lateral medial flow of PSM cells; 3. Cell polarity mediated convergent intercalation.

We thank NIH and HHWF-HHMI for support of this project.

Program Abstract #46

From Matrix stiffness to the Nuclear Lamina, starting with Heart & Brain Development

Dennis Discher

University of Pennsylvania, USA

Tissues such as brain and fat normally have a characteristic softness while tissues such as muscle have a characteristic stiffness, but tissue and matrix physical properties emerge in development, with ill-characterized effects on cells. We have begun to uncover systematic relationships between tissue physical properties in development and differentiation as well as disease, having first shown that matrix elasticity helps specify tissue lineages [1]. Broad analyses of protein levels in embryonic, mature, and fibrotic tissues [2, 3, 4] have revealed that fibrous collagen polymers increase with tissue elasticity *E*, as does nuclear lamin-A (related to keratins) in following polymer physics-type scaling. Lamin-A assembly controls nuclear plasticity, and differentiation of various cell types is modulated by lamin-A levels downstream of matrix *E* and soluble factors such as retinoids [2,4], with pathways such as SRF also being co-regulated by lamin-A. Complementary insights are obtained in structure-property analyses of cell migration, from stem cells to cancer cells [5], with surprising new results emerging for genomic changes.

1. A. Engler ... D.E. Discher. Matrix elasticity directs stem cell lineage specification. ***Cell*** 126: 677-689 (2006).

2. Majkut ... D.E. Discher. Heart-specific stiffening in early embryos parallels matrix and myosin levels to optimize beating. ***Current Biology*** 23: 2434-2439 (2013).

3. J. Swift ... D.E. Discher. Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-directed Differentiation.

Science341: 1240104-1 to 15 (2013).
4. P.C.D.P. Dingal ... D.E. Discher. Fractal heterogeneity in minimal matrix models of scars modulates stiff-niche stem-cell responses via nuclear exit of a mechanorepressor. ***Nature Materials*** 14: 951–960 (2015).
5. T. Harada ... D.E. Discher. Nuclear lamin stiffness is a barrier to 3D-migration, but softness can limit survival. ***Journal of Cell Biology*** 204:669-682 (2014).

Program Abstract #47

Nr2f-mediated prolonged progenitor maintenance sculpts the upper face

Lindsey Barske, Pauline Rataud, Samuel Cox, Ruchi Bajpai, Gage Crump
University of Southern California, USA

Heterochrony in skeletal differentiation has been recognized as a key mechanism underlying morphological evolution between species. This phenomenon also manifests within individuals during development, where regulated variation in the timing of differentiation across a field of skeletal progenitors can influence the formation of distinct skeletal shapes. We recently showed that heterochrony in the developing zebrafish face is achieved by antagonistic interactions between Jagged-Notch and Endothelin1 (Edn1) signaling: Notch selectively maintains undifferentiated skeletal progenitors in the upper face, restricting their differentiation into cartilage, whereas Edn1 opposes this activity in the middle/lower face. From an RNAseq analysis of facial skeletal precursors, we identified the Nr2f nuclear receptors (*nr2f1a*, *nr2f1b*, *nr2f2*, *nr2f5*) as positive Notch and negative Edn1 targets with enriched expression in undifferentiated progenitors. Consistent with a role for these genes in resisting skeletal differentiation, overexpression of *nr2f5* suppresses Edn1 target genes and cartilage differentiation in the middle/lower face, a phenotype nearly identical to that of *edn1* mutants. In Nr2f mutants, ectopic expansion of Edn1 target genes correlates with accelerated chondrogenesis in the upper face, with the small upper jaw transforming into a much larger cartilage resembling the lower jaw. This phenotype is the inverse of the homeotic phenotypes seen in Edn1 pathway mutants. Finally, reduction of Nr2f gene dosage in *edn1* mutants restores the imbalance between these antagonistic pathways and partially rescues the shape of the lower jaw. This mechanism for craniofacial patterning is thus a striking instance of the broader paradigm of antagonistic pathways providing exquisite spatiotemporal control over differentiation to build three-dimensionally complex organs during development. This work was supported by the A.P. Giannini Foundation and the NIDCR.

Program Abstract #48

Spatial and temporal coordination of ventral folding morphogenesis (VFM) with axial extension: role of BMP signaling

Svetlana Gavrilov, Elizabeth Lacy
Memorial Sloan-Kettering, USA

Upon completion of gastrulation in amniotes, the endoderm comprises a single cell layered epithelial sheet. Ventral folding morphogenesis (VFM) is a multi-step morphogenetic process that achieves internalization of the gut endoderm epithelium; concomitantly, VFM coordinates gut tube development with linear heart tube formation, ventral body wall closure and encasement of the fetus in extraembryonic membranes. Initiating at the early somite stage, VFM proceeds concurrently with the extension of the rostral-caudal axis driven by caudal progenitors in the node/tail bud. In human, ventral body wall birth defects result from abnormalities in VFM and consistent with the co-occurrence of VFM and axial elongation, an array of organ malformations often accompanies body wall defects. Despite their high prevalence (1:2000 births), this class of birth defects remains under investigated. Our studies in mice identified BMP signaling as a key pathway mediating VFM, and hence proper organ placement in the post-gastrulation embryo. Characterization of mutant embryos lacking *Bmp2* exclusively in visceral endoderm or in all epiblast derivatives identified anterior visceral endoderm as the source of the BMP2 signal that initiates early/anterior VFM (0-to-8 somite stage), mechanistically linking formation of foregut invagination with concurrent positioning of the head anterior to the heart. To investigate this model and define subpopulations of epiblast-derived cells responding to AVE-expressed BMP2, we applied a conditional *Bmpr1a* allele and lineage-specific Cre transgenes to generate mutant embryos lacking BMP receptor function in subsets of mesoderm, ectoderm or endoderm cells. Our analysis of embryos devoid of BMPR1A activity informs on BMP2 target populations anteriorly and reveals a role of BMP signaling in coordinating late/posterior VFM (8-to-20 somite stage) with concurrent axial extension.

Program Abstract #49

Stat3 is required for proper osteochondro differentiation and defects are central to multiple bent bone syndromes

Michael Hall, Caroline Murray, Michael Valdez, Alan Perantoni

National Cancer Institute, USA
Mice lacking *Stat3* die early in embryogenesis due to a failure at gastrulation and as such, little is understood about Stat3's wider role in post-gastrulation developmental processes. In mice, we found post-gastrulation *Stat3* expression in tissues of mesodermal origin and targeted conditional ablation of *Stat3* in mesoderm, causing dramatic postnatal axial and appendicular skeletal abnormalities, dwarfism, and perinatal lethality. Histologically, *Stat3* mutant mice harbor growth plates with an expanded hypertrophic chondrocyte zone which when coupled with the observed dwarfism and bowed tibiae, are reminiscent of the disease campomelic dysplasia (CD). CD is an often-fatal human congenital abnormality characterized by bending of the long bones and cytogenetically, arises from lesions on chromosome 17q that typically result in a reduced or non-functional SOX9 protein. Ablation of *Stat3* in Sox9-expressing lineages (chondroprogenitors) produces palate and tracheal irregularities found in the *Sox9*$^{+/-}$ mouse model of CD. Furthermore, mesodermal deletion of *Stat3* causes global embryonic down regulation of *Sox9* expression and function *in vivo*. Mechanistically, Stat3 specifically and directly activates the expression of *Sox9* by binding to discrete elements within its proximal promoter following activation. Additionally, *Stat3* mutant bones have reduced mineralization, regional restriction of osteoblast precursors, and reduction in markers of the osteoblast lineage, features inconsistent with CD but reminiscent of Stuve-Wiedemann Syndrome (STWS), a bent bone dysplasia with suspected defects in upstream JAK/STAT signaling. These findings illuminate a dual role for Stat3 signaling in bone development, the modulation of *Sox9* to instruct chondrocyte maturation, and separately in the regulation of the osteoblast lineage. We conclude that *Stat3* is central to normal bone development and that defective signaling is likely operative in multiple human bent-bone pathologies

Program Abstract #50

Lipid-GPCR signaling regulates developmental hematopoiesis and marrow transplant

Jamie Lahvic[1,2,3], Michelle Ammerman[1,3], Pulin Li[1,2,3], Song Yang[1,3], Nan Chiang[4,5], Paul C Norris[4,5], Michael Chase[1,3], Olivia Weis[1,3], Yi Zhou[1,2,3], Charles N Serhan[4,5], Leonard I Zon[1,2,3]

[1]Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital, USA; [2]Harvard Stem Cell Institute, Harvard Medical School, USA; [3]Howard Hughes Medical Institute, USA; [4]Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, USA; [5]Harvard Institutes of Medicine, Harvard Medical School, USA

11,12-epoxyeicosatrienoic acid (EET) signals via an unknown G-protein coupled receptor (GPCR) to mediate a variety of physiological processes, including vasodilation and cardioprotection. We identified EET in a screen for chemical enhancers of hematopoietic stem and progenitor cell (HSPC) transplant in the zebrafish. EET's pro-hematopoietic effects were conserved in mouse transplants as well as zebrafish embryos, where time-lapse imaging demonstrated that EET promoted specification of HSPC from endothelial cells of the embryonic niche. We used chemical and genetic inhibition of various Gα proteins to demonstrate that EET's enhancement of HSPC birth relies on Gα12/13 family members. Tissue-specific transgenics revealed that downstream signaling occurs in endothelial niche cells, rather than in HSPC themselves. These data confirmed that, like EET's known cardiovascular phenotypes, its hematopoietic roles are mediated by a GPCR and work through endothelial cells. Of 10 bioinformatically identified candidate EET receptors, only GPR132 was able to recruit β-arrestin in response to EET. This protein is known to be expressed in endothelial cells and monocytes, and has been shown to couple to Gα12/13. Morpholino knockdown of GPR132 prevented the EET-induced upregulation of HSPC markers. Additionally, GPR132 is a reported receptor for various oxygenated fatty acids. Like EET, these GPR132 ligands increased staining for HSPC markers in the zebrafish. Finally, we performed competitive HSPC transplant using wildtype and GPR132 knockout mice as donors and found that while treatment with EET increases engraftment of wildtype donor cells, no such improvement is seen in GPR132 knockout cells. These data indicate that EET and other small oxygenated fatty acids bind to GPR132 to activate hematopoiesis and improve marrow transplant. Funding: NIH grants 1F31HL129517-01, R01-HL04880, PPG-P015PO1HL3226232, 5P30-DK49216, 5R01-DK53298, 5U01-HL10001-05, R24-DK092760.

Program Abstract #51

Mechanistic Insight into Chromatin Opening and Cell Fate Changes by Pioneer Transcription Factor FoxA

Makiko Iwafuchi-Doi[1], Isabel Cuesta[1], Mark Magnuson[2], Kenneth Zaret[1]

[1]University of Pennsylvania, USA; [2]Vanderbilt University, USA

Gene regulation occurs in the context of chromatin, the complex of DNA and histone proteins that make up nucleosomes. Linker histones bind to nucleosomes and stabilize a condensed, repressive state. A distinct combination of transcription factors is necessary to elicit cell fate changes in embryonic development and cellular reprogramming. Within each group of fate-changing transcription factors, a subset called "pioneer factors" are dominant in their ability to engage silent,

unmarked chromatin and recruit other factors, thereby imparting new function to regulatory DNA sequences. However, the molecular mechanisms by which pioneer factors open chromatin remains unclear. Interestingly, pioneer factor FoxA and linker histone have similar DNA binding domain, and both factors bind near dyad axis of nucleosomes. However, FoxA recognize specific DNA sequences but linker histone does not. We knocked-out *FoxA1/A2* in mouse liver and found that FoxA replaced linker histones from chromatin around FoxA binding sites, thereby locally opening chromatin structure. Furthermore, we identified a FoxA domain that directly interacts with core histones and is required for chromatin opening. When we deleted this chromatin opening domain in the mouse genome, but kept the other FoxA2 parts intact, including DNA binding domain and transactivation domain, a portion of embryos were severely impaired their development, where no embryonic tissues are developed. We are analyzing the chromatin configuration of these mutant embryonic cells by ATAC-seq. Furthermore, we are now modifying the chromatin opening domain and engineering a "super pioneer factor" to enhance chromatin opening ability and cell reprogramming efficiency. These studies provide mechanistic insight into cell fate changes and can ultimately enhance our ability to control cell fate at will.

Program Abstract #52

Gene regulatory control of neural crest axial identity and fate

Marcos Simoes-Costa, Marianne Bronner
California Insitute of Technology, USA

The neural crest is a remarkable cell population characterized by its multipotency and migratory ability. In the embryo, neural crest populations along the body axis differ in developmental potential and cell fate. Whereas the cranial neural crest forms much of the craniofacial skeleton, the trunk crest fails to do so, even when grafted to the head. Here, we explore the regulatory program that imbues the cranial neural crest with its unique features. Using axial-level specific enhancers to isolate and perform genome-wide profiling of pure populations of cranial or trunk neural crest, we identified and characterized the regulatory relationships between a set of cranial-specific transcription factors. This transcriptional circuit conveys anterior regulatory information from gastrula stages to the migratory neural crest that initiates craniofacial development. We employed this regulatory program as a platform to reengineer the trunk neural crest to acquire cranial-like properties. By introducing components of the circuit into the trunk neural crest, we were able to manipulate their axial-identity and endow these cells with the ability to give rise to chondroblasts *in vivo*. Our discovery of a gene regulatory circuit that supports formation of particular neural crest derivatives holds the promise of enabling engineering and replacement of specific neural crest derived cell types.

Program Abstract #53

Identification of genes involved in oocyte specification and differentiation in *Drosophila*

Julie Merkle, Trudi Schupbach
Princeton University, USA

A fundamental question in biology is how cell fates are specified and maintained. In particular, the intricate process by which functional gametes are formed from the germline stem cells has yet to be unraveled. In *Drosophila*, oogenesis begins by asymmetric division of the germline stem cells, and after four mitotic divisions, produces a 16-cell cyst. One of these cells is selected as the oocyte, the future egg, while the remaining 15 become supporting nurse cells. In a genetic screen of lethal mutations in *Drosophila* using mosaic techniques, we identified mutations in several evolutionarily conserved genes that result in a failure of oocyte fate determination. Strikingly, egg chambers in which the germline cells are mutant produce cysts with 16 nurse cells and no oocyte. Two genes identified in this screen are *asteroid* (*ast*) and *Sec24CD*. Although mutation of each gene results in loss of oocyte identity, the stages at which they exhibit defects are different. This suggests that *ast* and *Sec24CD* are important during different steps of the oocyte fate differentiation process. The protein encoded by *ast* is predicted to be involved in DNA repair. Indeed, we observe persistence of double-strand breaks in *ast* mutant clones, indicating that meiosis-induced DNA damage is not properly repaired when *ast* is disrupted. *Sec24CD* mutant clones complete oocyte selection, however oocyte identity is not maintained and the selected oocyte reverts to a nurse cell fate. *Sec24CD*, which encodes a COPII secretory coat component, is required for the generation of secretory vesicles at the ER. Further investigation of *asteroid*, *Sec24CD* and other previously unreported genes involved in various steps along the path to oocyte differentiation will shed much needed light on this multistep cell fate decision. Supported in part by Postdoctoral Fellowship 125598-PF-14-041-01-DDC from the American Cancer Society.

Program Abstract #54

Two developmentally distinct populations of neural crest cells contribute to the zebrafish heart

Ann Cavanaugh[1,2], Jie Huang[1], Jau-Nian Chen[1]

[1]University of California, Los Angeles, USA; [2]Stowers Institute for Medical Research, USA

Cardiac neural crest cells are essential for outflow tract remodeling in animals with divided systemic and pulmonary circulatory systems, but their contributions to cardiac development in animals with a single-loop circulatory system are less clear. We genetically labeled neural crest cells and examined their contribution to the developing zebrafish heart. We have identified two populations of neural crest cells that migrate to the heart at different developmental stages and contribute to distinct compartments of the heart. First, a stream of neural crest cells migrates through pharyngeal arches 1 and 2 and integrates into the myocardium of the primitive heart tube between 24 and 30 hours post fertilization. These neural crest cells give rise to cardiomyocytes. A second wave of neural crest cells migrates along aortic arch 6, envelops the endothelium of the ventral aorta, and invades the bulbus arteriosus after three days of development. Interestingly, while inhibition of FGF signaling has no effect on the integration of neural crest cells to the primitive heart tube, it prevents these cells from contributing to the outflow tract, demonstrating disparate responses of neural crest cells to FGF signaling. Furthermore, neural crest ablation in zebrafish leads to multiple cardiac defects, including reduced heart rate, defective myocardial maturation and a failure to recruit progenitor cells from the second heart field. These findings add to our understanding of the contribution of neural crest cells to the developing heart and provide insights into the requirement for these cells in cardiac maturation.This work was supported by Grants from NIH to JNC (HL081700 and HL096980) and predoctoral fellowships to AMC (T32 GM07104 and T32 HL69766).

Program Abstract #55

Integrating Inquiry Based Learning into the Undergraduate Curriculum

Rebecca Landsberg[1], Ed Freeman[2]

[1]The College of St. Rose, USA; [2]St. John Fisher College, USA

This panel discussion will include three individuals with broad expertise in developing courses and experiences that emphasize engaging students in research. They will share their expertise while speaking to the challenges and successes in implementing inquiry-based learning into each of their home institutions. Dr. Mary Tyler: Beginning in the freshman year, biology students at the University of Maine-Orono are exposed to inquiry-based research through an innovative curriculum developed by Dr. Mary Tyler. Dr. Tyler will discuss working as a faculty member to develop a new curriculum focusing on student-designed research experiences that increase student understanding and interest in biology. She will also discuss developing resources for the greater scientific community. Dr. Sarah Elgin: As the Director of the Genomics Education Partnership (GEP) at Washington University in St. Louis, Dr. Elgin works with faculty from across the country to engage undergraduate students in genomic research projects. Dr. Elgin will discuss the success of the project in increasing students' understanding of the research process, and their knowledge of genes and genomes. The GEP has recently published a paper in G3 Genes|Genomes|Genetics with over 1000 co-authors, 940 of them undergraduates. Dr. Gita Bangera: As Dean of Undergraduate Research at Bellevue College Dr. Bangera began the RISE Program (Research, Innovation, Service, Experiential) while developing the institutional infrastructure to promote a culture of student-centered learning across varied academic areas, beginning in the sciences and moving into other disciplines as well. She will discuss this journey as well as touch on the challenges she faces as a Dean attempting to promote broad undergraduate research experiences

Program Abstract #56

Choose Development! - A long-term and continuing mentoring program to increase the diversity of undergraduates entering research careers in developmental biology

Graciela Unguez[1], Karen Bennett[2], Christine Weston[3], Ida Chow[4]

[1]New Mexico State University, USA; [2]Univ. of Missouri-Columbia, Columbia, MO, USA; [3]Johns Hopkins Univ., Baltimore, MD, USA; [4]Soc For Developmental Biol., Bethesda, MD, USA

The Choose Development! program from the Society for Developmental Biology (SDB) is on its fourth year of active recruitment of undergraduate students from underrepresented (UR) groups and students with disabilities into fields related to developmental biology. Choose Development! Fellows carry out an intensive summer research project in the lab of an established developmental biologist to enhance their interest in science and polish their communication skills, dissemination proficiency, and problem solving capabilities under the supervision of the research faculty and a postdoctoral fellow or advanced graduate student. This multi-level mentoring approach supports professional development activities and emphasizes the significance of the social, academic and scientific enculturation towards their scientific

training. Fellows recognize that establishing, cultivating and utilizing a professional community network is essential to their career productivity and success. Choose Development! provides forums for professional interactions between Fellows and successful scientists in their summer institution and at SDB meetings. 19 Fellows have worked for ≥1 year in laboratories of active SDB members (http://www.sdbonline.org/choose_development). Fellows present their findings at the annual SDB meeting and meet the SDB scientific community. 9 Fellows have completed their degree, 6 have been accepted into graduate programs (Stanford, NYU, Lehigh, Columbia U, UC-Berkeley, and San Francisco State), 1 into medical school (Thomas Jefferson), and 1 works in a research lab. All faculty and lab mentors report multiple advantages gained from working with the Fellows. These outcomes strongly support our hypothesis that long-term advocacy (and mentoring) is successful. Supported by grant NSF-IOS 1239422, with additional funds from SDB.

Program Abstract #57

Short research courses to increase the participation of American Indian students in STEM research

Christa Merzdorf, Jennifer Forecki
Montana State University, Bozeman, MT, USA

American Indians are extremely underrepresented in the STEM fields despite efforts by universities like Montana State University to recruit and retain American Indian students. One way to address this issue is to promote the involvement of American Indian students in undergraduate research, which creates a support network for students. Despite such efforts, it is difficult for student to leave family and home to attend college in the drastically different environment of even a small college town like Bozeman. Thus, we are in the process of developing a stepping stone approach that will provide an introduction to research for tribal college students before they eventually transition to a 4-year university. This program seeks to both introduce students attending tribal colleges to a research mindset and provide necessary exposure to research skills in a laboratory setting, along with extensive mentoring. Integral to the development of this program are visits to tribal colleges around the state to create working relationships and a better understanding of the needs of the students. With the input of tribal college faculty, we developed a one-week intensive research-based course, which was piloted at Montana State University in summer 2015. The students conducted mini-research projects that were centered on the early development of zebrafish, which are an ideal model in the classroom. Students learned how to design and plan experiments, formulate hypotheses, use laboratory equipment, and present data. The week spent on campus, living in dorms, and attending the course also provided invaluable exposure to campus life. Our future goals focus on expanding our program to serve more students by implementing the course at tribal college campuses, developing a more advanced course, and offering alumni of the courses positions as student teachers for deep immersion and as role models.

Program Abstract #58

Development of a course-based undergraduate research experience (CURE) using RNA-seq data from *Drosophila* ovarian tumors

Jessica Seifert[1], Felipe Karam Teixeira[2], Carlos Sanchez[2], Martyna Okuniewska[2], Colin Malone[2,3], Ruth Lehmann[2]
[1]Farmingdale State College, USA; [2]The Skirball Institute, NYU School of Medicine, USA; [3]Institute for Genomic Medicine, Columbia University Medical Center, USA

High-need undergraduate students which include low income, minority, first-generation college and adult learners have lower levels of college success, including 4 year graduation rates, than traditional students. In an attempt to increase the success rate of these students, Farmingdale State College was recently awarded a FITW grant from the USDOE to determine if institutionally-supported undergraduate research opportunities have a meaningful impact on the 4 year graduation rates of high-need learners. One approach being taken by the Biology Department is to develop a course-based undergraduate research experience (CURE) that will be taken by all Bioscience majors. This course will provide an authentic yet, structured, learning experience with specific processes to facilitate student learning outcomes. In collaboration with Ruth Lehmann's research group at NYU, we have generated RNA-seq libraries from the knockdown of 6 independent genes that each gives rise to ovarian tumors in *Drosophila*. Ovarian tumors occur when the germ-line stem cell or a germ-line progenitor cell fails to differentiate and/or continues to undergo cell division. The regulation of a cell's decision to self-renew versus differentiate is fundamental to tissue homeostasis and cancer biology. Students will develop testable hypotheses based on this essential biological question. In addition, students will engage in all aspects of the RNA-seq data analysis pipeline using publically accessible bioinformatics platforms from the iPlant Collaborative and Cold Spring Harbor DNA Learning Center. The goal of the course is to allow students to have an authentic yet structured research experience. Students will engage in the process of hypothesis formation and scientific method. Finally, students will be evaluated based on meeting specific learning outcomes within the disciplines of Genetics, Developmental Biology and Bioinformatics.

Program Abstract #59

Viktor Hamburger & Planarian Regeneration: Peer Education in an Elementary School Setting

Esperanza Evsikova[1], Rafael Evsikov[1], Anne Scott[1], Caralina Marin de Evsikova[2]

[1]Lee Academy, United States; [2]University of South Florida, United States

Science, technology, engineering, art and mathematics (STEAM) education is a current focus in K-12 educational settings to improve participation and cultivate future generation in critical and analytical thinking. While a variety of approaches can be integrated in the elementary school setting, we used a peer mentoring in an experiential setting of the daily science classroom to achieve greater understanding of basic scientific principles, such as regeneration, in young children. Another goal this peer learning activity was to reinforce the scientific concepts of making a hypothesis, and the use of experimental design and control, to answer the question posed by the hypothesis. The primary author, a 8-year-old student, taught her peers (children ranging from 4-9 years of age) to understand the principle of regenerative biology by repeating the experiments of Viktor Hamburger and extending this original research by conducting a group experiment on to test two hypothesi: 1. complex body parts, such as a head, vs. more than simple body parts, like a tail, will take longer time to regrow 2. It will take longer time to regrow multiple body parts than just one body part. Methods. Environment: the classroom, level C, was a mixed age, 7-9 years plus 4 year old, in their school science room, where provided a dissecting stereomicroscope, jars, paint brushes and planria (species). Next she gave a short lecture on regeneration, Dr. Viktor Hamburger, and a summary on his work with planaria. She explained the two hypothesi and taught the proper use of the equipment. All students participated in generating specimens for the experimental and control groups. The outcome was measured by students recalling both orally and in written assessment the scientific concepts of hypothesis, experiment, control, instruments, observation. Overall, the students had greater recall accuracy and retention in questions regarding these concepts more than other lessons taught in the academic year.

Program Abstract #60

Learning through research: Student-driven screen for genes involved in sperm development using planarian flatworms

Labib Rouhana

Department of Biological Sciences, Wright State University, USA

Involvement in undergraduate research has a positive influence on student retention and coursework performance. However, the ability to provide every student with the opportunity to perform undergraduate research is limited by several factors, which include: 1) time and space constrains on faculty to mentor several undergraduate students in the laboratory; 2) timely student awareness of research experience opportunities; and 3) funds to provide students with resources to perform modern research. A 3-week summer course (BIO4490) for advanced undergraduates at Wright State University was developed to identify genes involved in spermatogenesis. During the course, groups of 6-12 students were provided with cDNA constructs, which they used in experiments to determine the tissue of expression (by *in situ* hybridization) and function (by RNA-interference) of each gene in planarian flatworms. Students also formulated and presented hypotheses on putative developmental requirements for some of these genes, based on information from BLAST analyses and literature research. BIO4490 provides undergraduate students with an authentic research experience under short-term commitment. However, discoveries made during the summer course can be furthered by committed students in the form of an undergraduate honors thesis or as a research publication.

Support for this work is provided by grant no. 1R15HD082754-01 from the NIH NICHD.

Program Abstract #61

Guiding Education through Novel Investigation (GENI): Using a reverse genetic screen in *Caenorhabditis elegans* to engage undergraduates in the research cycle

Jennifer Tenlen, Daihong Chen, Kathryn Houmiel, Andrew Lumpe, Derek Wood

Seattle Pacific University, USA

Student participation in authentic, original research has been demonstrated to enhance their engagement in biology courses, and to improve their processing and critical thinking skills. Integration of faculty research into lab courses allows more students the opportunity to engage in research than could be accommodated by individual faculty labs. The GENI-ACT program (http://www.geni-science.org/) facilitates learning in undergraduate science courses through shared and collaborative authentic research projects in biology and biochemistry. Through the GENI-ACT website, detailed protocols and reagents are available to instructors who wish to implement these projects in their own courses. The results generated in this program are intended for publication in peer reviewed literature or scientific databases. We describe here a quarter-long research project in an upper-division Genetics course, in which students use RNA interference (RNAi)

in a sensitized genetic background to identify novel genes involved in endoderm specification in the nematode *Caenorhabditis elegans*. Candidate genes identified in this primary screen are further characterized in a subsequent Developmental Biology course, allowing students to experience an extended research cycle. Formal assessment of students' feelings of efficacy in research, and engagement in the course was conducted using a validated Student Science Learning Gains (SSLG) instrument. Students reported statistically significant enhancement in several areas, including their attitudes toward science and feelings of self-efficacy in conducting research, their ability to transfer prior research experiences to new problems, and their understanding of core concepts. These findings strongly support the value of implementing authentic research in undergraduate courses, and demonstrate the efficacy of the GENI-ACT platform in promoting collaborative research. This research was supported by NSF TUES DUE-1322848.

Program Abstract #62

Drawing Embryos Together: Seeing *Crepidula fornicata* Development Under the Microscope and in Virtual Reality

Beatrice Steinert

Brown University, USA

Seeing microscopic, dynamic, and highly spatially complex objects such as developing embryos involves myriad kinds of senses, skills, materials, and instruments. Here I present two processes of seeing embryos of the marine slipper limpet *Crepidula fornicata*: that of the late 19th and early 20th century embryologist Edwin Grant Conklin and my own process of working with *Crepidula* embryos to create interactive, virtual reality 3-D models to see them in a new way. Conklin was one of the first to harness technologies such as histological staining and the camera lucida to meticulously investigate the very early stages of development with a particular focus on tracing lineages and establishing identities of individual cells. For Conklin, the multisensory process of producing hundreds of camera lucida sketches and drawings played a central role in his ability to see and produce knowledge about *Crepidula* development. Motivated by exploring new ways of seeing embryos and to build on this history, I generated digital 3-D models of *Crepidula* embryos from confocal data sets that could be visualized in the YURT, the virtual reality theater housed at Brown University. Virtual reality tools such as the YURT can transform the way we see developing embryos by bringing them out from under the microscope and into our world. The YURT engages the entire body, and thus all the senses, in the act of seeing and allows the user to closely examine the embryo from every possible perspective, to move it around, and to walk around and even inside of it. These tools have the potential to be especially powerful for teaching development, giving students a more comprehensive and intuitive understanding of the spatial complexities of developing embryos.

Program Abstract #63

Inexpensive fluorescent staining techniques to enhance student experiments in zebrafish development

Judith Cebra-Thomas, Matthew Smith, Moira Dougherty, Taylor Parker, Colette Sweitzer

Millersville University, USA

The inexpensive NIGHTSEA^{TM} stereo microscope fluorescence adapter is expanding the possibilities for incorporating fluorescence microscopy into student laboratories in developmental biology. Many exciting experiments can be performed using zebrafish lines in which specific lineages have been tagged by expression of fluorescent proteins, or GloFish^{TM}. To complement these approaches in a way that does not require maintaining special zebrafish stocks, we have explored the use of fluorescent stains for cell death, lateral line neuromasts, and calcified bone formation. The hair cells of the zebrafish lateral line provide an excellent model for the hair cells of the mammalian inner ear, and are susceptible to ototoxic chemicals including environmental toxins (e.g. copper) and antibiotics (e.g. neomycin). These staining techniques can be used in conjunction with Developmental Biology laboratories or student projects that explore the effect of teratogens (e.g. ethanol or valproic acid exposure) or environmental perturbations (e.g. heat shock) on embryonic development.

Funding source: National Science Foundation, Millersville University

Program Abstract #64

The Mystery Mouse: Can your undergraduates solve the mystery and identify their transgenic mouse?

Barbara Murdoch

Eastern Connecticut State University, United States of America

There is a projected shortfall over the next decade of more than 1 million science, technology, engineering and math (STEM) graduates. In fact, less than 40% of STEM majors graduate with a STEM degree. To retain a stronghold in the STEM fields and the associated economic, social and security benefits, strategies for increased retention of STEM students are needed. Research experiences within the first few years of university are known to increase STEM retention.

But typically there are limited research opportunities for undergraduates, especially in stem cell and developmental biology. Here I demonstrate how collaborations between research-based and teaching-based universities can provide precious materials that are not normally accessible to students at primarily undergraduate universities. During the *Mystery Mouse* laboratories, students are guided through a series of inquiry-based experiments that allow them to elucidate from their own research, the presumed identity of their *Mystery Mouse*. Students interrogate wholemounts and/or tissue from transgenic mice (that was generously provided by colleagues residing at research-based universities) using a variety of molecular and genetic techniques, including histochemistry, immunohistochemistry and genotyping. From their accumulated data and after literature review, they prepare a research paper outlining their experiments, conclusions and identity of their *Mystery Mouse*. Students who participate in these inquiry-based, rather than "canned" laboratories are more engaged in their learning, show increased retention of course material and demonstrate critical thinking skills. These types of hands-on undergraduate laboratories present unique opportunities for collaboration with research-driven laboratories and primarily undergraduate institutions, while simultaneously addressing the shortfall of STEM graduates.
Funding was provided by the CSU-AAUP and the Eastern Connecticut State University Biology Department.

Program Abstract #65
Incorporating Experimental Design, Research Protocol Writing, and Research Practice in the Traditional Developmental Biology Laboratory
Gary Lange
Saginaw Valley State University, USA
With its broad scope and diverse organism focus, the traditional developmental biology laboratory can be an excellent place to incorporate instruction about techniques designed to help the undergraduate biology major think more like a practicing researcher. The traditional instructional laboratory in development examines an array of different organisms, and each is briefly observed for specific, observable processes defining concepts of development. Frequently seen in the semester's laboratory of this course, are labs observing organisms such as ferns, rockcress, slime molds, urchins, flies, amphibians, chickens, zebrafish, and mammals. Developmental processes examined with this range of organisms includes fertilization, growth, specification, morphogenesis, differentiation, regeneration, and overall control and timing of development. Students who study development in this traditional format acquire a broad range of knowledge and skills. In this poster, a model is shown suggesting how traditional aspects of the developmental biology laboratory can serve as an effective vehicle for a semester-long focus on research practice is highlighted. Shown is how instruction in this lab can help students develop skills in experimental design, research protocol writing, and strong research practice. In my course, the first quarter of the semester focuses on making decisions about interesting developmental questions to study. The second quarter has students writing and submitting persuasive research protocol applications. And, the last half of the semester has students execute their experiments, analyze data, and report their findings.
With this focus on experimental design, writing, and research practice, undergraduate students improve their skills on how laboratory-based biology research is conducted. Through this effort, students become better prepared for the next steps in their journey to their future career.

Program Abstract #66
Using Concept Mapping to Improve Comprehension of Scientific Literature Among Undergraduates
Jialiang Wang
The University of Georgia, United States
Concept Mapping is a visualized illustration of concepts and their relationships. A PhD program in biology requires comprehension of scientific papers for the development of one's dissertation project. In many undergraduate programs, no systematic curriculum exists to help students acquire skills of scientific reading. Due to this area of need, I focus on the use of concept mapping when reading scientific literature. This study is specifically narrowed to biology major undergraduates who are planning to go to graduate school. A three-step training is incorporated to help students organize substantial information from scientific papers. The first phase was observing and understanding, where the definition and importance of concept mapping in biological research was introduced. Students were shown how to draw concept maps by extracting key words from a topic and then building connections. The second phase was trial and experiencing, where students were divided into two groups to test the efficacy of concept mapping. Group one read a selected paper with the help of a pre-made concept map, while group two read the same paper without the concept map. Two groups then took the same quiz designed based upon the paper they read. Students from group one showed an increased understanding of the paper as evidenced by getting a higher average score. The final phase was teaching and evaluation, where students were assigned a review paper and instructed to make their own concept maps and then presented to the class. Based on the data

collected as well as student evaluations, it shows that concept mapping can help biology major undergraduates distinguish the primary idea (core concept) and secondary ideas (key concepts) within a paper. This approach can also strengthen their ability to comprehend large amounts of information. Therefore, concept mapping is an effective and directed training for undergraduates who want to pursue careers in research and academia.

Program Abstract #67
Criteria about terminology and concepts of oocytes and embryos covers
Ana Sanz Ochotorena, Yamilka Rodríguez Gómez
Faculty of Biology. University of Havana, Cuba
Protective formations that surround the eggs vary in structure of most animals. Oocyte's covers or egg membranes are clasiffied as primary, secondary and tertiary according to their characteristics and the site of its synthesis and deposition. The often called primary membranes are those developed in the ovary and cover the surface of the egg outside the plasma membrane. The primary membrane is called vitelline membrane in insects, mollusks, amphibians and birds, chorion in tunicates and fish and it is the zona pellucida in mammals. Insects have a second thick membrane also called chorion, which is classified as primary or secondary by different authors. Secondary membranes are secreted by the oviducts and parts of the genital system while the egg is passing to the outside. They include the jelly coat of frog's eggs and the albumen and shell of bird's eggs. However you can find the same definition for tertiary membranes. On the other hand, chorion is one of the two membranes that surround the fetus, it is the outer membrane of amniote`s embryo serving as a protective envelope and forms fetal placenta in mammals. Because of these inaccuracies and the use of similar names for different structures, the aim of this paper is to analyze these concepts. Experiences with students of the Biology major at University of Havana are exposed in this work. Results about surveys of teaching process applied to students in the courses of Developmental Biology are shown. There are difficulties in the assimilation of these concepts in students due to the contradictions in the historically used terminology. We proposed for primary oocyte membranes the use of the term zona pellucida at least in vertebrates and reserve the term chorion to the cover of amniote embryo.

Program Abstract #68
Virtual Museum of Natural History
Daniel DiCorpo[1], Eric Edsinger[2], Lydia Mathger[2], Rudolf Oldenbourg[2], Linda Amaral-Zettler[2], Erik Zettler[4], Thor-Seng Liew[5], James C Weaver[6], David Rich[7], Steve Pieper[8], Eliot Michaelson[1], Charlotte Frank[1], James S. Michaelson[1], Christos Arvanitidis[3]
[1]Pathology / Surgery Depts., Massachusetts General Hospital, United States; [2]Marine Biological Laboratory, United States; [3]Hellenic Center for Marine Research, Greece; [4]Sea Education Association, United States; [5]Institute for Tropical Biology and Conservation Universiti Malaysia Sabah, Malaysia; [6]Wyss Institute, Harvard University, United States; [7]MathWorks, United States; [8]Surgical Planning Lab, Brigham & Women's Hospital, United States
The "Virtual Museum of Natural History"™(VMNH) is a new, multi-institutional project for sharing and viewing high resolution 3D data of objects of interest, especially biological organisms, for naturalists, developmental biologists, medical scientists, archaeologists, anthropologists, geologists, etc. Scientists can freely use the VMNH web site to upload, download, and discuss a wealth of 3D data. To motivate participation, users who prepare a publication using the VMNH will offer co-authorship to the original up-loaders. Viewing of 2D cross sections is available to anyone and videos of 3D renderings are available on our YouTube channel youtube.com/channel/UCGCBXNkhm6BMQxtV_Thvkmw. Volumetric data acquired from Micro Computed Tomography (Micro CT) devices (SkyScan1275 and Nikon-XTH225) has provided our initial 1200 datasets from about 900 specimens. The largest phylum represented is mollusks, for which we have 722 shell specimens of 198 species in 172 genera. Of special interest are 598 shell specimens in graded sizes (growth series) of 88 species in 75 genera. For example, 23 specimens of *Nautilus pompilus* range from 10 mm to 22 cm. Other specimens include animal eyes, marine eyes, de-identified human autopsy specimens, brittle worms, annelids, cephalopods, and various vertebrates. The Marine Biological Lab Marine Resources Center also provided 111 specimens including 32 animals, 31 invertebrates, and 2 seaweeds (*S. fluitans* and *S. natans*). All organisms were first imaged unstained to reveal the radiographically dense anatomical features, and imaged again after staining with Iodine to reveal soft tissues. Plans are underway to add datasets from embryos at graded stages of development in chickens, zebrafish, mice, humans, and marine invertebrates. Those with a particular interest in the VMNH are encouraged to participate on a yearly basis as a "virtual curator" representing a particular group of datasets. Find us at: virtualmuseumnaturalhistory.org and virtnat.org

Program Abstract #69

The Developmental Consequences of the Evolution of Transcription Factor Function

Greg Cary, Rene Francolini, Alys Jarvela, Veronica Hinman

Carnegie Mellon U, USA

It is well documented that GRNs can evolve extensively through mutations to cis-regulatory modules. Transcription factor proteins that bind these cis-regulatory modules may also evolve to produce novelty. Coding changes, however, are considered to be more rare, because transcription factors are highly pleiotropic and hence are more constrained to evolve in ways that will not produce widespread detrimental effects. Recent technological advances have unearthed a surprising variation in DNA-binding abilities, such that individual transcription factors may recognize both a preferred primary motif and an additional secondary motif. This provides a source of modularity in function. In this talk, we will present recent work that shows that orthologous transcription factors can also evolve a changed preference for a lower affinity secondary binding motif, thereby offering an unexplored mechanism for GRN evolution. We demonstrate that this difference may allow for greater evolutionary change in timing of regulatory control and provide a mechanism through which organisms can evolve a changed response to signaling gradients. . This uncovers a layer of transcription factor binding divergence that could exist for many pairs of orthologs.

Program Abstract #70

Evolution of gastrulation: fate mapping, live-imaging, and gene expression analyses in the snail *Crepidula fornicata*

Deirdre Lyons[1,3], Kimberly Perry[2], Jonathan Henry[2]

[1]Duke University, USA; [2]University of Illinois, Urbana-Champaign, USA; [3]Scripps Institution of Oceanography, USA

The superphylum Spiralia is the largest clade of bilaterian metazoans. The Spiralia exhibit extremely diverse larval and adult body-plans, yet many species share a stereotypical mode of development including a conserved fate map and cleavage pattern, referred to as spiral cleavage. The spiral cleavage pattern offers a unique opportunity to discover how diverse body-plans evolved, by studying homologous cell lineages at the single-cell level. Yet, compared to deuterostomes and ecdysozoans, spiralians have received much less attention as subjects for uncovering the cellular and molecular mechanisms controlling development. Gastropod slipper snails in the genus *Crepidula* have emerged as models for functional developmental studies. Work on *C. fornicata* has led to key insights into the role of an embryonic organizer, high-resolution fate maps, and the first demonstration of CRISPR/Cas9 genome editing in the Spiralia.

Recently, we used this species to make the first comprehensive study of spiralian gastrulation, using cell-labeling and live-imaging. Gastrulation is critical for metazoan development, directly linked to germ-layer segregation, axis establishment, and gut formation. The fate of the blastopore (the site of gastrulation), varies among metazoans: it can become the mouth, the anus, both, or neither. Thus, changes in gastrulation likely influenced body-plan evolution, but the mechanism is debated, partly because it is difficult to homologize the blastopore across distantly related taxa. Spiralians offer a solution, because the homologous cleavage pattern allows direct comparison of cells around the blastopore.

Combining lineage studies with gene expression analyses, we constructed a fate map linking regulatory factors to specific cell lineages. These data help explain observed variation in blastopore morphogenesis among spiralians, and have profound implications for hypotheses about gastrulation evolution in metazoans in general. Funding source: NSF.

Program Abstract #71

The molecular evolution of tissue polarity: Insights from early embryogenesis of *Nematostella vectensis*

Miguel Salinas-Saavedra

University of Florida, USA

In most bilaterian animals, embryonic and cell polarity are set up during embryogenesis with the same molecules being utilized to regulate tissue polarity at different life stages. aPKC, Lgl, and Par proteins are conserved components of cellular polarization, and their role in establishing embryonic asymmetry and tissue polarity have been widely studied in model bilaterian groups. However, the role of these proteins in animals outside Bilateria has been scarcely studied. We addressed this by characterizing the localization and interactions of different components of the Par system during early development of the sea anemone *Nematostella vectensis*, a member of the clade Cnidaria. Immunostaining using specific *N. vectensis* antibodies and the overexpression of mRNA-reporter constructs show that components of the *N. vectensis* Par system distribute throughout the microtubule cytoskeleton of pre-blastula stages without clear polarization along any embryonic axis. However, they become asymmetrically distributed at later stages, when the embryo forms an ectodermal epithelial layer, and the interaction between them maintain the stability of cell-cell contact in a manner seen in bilaterian animals. Interestingly, the protein-protein interactions described for this system in Bilateria is present during the whole development of *N. vectensis*, suggesting possible integration of other pathways to generate symmetry breaking. *N.*

vectensis exhibits clear polarity at all stages of early embryonic development, which appears to be established independent of the Par system reported in many bilaterian embryos. However, in *N. vectensis*, components of this system are deployed to organize epithelial cell polarity at later stages of development. This suggests that Par system proteins were co-opted to organize early embryonic cell polarity at the base of the Bilateria and that, therefore, different molecular mechanisms operate in early cnidarian embryogenesis. Thanks to NSF

Program Abstract #72

Archenteron cilia are required for symmetry breakage in the sea urchin

Martin Blum, Matthias Tisler, Sabrina Mantino, Franziska Wetzel, Philipp Vick
University of Hohenheim, DE

Left-right (LR) organ asymmetries are a common feature of metazoan animals. In most cases, laterality is established by a conserved asymmetric Nodal signaling cascade during embryogenesis. In fish, amphibians and mammels, asymmetric nodal induction results from a cilia-driven leftward fluid flow at the left-right organizer (LRO), a ciliated epithelium present during gastrula/neurula stages. Conservation of LRO and flow beyond the vertebrates has not been reported yet. Here we study sea urchin embryos, which use nodal to establish larval LR asymmetry as well. Cilia were found in the archenteron of embryos undergoing gastrulation. Expression of foxj1 and dnah9 suggested that archenteron cilia were motile. Cilia were polarized to the posterior pole of cells, a prerequisite of directed flow. High-speed videography revealed rotating cilia in the archenteron slightly before asymmetric Nodal induction. Removal of cilia through brief high salt treatments resulted in aberrant patterns of nodal expression. Our data demonstrate that cilia - like in vertebrates - are required for asymmetric nodal induction in sea urchin embryos. Based on these results we argue that the anterior archenteron represents a bona fide LRO and propose that cilia-based symmetry breakage is a synapomorphy of the deuterostomes.

Program Abstract #73

Ancient deuterostome neurogenic gene regulatory networks revealed by sea urchin neurogenesis

Leslie Slota, David McClay
Duke University, USA

A common theme of metazoan neurogenesis is the use of conserved classes of transcription factors to build anatomically diverse nervous systems. However, in many cases there are critical differences in expression and function of neural transcription factor orthologs between protostome (especially *Drosophila*) and deuterostome model organisms, leaving their ancestral functions unknown. Here we use a basally branching deuterostome, the sea urchin *Lytechinus variegatus*, to determine how three neurogenic transcription factors contribute to neurogenesis and to the generation of neural subtypes. We focus on the roles of two bHLH transcription factors, *achaete-scute* and *neurogenin*, and one homeobox transcription factor, *orthopedia.* We show for the first time that subcircuit gene regulatory networks which give rise to different neural subtypes are conserved between echinoderms and vertebrates. Similar to their role in vertebrates, *achaete-scute* and *neurogenin* are used in a proneural fashion in *L. variegatus*, with *Lv-achaete-scute* required for specification of serotonergic neurons in the apical organ and *Lv-neurogenin* for the specification of a population of neurons in the ciliary band. Furthermore, we find that *Lv-orthopedia* is required for the downstream differentiation of a population of dopaminergic/ cholinergic neurons, a role strikingly similar to that in vertebrates. We propose that these subcircuit GRNs are ancient components of the deuterostome lineage, possibly of all bilaterians, and were employed by the common ancestor of chordates and ambulacrarians during neurogenesis. Funding: NSF GRFP Fellowship; NIH RO1-HD-14483; NIH PO1-HD-037105.

Program Abstract #74

Comparative analysis of global regulatory gene deployment reveals tempo and mode of alterations to developmental gene regulatory networks in echinoids

Eric Erkenbrack[1,2]
[1]*California Institute of Technology, USA;* [2]*Yale University, USA*

Developmental gene regulatory networks (GRNs) are assemblages of regulatory genes directing embryonic development of animal body plans. Alterations to GRN circuitry cause variation in developmental programs both during an individual's development and evolution of individual lineages. These networks are best exemplified by the global embryonic GRN directing early development of the euechinoid sea urchin *Strongylocentrotus purpuratus*. Notably, research on closely-related euechinoids in the sea urchin order Camarodonta, e.g. *Lytechinus variegatus* and *Paracentrotus lividus*, has revealed marked conservation of circuitry in this global GRN, suggesting little appreciable alteration has occurred since

the divergence of camarodonts at least 90 million years ago (mya). To test whether this observation extends to all echinoids, we undertook a systematic survey of global spatiotemporal activity and GRN circuitry of 65 regulatory genes in the cidaroid sea urchin *Eucidaris tribuloides*, which diverged from euechinoids at least 268 mya. Our data reveal alterations to all levels of echinoid GRN architecture since the cidaroid-euechinoid divergence. Alterations to mesodermal subcircuits were particularly striking, including functional differences in specification of non-skeletogenic mesoderm, skeletogenic mesoderm and endomesodermal segregation. While GRN circuitry specifying endomesodermal embryonic domains had clearly diverged, intriguingly these domains became populated by remarkably similar transcription factors and regulatory states in pregastrular embryos of these two groups. Analyses of *E. tribuloides* dorsal-ventral (aboral-oral) specification further suggested that mesodermal regulatory genes incurred more alterations to deployment than those in endoderm and ectoderm. Collectively, our data highlight the remarkable lability of GRNs in developmental evolution and suggest that mesodermal regulatory genes have undergone disproportionate and extensive rewiring in this clade.

Program Abstract #76

The role of toolkit genes in the evolution of the complex abdominal color pattern of *Drosophila guttifera*

Komal Kumar Bollepogu Raja, Peter Nouhan, Evan Bachman, Alexander McQueeney, Elizabeth Mundell, Amber Peabody, Alexandri Armentrout, Thomas Werner

Michigan Technological University, USA

How gene-regulatory networks drive morphological diversity is an intriguing evo-devo question. Animals share a common genetic toolkit, which orchestrates the building of the basic body plan. Some toolkit genes have been coopted into new developmental pathways, thereby leading to many evolutionary novelties. One example is the formation of color patterns in animals. We developed the fruit fly *Drosophila guttifera* as a model to study complex wing and body color patterns. The coloration of *D. guttifera* is striking, as this species displays spot and stripe patterns on its wings, thorax, and abdomen. Previously, we have shown that the Wingless morphogen is sufficient to induce the pigmentation gene *yellow* (*y*), which is necessary for the production of black melanin spots on the wings of *D. guttifera*. Our current research focuses on the abdominal pigmentation pattern of the same species, which consists of four distinct sub-patterns: one pair of dorsal, median, and lateral rows of spots, plus a dorsal midline shade. Our *in situ* hybridization and immunohistochemistry data in developing pupae show that the pigmentation gene *y* is expressed as mRNA and protein in the same pattern as the adult melanin pattern. In our search for regulators of *y* expression on the abdomen, we found that the toolkit gene *wingless* foreshadows the entire spotted pattern just before *y* transcription starts, while *abdominal-A*, *decapentaplegic*, *hedgehog*, and *zenknullt* are also expressed at that time, but only in distinct subsets of the abdominal pattern. Using a transgenic reporter assay in *D. guttifera* pupae, we have identified a ~1 kb fragment within the *y* intron that drives DsRed in a pattern closely resembling the adult abdominal spot pattern. We are currently sub-dividing this 1 kb fragment to identify the core enhancer sequence to allow us to narrow down on the putative transcription factor binding sites that may activate *y* in the spotted pattern.

Program Abstract #77

Resolving the molecular mechanisms by which DNA mutations alter the function of a genetic switch

Emily Wey, Thomas Williams

University of Dayton, United States

An individual genome can be anticipated to possess thousands to upwards of a million mutations that are genetic baggage from DNA replication mistakes or "mutations" that occurred in the past. Each mutation can have one of three outcomes on an individual, these are to improve, reduce, or have no effect on fitness. Moreover, the effects of such mutations can depend on the presence or absence of other mutations, so called epistatic interactions. A major goal of genomic medicine is to glean diagnostic or predictive health information from the genome sequences of individuals. However, this goal remains out of reach as the effects of mutations and epistatic interactions are difficult to predict without knowing the function of the DNA sequence they reside in. This difficulty is especially heightened for mutations occurring in enhancer sequences that act as switches to control gene transcription. Our research uses a fruit fly model to test hypotheses about the molecular mechanisms by which mutations alter a genetic switch's activity and whether these mutations are subjected to the tyranny of epistatic interactions. Specifically, we are investigating the *Drosophila* (*D.*) *melanogaster* dimorphic element which is a transcription-regulating enhancer for the *bric-à-brac* genes. Three mutations in the dimorphic element were identified that individually alter the level of the enhancer's activity. The presence or absence of epistatic interactions will be determined by measuring the activity of dimorphic elements from related species that have been engineered to possess the *D. melanogaster* mutations. We are also testing the hypothesis that these mutations impart their effects by

creating or destroying binding sites for transcription factor proteins. The results will provide a sorely needed example where an understanding of molecular mechanisms bridges the gap between a DNA sequence and its *in vivo* function.

Program Abstract #78

Understanding gene expression regulation and its evolution through genome editing and transgenesis approaches

Alexandra Hallagan, Thomas Williams
University of Dayton, United States

Trait development occurs by temporally and spatially regulated gene expression, and changes in gene expression play a major role in the origination, diversification, and loss of traits. Gene expression is controlled by cis-regulatory elements (CREs), and a CRE's pattern of gene regulation results from its combination of transcription factor binding sites that are realized in certain cell types and developmental stages. Furthermore, patterns of gene expression are often driven by the collective input of multiple CREs, including ones that appear functionally redundant. It remains inadequately understood how evolved combinations of transcription factor binding sites drive new gene expression patterns and to what extent gene expression evolution is shaped by the input of multiple CREs. One approach to study CREs is reporter transgene assays, where a CRE is coupled to an easy to monitor reporter gene, such as GFP. However, this method evaluates CREs outside of their endogenous context that may include other, perhaps redundant, CREs. Also, the necessity of a CRE often remains unexplored as the endogenous CRE is not perturbed in reporter assays. Moreover, orthologous CREs thought to drive divergent patterns of gene expression are typically tested in a convenient model organism, which cannot resolve to what extent differences in gene expression result from the mutational modification of the orthologous CREs and by mutational changes in another gene or genes. We have been utilizing the diverse patterns of fruit fly abdominal pigmentation as a model trait to understand gene expression regulation and its evolution. Here we present our early results for tests of CRE necessity by a genome editing approach and tests for CRE sufficiency in reporter transgene assays in multiple fruit fly species.

Program Abstract #79

The evolution of partially redundant shadow enhancers underlies the origins of a signaling center essential for a novel morphological feature

Sarah Smith, William Glassford, Winslow Johnson, Mark Rebeiz
University of Pittsburgh, USA

The deployment of signaling pathways in new patterns is thought to contribute to the origins of novel anatomical formations. Many developmental loci, including signaling pathway ligands tend to have a complex regulatory architecture, which often includes redundant transcriptional regulatory sequences (i.e. shadow enhancers) that drive their developmental expression. However, the underlying selective pressures that drive the evolution of these architectures remain obscure. To investigate this, we characterized the regulatory region of the JAK/STAT ligand *unpaired* (upd), which is highly expressed in the posterior lobe, a recently evolved appendage-like structure on the genitalia of members of the *Drosophila melanogaster* clade. Dissecting the 70 kilobase *upd* locus revealed a pair of partially redundant shadow enhancers that can account for its heightened expression in species that develop a posterior lobe. Tracing the evolutionary history of these enhancers uncovered unique trajectories of their origin. One enhancer is utilized to drive gene expression in two ancestral JAK/STAT signaling centers, the posterior organizing center of the eye and the hinge of the wing imaginal disc. Our results indicate that *cis* changes have occurred in this signaling center enhancer to generate novel activity in the developing posterior lobe. In contrast, the second enhancer is conserved between lobed and non-lobed species, suggesting that *trans* changes upstream of this element have occurred. This work highlights how a unique shadow enhancer arrangement may have facilitated the origin of a novel anatomical structure, a new mechanism that may shed light on why such redundant enhancers evolve.

Program Abstract #80

Effect of Wolbachia on the Reproductive Output of Oregon Field Collected *Drosophila melanogaster*

Mallory Hiefield, Kelsey Lee, Joanne Odden
Pacific University, USA

Wolbachia is an endosymbiotic bacterium that infects approximately 60% of insects globally and can cause a variety of reproductive or developmental effects depending on the host, including cytoplasmic incompatibility (CI), male killing, and parthenogenesis. CI occurs when an uninfected female is crossed with an infected male and the embryos produced are not viable. Due to this phenomenon, *Wolbachia* has been explored as a mechanism of biocontrol for tropical mosquito borne viruses, such as Dengue and Zika. Specifically, the *wMel* line is primarily used in biocontrol of Dengue virus was

transferred from *Drosophila melanogaster*. Despite substantial interest in *Wolbachia* based biocontrol, the mechanism of how *Wolbachia* causes CI is unknown. Specifically, there are no known *Wolbachia* strains that cause CI in *D. melanogaster*. Due to the plethora of genetic tools, it would be ideal to study CI in *D. melanogaster.* In our research, we collected *D. melanogaster* from two sites in Oregon. We established 21 isofemale lines, identified that 11 were infected with *Wolbachia*, and set up pair-wise reproductive assays for 7 isofemale lines. In this assay, one infected male was crossed to an uninfected *D. melanogaster* female, and hatch rate of embryos was quantified. We found a strong CI effect of 5% mean hatch rate in isofemale line, 04JO, and a partial CI effect of 26% and 28% hatch rate in two additional isofemale lines, 17JO and 08JO, respectively. The presence of CI indicates the possibility of new *Wolbachia* lines to use for biocontrol, and may help to identify the mechanism of CI.
Funding: M.J. Murdock Charitable Trust Start Up Funds and Pacific University

Program Abstract #81
Using CRISPR/Cas9 Technology to Investigate the Role of Dlx Genes in Zebrafish
Allisan Aquilina-Beck, Neal Macdonald, Sebastian Bilitza, Mariam Awad, Thomas Sargent
National Institutes of Health, USA
The Dlx gene family encodes homeobox transcription factors that regulate early patterning of the brain, limb and craniofacial development in vertebrates. Dlx genes (homologs of the Distal-less gene in *Drosophila*) are present in a number of species across evolutionary phyla. Previous studies on Dlx genes in mouse show loss of function of both dlx5 and dlx6 result in severe abnormalities in jaw development. Patterning of the pharyngeal arches (precursors to vertebrate cranial facial features) depends on highly conserved interactions between Dlx genes. There are 6 Dlx genes in zebrafish organized into three bigene clusters, similar to mammals, with an additional two genes present at unlinked loci. Using zebrafish as a model organism to study these genes has mainly been possible with the use of knock down morphlinos; however, the potential for generating ancillary artifacts has recently become evident. With the advent of CRISPR/Cas9 technology, it is now possible to knock out specific genes in order to examine function in zebrafish. In this study, we have examined the effects of both single and double gene knockouts on craniofacial morphological patterning. Preliminary results suggest that loss of individual dlx genes has negligible effect on development, including survival to adulthood. Since there are a number of dlx genes in zebrafish and many of them are compensatory, work is ongoing to look at the combined effects of multiple gene knockouts.
This research is made possible through NIH funding.

Program Abstract #82
Heat shock Proteins and Stress Granules: Key Players for Marine Organisms Resilience in a Changing Environment
Anastazia T. Banaszak[1], Emma Rangel-Huerta[1], Enrique Lozano-Alvarez[1], Carlos Rosas[2], Ernesto Maldonado[1]
[1]EvoDevo lab. Unidad Académica de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, UNAM, Puerto Morelos, Quintana Roo., México; [2]Unidad Multidisciplinaria de Docencia e Investigación, Facultad de Ciencias, Universidad Nacional Autónoma de México, UNAM. Puerto de abrigo, Sisal, Yucatán, México
In the context of climate change marine animals are equally affected as land animals, however, living at sea, marine organisms are particularly affected by hydrology related changes as ocean currents shifting, water mass distribution, sea level change and global warming. Coral reefs are particularly sensitive, for example the ocean current El Nino, that started in 2014, has brought massive amounts of unusual warm water to the equatorial pacific and in consequence corals were bleached at a rate that is now considered the longest bleaching event ever. However, Natural Selection Theory states that thermal resistant animals could prevail and with time (geological time nevertheless) coral reefs may fully recover. Not only corals but many other marine organisms, like sea anemones, octopuses and many others are sensitive to sudden changes in water temperature. Our question is: How organisms respond to a temperature increase in the long term? In the short term, they may physiologically acclimatize using heat shock resilience systems, among others; Heat shock proteins and Stress granules. Hsp90 and Hsp70 chaperones stabilize more than 200 proteins involved in homeostasis and Stress granules that are formed under elevated temperatures, stall translation and maintain the mRNA pool ready to be translated when conditions return to normal. We believe that these systems could also be used for organisms to adapt, even for several generations, as a response to environmental stress. It has been proved already that Hsp90 is the capacitor for genetic assimilation and we aim to probe that Hsp90 capacitor is redundant with a mRNA responsive module under heat shock conditions and our candidate is stress granules. We aim to do experiments about induced phenotypic plasticity in

embryos from corals, like *Acropora palmata*, sea anemones, like *Aiptasia pallida* and octopuses like *Octopus maya* and *Octopus briareus*, in order to test our hypothesis.

Program Abstract #83

Leveraging genome editing to uncover the genetic basis of trait evolution in an evo-devo model, the Mexican cavefish *Astyanax mexicanus*

Johanna Kowalko, Hannah Klaassen, Hanna Greiner
Iowa State University, USA

There is an immense amount of diversity observable in the living world today that arose incrementally through the accumulation of trait changes that generated new species over hundreds of millions of years. Understanding the genetic basis of trait evolution is critical to identifying the developmental mechanisms that generated this diversity. Towards this end, a substantial amount of work has been done to identify loci responsible for natural variation. However, a significant challenge that remains is testing for the functional consequences of candidate genes within these loci in an ecologically and evolutionarily relevant organism. *Astyanax mexicanus*, the blind Mexican cavefish, exists in two interfertile forms, a surface-dwelling form and multiple independently evolved cave-dwelling forms. Cavefish have evolved a number of morphological and behavioral traits, including loss of eyes and pigmentation, increase in number of taste buds and neuromasts, and decrease in schooling and shoaling behaviors. Multiple quantitative trait loci (QTL) analyses have been performed to identify QTL for these traits. These studies, combined with the recent sequencing of the cavefish genome, provide a unique opportunity to identify and test candidate genes for these cave-specific traits. We have leveraged recently developed genome editing techniques to test the role of candidate genes in the evolution of traits in *Astyanax mexicanus*. Specifically, we modified genes hypothesized to be responsible for the evolution of pigmentation and behavior in surface fish and zebrafish. This work has allowed us to evaluate the role that these genes play in the development of cave traits.

Funding – Department of Genetics, Development and Cell Biology

Program Abstract #84

The evolution of temperature preference in the Mexican cave fish *Astyanax mexicanus*

Julius Tabin[1], Nicolas Rohner[2], Alexander Haro[3], Johanna Kowalko[4], Brian Martineau[1], Richard Borowsky[5], Cliff Tabin[1]
[1]Harvard Medical School, USA; [2]Stowers Institute for Medical Research, USA; [3]S.O. Comte Anadromous Fish Research Laboratory, U.S. Geological Survey, USA; [4]Iowa State University, USA; [5]New York University, USA

Even though a human can survive equally well in both fifty degree weather and seventy degree weather, why is it that most would prefer to be in the latter? Everybody has preferences, but not much is known about the genetics of preferences or how they evolve. We have been studying the temperature preference of *Astyanax mexicanus* populations from different caves and of the surface population living in adjacent rivers. In principle, the temperature in the caves differs from, and is more stable than, the temperature in rivers, which could lead to selection for different preferences. We are in the process of measuring the actual temperatures throughout the year at a series of caves and the adjacent rivers in Mexico. It would make sense that the caves have different temperatures, as we have indeed found that the cave populations have different temperature preferences. In particular, the fish from the Molino cave like warmer temperatures, the Pachon fish like cooler temperatures, and the Tinaja fish and river fish both seemed to like moderate temperatures. Genetic analysis indicates that these are inherited traits. For example, when we crossed the Molino and Pachon fish, the hybrids overwhelmingly went to the warmer temperatures like the Molino fish. Analysis of F2s suggest that the trait is not Mendelian but rather is more complex. We are now attempting to map the genes underlying temperature preference in *Astyanax* by QTL analysis.

Program Abstract #85

The eyeless cavefish Astyanax mexicanus as a model to investigate evolution and development of the gastrointestinal tract

Misty Riddle[1], Ariel Aspiras[1], Werend Boesmans[2]
[1]Harvard Medical School, USA; [2]KU Leuven, Netherlands

To flourish in unique environments, animals have evolved a host of mechanisms to maximize the intake, storage, and use of energy. The teleost fish *Astyanax mexicanus* is a proven model for understanding the genetic basis of adaptation and represents a particularly strong system to investigate evolutionary changes in the development of the digestive tract. This fish exists as two interfertile morphotypes, a river-dwelling form with abundant food and predators, and multiple independently derived cave-dwelling forms that thrive in perpetual darkness with limited food and no predators. Cavefish store large amounts of fat compared to surface fish and can survive long periods of starvation. Surprisingly, cavefish are

more active than surface fish. We have quantified differences between the river and cavefish digestive system that may contribute to cave-adapted metabolism. Adult cavefish have a longer and wider digestive tract with fewer structures called pyloric caeca, collectively resulting in greater surface area for nutrient absorption. Cavefish also appear to maintain a larger gut during periods of starvation, perhaps representing altered cell turnover that conserves energy. We have measured digestive tract motility through live imaging of transparent larvae and found that cavefish exhibit frequent "stomach like" churning contractions that are largely absent in the surface fish. To understand if altered motility represents an adaptive advantage we are currently measuring digestive transit time and fatty acid absorption directly. We are beginning to understand the developmental basis of motility differences by examining the formation of smooth muscle and the enteric nervous system. We will quantify these characteristics in F2 cave/surface hybrids to identify quantitative trait loci (QTL) with the aim of uncovering the developmental and genetic underpinnings of adaptation to low nutrient environments.

Program Abstract #86

3D modeling of the spiral valve intestine in the skate, *Leucoraja erinacea*.

Nicole Theodosiou, Samantha Frye, Jordan DeFelice
Union College, USA

In vertebrates, the organs of the digestive tract have evolved differences in function and shape depending on an animal's diet and habitat. The Elasmobranchii class of cartilaginous fish have a structurally unique gut morphology. Unlike mammals that have long coils of intestine to assist in absorbing nutrients from high protein diets, elasmobranchs have short intestines that form an internal spiral valve. The spiral valve structure allows elasmobranchs to compensate for having a short intestine by increasing the absorptive surface area. The goal of our work is to understand how the structure of organs evolved to take on different morphologies. To better understand formation of the spiral valve intestine in the skate*, Leucoraja erinaea*, we are creating 3D-printed models from micro-CT scan data. Analysis of the 3D structure of the spiral valve intestine during development provides insight into how the spiral valve takes shape. In addition, we are continuing work to characterize the expression of genes that pattern the intestines. Previous work from our lab demonstrated that different regions of the spiral valve intestine have different functions. In light of this, how are regional differences in function specified by gene expression when the developing structure is not linear? By overlaying gene expression patterns with 3D models, we hope to gain insight into how the shape and function of intestines evolved in vertebrate animals.

Program Abstract #87

Vertebral skeletal development in the little skate, *Leucoraja erinacea*, a cartilaginous fish

Katharine Criswell[1], Michael Coates[1], Robert Ho[1], Andrew Gillis[2]
[1]University of Chicago, USA; [2]University of Cambridge, UK

The vertebral column is the defining feature of vertebrates, but ancestral character estimations reveal multiple origins of different components of the axial skeletal complex. There are at least nine independent originations of centra among jawed vertebrates, and the developmental processes contributing to centrum formation vary accordingly. Centra in teleost fishes can form from notochord contributions, from migrating sclerotomal cells, or from both, whereas amniote centra develop exclusively from the sclerotome. To reconstruct the early evolution of centra, we examined embryonic morphology, gene expression and sclerotome fate in a cartilaginous fish, the little skate (*Leucoraja erinacea*). MicroCT scans of a series of skate embryos show a continuous condensation of mesenchymal tissue that surrounds the notochord and neural tube and that subsequently differentiates into discrete centra. When vertebral skeletal elements begin to differentiate, centra appear to form from two separate components: one inside and one outside the notochord sheath. Histological analysis reveals a substantial thickening of the fibrous notochord sheath prior to centrum formation. To investigate the embryonic origin of skate centra, we performed sclerotome fate mapping experiments. Presumptive sclerotome cells were labeled with the lipophilic dye CM-DiI prior to their emigration from the somite and embryos were allowed to develop for seven or twelve weeks post injection. DiI-labeled cells were recovered both surrounding, and within, the notochord sheath and incipient centra, as well as in the haemal arches, in both seven and twelve week experiments. These results suggest that both arches and centra derive from sclerotomal cells in chondrichthyans, and point to the sclerotome as the primitive source of skeletogenic mesenchyme for gnathostome vertebrae. This work was supported by NSF DDIG DEB-1501749, the Company of Biologists, and an MBL/UChicago Graduate Student Research Award.

Program Abstract #88

Molecular evolution of HoxA13 in Snakes: differential modulation of signaling pathways Wnt, FGF, Notch and RA during the elongated body axis.

Marina Singarete[1], Tainá Raiol[6], Juliana Issa Hori[1], Julien Jourde[2], Hozana Andrade Castillo[5], José Xavier-Neto[5], Scott Stadler[4], Gunter Wagner[3], Tiana Kohlsdorf[1]

[1]Universidade de Sao Paulo, BR; [2]Universidade de Brasília, BR; [3]Yale University, USA; [4]Oregon Health and Science University, USA; [5]Brazilian National Laboratory for Biosciences, BR; [6]Fundação Oswaldo Cruz (Fiocruz), BR

The evolutionary origin of snakes was characterized by remarkable morphological changes including extension of the body axis. Among the leading genetic candidates underlying such changes, much attention has been given to Hox genes as they play a central role in the specification of structures along the anterior-posterior body axis in vertebrates. Posterior Hox genes have recently been associated with the control of the body axis elongation. Studies in chicken embryos have shown that activation of the posterior HoxA13 in the tail bud is related to the reduction of the axial elongation rate. Specifically in snakes, five amino acid sites located in the exon-1 of HoxA13 have been detected as evolving under positive directional selection; the functional relevance of such mutations, however, remained obscure. Here we evaluate the functional relevance of the molecular signature detected in the HoxA13 of snakes, with focus on molecular interactions in the context of signaling pathways associated with segmentation and somitogenesis process. Functional in vitro assays were conducted to investigate the differential expression of protein coding genes in embryonic cells, transfected with an expression plasmid containing the HoxA13 sequence with the characteristic mutations detected in the group of snakes. Subsequently gene expression was assessed by large-scale sequencing (RNA-seq). The analysis of differential expression of protein-coding genes and functional annotation show the induction of genes belonging to the pathways Wnt, FGF and Notch, and inhibition of the RA. Differential modulation of these pathways suggests that the molecular signature detected in the coding region of HoxA13 transcription factor is associated with regulatory changes. These can prolong the cellular mechanisms that culminate with extension of the process of body elongation in snake embryos, thus contributing to the development of the snakelike morphology.

Financial support: CAPES, FAPESP

Program Abstract #89

Development and evolution of mesodermal components of early vertebrates

Rie Kusakabe, Shigeru Kuratani

RIKEN, Evolutionary Morphology Laboratory, Japan

Jawed vertebrates are characterized with particularly complex mesodermal organization in the head/trunk interface region that delineates the posterior boundary of the pharynx. At this interface, during development, mesodermal cells with different origins (somites, lateral plate mesoderm, and unsegmented head mesoderm) and neural crest-derived cells lie in close proximity and give rise to muscles and cartilages. These tissues comprise vertebrate-specific, functionally complicated morphology, such as forelimb/shoulder, head muscles/cartilages including those of the tongue, and the heart. The lamprey, one of the only two extant cyclostomes, retains a variety of ancestral features of the vertebrates, including absence of jaws, paired fins and epaxial/hypaxial distinction of the trunk skeletal musculature. We have studied the developmental mechanisms underlying the myogenesis of the Japanese lamprey, and have discovered that the hypobranchial muscle of the lamprey undergoes a similar developmental process to that found in the tongue muscles of jawed vertebrates. On the other hand, the elasmobranchs possess paired fins and other gnathostome-like body plan, yet the myogenetic pathway of each muscle has yet to be clarified. Using these animals, we have examined the expression of developmental markers and delineated the temporal order of differentiation of various skeletal muscles, such as the hypobranchial, posterior pharyngeal and cucullaris (trapezius) muscles, all located near the head/trunk interface. Our analysis has provided new insights regarding cellular and molecular characteristics of each musculature and illustrated how they have contributed to the complexity and diversification of vertebrate morphology.

Program Abstract #90

How to remake a head or a tail? The polarized whole-body regeneration program of the sea-anemone Nematostella vectensis.

Uri Gat, Amos Schaffer, Michael Bazarsky, Karine Levy

Hebrew University in Jerusalem, IL

The ability of regeneration is essential for the homeostasis of all animals as it allows the repair and renewal of tissues and body parts upon normal turnover or injury. The extent of this ability varies greatly in different animals with the sea-anemone *Nematostella vectensis*, a basal cnidarian model animal, displaying remarkable whole-body regeneration

competence. In order to study this process we performed an RNA-Seq screen wherein we analyzed and compared the transcriptional response to bisection in the wound-proximal body parts undergoing oral (head) or aboral (tail) regeneration at several time points up to the initial restoration of the basic body shape. The transcriptional profiles of regeneration responsive genes were analyzed so as to define the temporal pattern of differential gene expression associated with the tissue-specific oral and aboral regeneration. The genes thus identified were characterized according to their GO (gene ontology) assignations with particular attention to their affiliation to the major developmental signaling pathways. While some of the genes and gene groups thus analyzed were previously known to be active in regeneration, we have revealed novel and sometimes surprising candidate genes that likely participate in this interesting developmental program. This work highlighted the main groups of genes which showed polarization upon regeneration, notably the proteinases, multiple transcription factors and the *Wnt* pathway genes that were highly represented, all displaying an intricate temporal balance between the two sides. In addition, the evolutionary comparison performed between regeneration in different animal model systems may reveal the basic mechanisms playing a role in this fascinating process and can potentially contribute to enhance injury treatments in man.

Program Abstract #91
Conserved role of muscle cells in bilaterian regeneration
Amelie Raz
MIT/Whitehead Institute, USA
Regeneration - the replacement of lost body structures – is a widespread phenomenon among metazoans; however, it is unknown whether there are common molecular hallmarks of this process across phyla. Just as in embryonic development, new structures created during regeneration require positional information that allows for the identification of the correct axial tissues to be regenerated. The planarian worm *Schmidtea mediterranea* is a classic model of regeneration, capable of replacing any amputated body part. A group of genes known as positional control genes (PCGs) are hypothesized to be responsible for conveying positional information to the proliferating planarian stem cells. PCGs 1) display regionalized expression along the planaria body axes, and 2) either display an aberrant regenerative patterning RNAi phenotype or encode a protein known to be in the pathways of such a patterning gene. Strikingly, all PCGs described to date in planarians are expressed primarily in a single differentiated tissue type: the muscle. Genes that fulfill criteria for PCGs exist in non-planarian regenerative species as well. These include the emerging acoel model *Hofstenia miamia*, an early-diverging bilaterian, cladistically distinct from both deuterostomes and protostomes and separated from all other bilaterians by over 550 million years of evolution. We demonstrate here that *Hofstenia* PCGs are coexpressed in a common cell type, consistent with a single source of positional information. Furthermore, analysis by *in situ* hybridization, single-cell qPCR, and single-cell RNA sequencing suggest that *Hofstenia* PCGs are specifically expressed in muscle cells. Such a similar positional control mechanism across vastly diverged regenerative species supports a conserved role for muscle as source of positional information guiding adult regeneration across the bilaterians.

Program Abstract #92
miR-1 regulation of muscle differentiation in the hemichordate *Saccoglossus kowalevskii*
Jessica Gray[1], Robert M. Freeman, Jr.[1], John Gerhart[2], Marc Kirschner[1]
[1]Harvard Medical School, USA; [2]University of California, Berkeley, USA
Since an individual miRNA can target a large number of genes, miRNAs have the potential for broad manipulation of signaling pathways or development processes. This potential makes miRNAs intriguing candidates for the study of developmental regulatory changes through evolution. But the function of miRNAs in the development of a wide range of animals outside of traditional models remains largely unknown. Understanding how miRNAs are integrated into different developmental signaling pathways will help determine whether changes in miRNAs and their targets play a role in developmental evolution or are uniquely regulated in different lineages. We are establishing the direct-developing hemichordate *Saccoglossus kowalevskii* as a model for addressing these questions in the deuterostome lineage. To that end we have generated miRNA expression profiles across development and genome-wide target predictions. We have also established methods for functional perturbations to identify miRNA-regulated developmental signaling pathways and processes in vivo, with an initial focus on the muscle miRNA miR-1. miR-1 expression begins after gastrulation and is restricted to the highly muscularized anterior proboscis. Animals injected with miR-1 inhibitors or mimics develop normally past hatching, but exhibit disrupted muscle patterning along with under and overgrowth of the muscle respectively, and these changes are accompanied by differences in MyoD expression levels. We also confirm changes in conserved predicted targets, as well as unique targets, and the shifting of targets to different genes within the same family. While this opens several avenues of investigation regarding muscle miRNA regulation and patterning in particular –

including evolved differences in regulation of head and trunk muscle genes, it also establishes the necessary tools for further inquiries into understanding the evolution of miRNA signaling in development. Support for this project was provided by NIHR01HD042724.

Program Abstract #93

Functional specialization of Mmp11 and Timp4 interacting at the myotome boundary in the developing zebrafish

Emma Matchett

UNB, CA

Myotome boundary maturation occurs in the zebrafish embryo between 24hr-48hr post fertilization, and is thought to be fundamentally similar to the maturation of the myotendenous junction (MTJ) in tetrapods. During this process extracellular matrix is remodelled both mechanically and biochemically, changing shape and composition from being a fibronectin dominated matrix to a laminin dominated matrix. Suggestively, both matrix metalloproteinase 11 alpha and beta (Mmp11α + β), as well as tissue inhibitor of matrix metalloproteinases 4 (Timp4) co-localize at the myotome boundary during this process. The MMPs are zinc-dependent proteinases that are responsible for much of the matrix remodelling that takes places during development and physiological and pathological processes like wound healing and tumour metastasis. MMP activity is regulated largely post-translationally, with the TIMPs playing fundamental roles both in the inhibition of MMP activity, and in co-ordinating the proteolytic activation of proMMPs. In order to understand better how MMP activity is regulated during maturation of MTJs, I have investigated if, when, where and how the various paralogs of Mmp11 and Timp4 encoded by the zebrafish genome interact at the MTJ during this process. Proximity ligation assays demonstrate that Mmp11β interacts with Timp4 and with fibronectin but not with laminin during the maturation of the myotome boundary. Depending on the domains interacting, the MMP-TIMP complex can either be inhibitory or modulatory. I use yeast-two-hybrid assays to tease apart domain interactions between these molecules; Timp4β interacts in an inhibitory way with the catalytic domain of Mmp11α. Timp4α however interacts with the hemopexin domain of Mmp11β, suggesting a modulatory interaction. Taken together, I conclude that Mmp11 and Timp4 paralogues are important effectors of matrix remodelling at the MTJ, and that functional specialization of these paralogs has likely occurred.

Funding: NSERC

Program Abstract #94

Ancient origin of lubricated joints in bony vertebrates

Amjad Askary[1], Joanna Smeeton[1], Sandeep Paul[1], Simone Schindler[1], Ingo Braasch[2,3], Nicholas Ellis[4], John Postlethwait[2], Craig Miller[4], Gage Crump[1]

[1]University of Southern California, USA; [2]University of Oregon, Eugene, USA; [3]Michigan State University, USA; [4]University of California, Berkeley, USA

We owe our flexibility to specialized 'synovial' joints that provide lubrication at connection sites between bones. Synovial joints are characterized by the presence of a cavity, which is filled with lubricating fluid, flanked by hyaline articular cartilage, and wrapped in synovium and joint capsule. Articular chondrocytes that line synovial joints produce the proteoglycan Prg4/Lubricin, a lubricant required to maintain joints. It is commonly thought that these types of joints first arose as vertebrates came onto land, in response to the newfound mechanical forces on weight-bearing limbs. Therefore ray-finned fish are considered to generally lack lubricated synovial joints. In contrast, we present histological, molecular, and functional evidence that the jaw joints of diverse ray-finned fishes in juvenile and adult stages share many properties with mammalian synovial joints. We find that the jaw joints of zebrafish, stickleback, and spotted gar have prominent synovial cavities lined by synovial membrane and flattened chondrocytes that express synovial joint markers. The lubricating function of Lubricin is also conserved in zebrafish, as deletion of *prg4b* results in the abnormal matrix deposition in joint cavity and synovial hyperplasia in the adult jaw, similar to what is reported for synovial joints in human and mouse lacking *Prg4* function. Interestingly, certain joints within the pectoral fin also show morphological and molecular features of synovial joints and degenerate in zebrafish *prg4b* mutants, suggesting that the lubricated joints within tetrapod limbs arose from these pre-existing fish joints. Our data provides the first molecular evidence that lubricated synovial joints evolved much earlier than currently accepted, at least in the common ancestor of all bony vertebrates. Moreover, we established the first arthritis model in zebrafish. In future this model can provide unique advantages in the study of synovial joint development, disease, and regeneration.

Program Abstract #95

Developmental Differences of the Pharyngeal Apparatus in Bluegill and Pumpkinseed Sunfish

Corrie Olson, Sofiya Bychkova, Sourabh Goyal, Julia Melnyk, Brad Nowosielski, Mike Vitullo, Greg Andraso, Kelly Grant

Gannon University, USA

The evolutionary divergence of the pharyngeal apparatus (PA) in sunfishes (Family Centrarchidae) allowed the fishes to exploit different niches. The PA is formed by the bones of the pharyngeal arches, specifically ceratobranchial 5 (cb5) and pharyngobranchials (pb2, 3, & 4). In adult sunfish, the morphologies of cb5 and pb3 are often quite different among species. Bluegills (*Lepomis macrochirus)* have a delicate bone structure with sharp, cardiform teeth; pumpkinseeds (*Lepomis gibbosus*) have larger, robust bones with molariform teeth used for crushing prey. We are investigating the developmental mechanisms underlying these differences. To analyze the growth of cb5, we fertilized embryos, including both sets of hybrids, and reared them in the lab and a facility on Lake Erie. cb5 cartilage appears similarly sized in both species when it forms at 8-9 dpf. Preliminary data suggests that the cartilage may grow faster in pumpkinseeds than bluegills. We are beginning to tease apart the relative roles of increased cell proliferation compared to increased chondrocyte size. Accelerated bone growth continues in juvenile and adult pumpkinseeds but cartilage position is not predictive of that growth. In addition, we are beginning to explore the expression of genes that might contribute to the morphological differences in bones of the PA. The literature suggests many candidate genes that might be responsible for accelerated bone growth in pumpkinseeds or alterations in tooth patterning. We have cloned fragments of *ectodysplasin (eda*), *Beta-catenin, BMP2, BMP4, and BMP6* and are continuing to clone other candidates. We plan to evaluate the expression of these genes in both species using qPCR, as well as, analyze allele specific expression in the hybrids.
Funded by the Cooney-Jackman endowed professorship to G. Andraso (GA), Regional Science Consortium Grant to GA, and Gannon University Faculty Research Grant to KG.

Program Abstract #96

GRNs for cartilage and bone formation overlap during cartilage maturation

Patsy Gomez Picos, Amir Ashique, Katie Ovens, Ian McQuillan, Brian Eames

University of Saskatchewan, CA

A cell type is a homogenous population of cells expressing a characteristic set of genes, termed its molecular fingerprint, organized into various gene regulatory networks (GRNs). Here, we hypothesize that two GRNs regulate differentiation of three main skeletal cell types: immature chondrocyte, mature (hypertrophic) chondrocyte, and osteoblast. A cartilage GRN, run by the transcription factor Sox9, regulates immature chondrocytes. A bone GRN, run by Runx2, drives osteoblasts. Mature chondrocytes, which undergo hypertrophy and mineralize, depend upon both cartilage and bone GRNs. To test this hypothesis in an unbiased fashion, we used laser capture microdissection (LCM) coupled to RNAseq, isolating high-quality RNA from each of the three skeletal cell types in the mouse embryo (E14.5) in order to identify their molecular fingerprints. Transcriptomic data were obtained from three samples of each specific skeletal cell type, allowing for analyses with statistical significance. Two main findings support the hypothesis. First, bioinformatic clustering analyses suggest that the mature chondrocyte molecular fingerprint shows an overlap between immature chondrocyte and osteoblast molecular fingerprints. Second, mature chondrocytes expressed the lowest number of "unique" genes of the three cell types (i.e., "unique"=gene counts above threshold only in one cell type). Two of these genes, Pde11a and Rhox8, were not identified in previous, biased transcriptomic studies, so we used in situ hybridization to confirm their expression in mature chondrocytes. In closing, our approach will provide unique insights into the molecular mechanisms underlying skeletal cell type differentiation, and future comparative studies will identify clade-specific changes to skeletal cell molecular fingerprints.

Program Abstract #97

From sticklebacks to humans: Evolving skeletal traits by cis-regulatory changes in bone morphogenetic proteins

Vahan Indjeian[1,2,3], David Kingsley[2,3]

[1]MRC Clinical Sciences Centre, GB; [2]Howard Hughes Medical Insitute, USA; [3]Stanford University School of Medicine, USA

Changes in bone size and shape are defining features of many vertebrates, and underlie many of the traits that distinguish humans from other primates. To uncover the major loci and genomic sequence changes that regulate skeletal traits, we did high-resolution mapping experiments in sticklebacks. We identified the gene for a secreted bone morphogenetic protein, *Growth/Differentiation Factor 6* (*GDF6*), as a major locus controlling flat dermal bone size in wild populations. Freshwater fish have a *cis*-acting regulatory change that increases *GDF6* expression, and transgenic overexpression

phenocopies evolutionary changes in dermal bone size. Comparative genomics revealed that the human *GDF6* locus also has undergone distinctive regulatory evolution, including complete loss of an enhancer that is highly conserved in other mammals. Functional tests show that the ancestral enhancer drives expression in hindlimbs but not forelimbs, in anatomic domains that have been specifically modified during the human transition to bipedalism. These results add to growing evidence that *cis*-regulatory modifications of BMP genes represent a common mechanism for evolving specific skeletal changes in humans and other vertebrates.

Program Abstract #98

Natural variation in mouse skin appendages gives clues to human evolution

Yana Kamberov[1,2], Elinor Karlsson[2,4], Daniel Lieberman[4], Pardis Sabeti[2,4], Bruce Morgan[1,3], Clifford Tabin[1]
[1]Harvard Medical School, USA; [2]Broad Institute of MIT and Harvard, USA; [3]Massachusetts General Hospital, USA; [4]Harvard University, USA

Humans are unique amongst mammals in their capacity for efficient evapotranspiration during vigorous activities such as long distance walking and running. This adaptive mechanism of thermoregulation relies on the dramatic increase in the distribution and density of eccrine glands coupled with the miniaturization of body hair relative to other primates. Despite the critical role of these changes in shaping our species, little is known about their genetic and evolutionary basis. Pursuit of the genetic underpinnings of the increased eccrine gland density in humans has been particularly complicated by difficulties in directly examining this trait in our species. We recently showed that the mouse serves as an outstanding model to pursue eccrine gland evolution in humans, and evidence suggests the basic molecular mechanisms governing the development of these appendages is highly conserved with man. We turned to this tractable system and used quantitative trait locus mapping to investigate how natural variation in eccrine gland density is generated in mice. We identified a major effect QTL on Chromosome 1 and mapped to the transcription factor *Engrailed-1* (*EN1*). We demonstrate that EN1 is critical for the specification of both eccrine gland and hair follicle densities in regions where these two appendages are interspersed, a situation similar to that in human skin. We further carried out comparative genomic analysis and identify a human-specific change at the *EN1* locus located in a putative skin enhancer. Our results yield novel insight into the development and patterning of eccrine glands and hair follicles, and have important implications for the molecular basis underlying adaptive changes in these organs in humans.

Program Abstract #99

Morphogenesis of Skin in 3D Co-cultures - Sorting and Mixing of Interspecific, Intraspecific, Differentiated and Stem Cells Traced with Lineage Specific Keratins.

Ferez Nallaseth[1,2], Bozena Michniak-Kohn[3], Ramsey A. Foty[4], Dale Woodbury[5], Dan Medina[6], Dongxuan Jia[7], Kelly Walton[6], Priya Batheja[3,8], Pei-Chin Tsai[3]
[1]Life Sciences Institute of New Jersey, 229 Parsons Court, Belle Mead, NJ 08502, USA; [2]Complex Biological Systems Alliance, Boston, MA, USA; [3]Center for Dermal Research, NJ Center for Biomaterials, Rutgers-The State University of New Jersey, Piscataway, NJ, 08854, USA; [4]Department of Biomedical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ, USA; Department of Surgery, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ,08901, USA; [5]Ira Black Center for Stem Cells, Rutgers Robert Wood Johnson Medical School, NJ, 08854, USA; [6]Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey, USA; Rutgers Robert Wood Johnson Medical School, NJ, 08901, USA; [7]Department of Surgery, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, 08901, USA; [8]Pfizer Pharmaceuticals, Boston, MA,, USA

In mouse models Inter-follicular Epithelial (IFE) and Hair Follicle (HF) stem cells exclusively yield the epithelial layer under homeostasis and wound healing conditions. *In vitro* studies in 3D were initiated due to regulatory constraints and functional differences between murine and human skin. Combinations (12) of intraspecific, interspecific, primary, established, differentiated and stem cells were analysed by 4 approaches. In hanging drop (HD) cultures, cells have the same opportunity to interact as they would *in vivo*. Published HD methods were extensively adapted to the analysis of skin morphogenesis which at least initially followed constraints imposed by the Differential Adhesion Hypothesis (DAH). HD aggregate formation displayed serum and cell type dependent spectrum of demixing behaviors. At equilibrium, human dermal cells and keratinocytes completely demixed from each other, thus exhibiting very low to non-existent levels of cross-adhesion possibly required for boundary formation. However, demixing of other interspecific and stem cell combinations was more variable even at equilibrium. This contrasts with embryogenesis when the differentiation of the single cellular embryonic epithelium is obligatorily dependent on contact with the underlying mesoderm in initiating the morphogenesis of skin. In Human Skin Equivalents (HSEs) morphology, with exceptions e.g. HF, but not morphogenesis, recapitulates *in vivo* skin. However, in 2D gel electrophoresis, proteins extracted from HSEs displayed differentiation

stage specific mobilities of keratins. In contrast in 2D co-cultures of HDFn and rat stem cells exhibited highly variable distributions, which were more consistent with isotypic affinities of cell types. These differences may identify a requirement for additional components for *in vivo* morphogenesis of these cell types into skin. Whether transient contact induces expression of HF, IFE and SC specific epitopes of keratin in all 3D cultures is being established.

Program Abstract #100
Role of 5'Hoxd genes and Gli3 in the dominance of primary limb axis polarity
Anna Trofka, Bau-Lin Huang, Susan Mackem
National Cancer Institute, USA
In most vertebrates, the primary limb axis runs through the posterior limb with the ulna/digit4 (d4) condensing first. One exception is the Urodele amphibians, which have anterior dominance (radius/d2 appear first), and also can regenerate limbs as adults. It has been proposed that the axis shift in Urodeles results from failure to expand 5'Hoxd gene expression in the late distal limb bud (LB). To test this hypothesis, we examined primary axis appearance in 5'Hoxd-Del mice (5'Hoxd genes Hoxd11-13 deleted) and found an anterior axis shift. The 5'Hoxd homeobox transcription factors play roles in replication licensing and cell adhesion. Few specific targets are known, but include the cell adhesion factor EphA3. Gli3 repressor, expressed anteriorly, also regulates proliferation and condensation, and antagonizes 5'Hoxd function. In compound 5'Hoxd-Del/Gli3 mutants, posterior axial polarity is restored. Based on early steps in condensation formation, either changes in proliferation rate and/or timing of condensation in anterior versus posterior LB could determine the polarity of the primary limb axis. Flow analysis of cell cycle reveals fewer anterior cells in G2/M in 5'Hoxd-Del than control LB just prior to appearance of primary limb axis condensation; an inverse correlation between proliferation and condensation. We are analyzing relative rates of cell movement and aggregation (over 24 hrs) in mixed cultures of fluorescently-tagged anterior or posterior cells from 5'Hoxd-Del and control LBs. Several stages are being analyzed to determine if a temporal shift in anterior versus posterior aggregation correlates with primary axis dominance. We propose that the anterior/posterior balance between antagonistic 5'Hoxd-Gli3 functions governs the polarity of primary limb axis formation.

Program Abstract #101
Molecular mechanisms underlying the exceptional adaptations of batoid fins.
Tetsuya Nakamura[1], Jeff Klomp[1], Joyce Pieretti[1], Igor Schneider[2], Andrew R. Gehrke[1], Neil H. Shubin[1]
[1]The University of Chicago, USA; [2]Universidade Federal do Para, Brazil
Extreme novelties in the shape and size of paired fins are exemplified by extinct and extant cartilaginous and bony fishes. Pectoral fins of skates and rays, such as the little skate (Batoid, *Leucoraja erinacea)*, show a strikingly unique morphology where the pectoral fin extends anteriorly to ultimately fuse with the head. This results in a morphology that essentially surrounds the body and is associated with the evolution of novel swimming mechanisms in the group. In an approach that extends from RNA sequencing to in situ hybridization to functional assays, we show that anterior and posterior portions of the pectoral fin have different genetic underpinnings: canonical genes of appendage development control posterior fin development via an apical ectodermal ridge (AER), whereas an alternative Homeobox (Hox)-Fibroblast growth factor (Fgf)-Wingless type MMTV integration site family (Wnt) genetic module in the anterior region creates an AER-like structure that drives anterior fin expansion. Finally, we show that GLI family zinc finger 3 (Gli3), which is an anterior repressor of tetrapod digits, is expressed in the posterior half of the pectoral fin of skate, shark, and zebrafish but in the anterior side of the pelvic fin. Taken together, these data point to both highly derived and deeply ancestral patterns of gene expression in skate pectoral fins, shedding light on the molecular mechanisms behind the evolution of novel fin morphologies. This work was supported by The Brinson Foundation and the University of Chicago Biological Sciences Division; JSPS Postdoctoral Fellowship for Research Abroad, Uehara Memorial Foundation Research Fellowship, and MBL Research Grant; NSF Grant IOS-1355057; Graduate Assistance in Areas of National Need Grant P200A120178; NIH Grant T32 HD055164 and NSF Doctoral Dissertation Improvement Grant 1311436 and Brazilian National Council for Scientific and Technological Development Grants 402754/2012-3 and 477658/2012-1

Program Abstract #102
Digits and Fin Rays Share Common Developmental Histories
Andrew Gehrke, Tetsuya Nakamura, Justin Lemberg, Julie Szymaszek
The University of Chicago, USA
Comparisons of fish fins with tetrapod limbs have been limited by 1 a relative lack of understanding of the cellular and molecular processes underlying the development of the fin skeleton. For example, knockout and cell lineage data of genes

essential for patterning skeletal structures in mice have been lacking for orthologous genes in fish. Here, we provide the first functional analysis, using CRISPR/Cas9 and fate mapping, of 5' hox genes in zebrafish that are indispensable for the development of the segments of endochondral bones of tetrapod limbs. We show that the fates of hoxa13 expressing cells, which mark the autopod in mice, exclusively mark elements of the fin fold including the osteoblasts of the dermal rays. Moreover, in hox13 knockout fish, we find a dramatic reduction and loss of fin rays which is associated with an increased number of endochondral distal radials. Our data reveal a developmental connection between fin rays and the digits of tetrapods, and suggest a mechanism of endochondral expansion in tetrapod origins by via the transition of distal cellular fates.

Program Abstract #103

These fish were made for walking: a single mutation reveals hidden capacity for the formation of limb-like structures in the zebrafish

Matthew Harris[1], M. Brent Hawkins[1,2,3], Katrin Henke[1]

[1]Harvard Medical School, USA; [2]Harvard University, USA; [3]Museum of Comparative Zoology, USA

The diversification and specialization of the paired appendages are hallmarks of vertebrate evolution. In the lineage leading to tetrapods, the appendicular skeleton was elaborated along the proximal-distal (PD) axis by adding articulated skeletal elements to form the stylopod (humerus), zeugopod (radius/ulna), and autopod (wrist/digits) of limbs. This tripartite skeletal pattern was key to the successful invasion of land and has remained constant during the 360 million years of tetrapod evolution. In contrast, the teleost fish lineage shows a reduced appendicular skeletal pattern, having a diminutive endochondral skeleton consisting of only proximal radials and small, nodular distal radials along the PD axis. This pattern is canalized and has persisted over 250 million years of teleost evolution. Using a forward genetic approach in the zebrafish, we have discovered an adult-viable, dominant mutation that results in the surprising acquisition of supernumerary long bones located between the proximal and distal radials. Unlike wild type radials, these extra elements have dual epiphyseal growth zones, and articulate and form joint-like structures with the proximal and distal radials. Ontogenetic analyses reveal that the new elements develop from the branching and splitting of cartilaginous condensations in a fashion similar to that seen in tetrapod limb development. Unexpectedly, unlike wild type fin radials, the extra elements are directly connected to musculature, analogous to the muscular integration of limb elements observed in tetrapods. Supporting our interpretation that these extra bones arise through a limb-like process, an analysis of early development revealed modification of known limb developmental gene networks in mutant fins. The genetic alteration in this mutant reveals the latent capacity for skeletal elaboration in the fins of fishes and may inform our understanding of 'limb-ness' and the fin to limb transition in evolution.

Program Abstract #104

Functional Divergence of a Tbx4 Enhancer and the Evolution of Hindlimb Reduction in Reptiles

Carlos Infante[1,2], Sungdae Park[1], Jialiang Wang[1], Shana Pau[1], Douglas Menke[1]

[1]University of Georgia, USA; [2]University of Arizona, USA

Vertebrate limbs are highly patterned structures, but show remarkable changes in size and shape between species adapted to different habitats and to different methods of locomotion. While many important components of the limb developmental pathway have been uncovered, the genetic and developmental mechanisms that drive the evolution of limb morphology remain largely unknown. We recently discovered that HLEB, a deeply conserved hindlimb and genital enhancer of the *Tbx4* gene, has lost hindlimb enhancer activity in snakes, a lineage which evolved limb loss more than 100 million years ago. In addition, deletion of the HLEB element from the mouse genome reduces the size of hindlimb bones and phallus. With over 130 described species in the Caribbean, *Anolis* lizards are an ideal system for studies of limbs evolution since short-limbed species have evolved independently several times on different islands. Our survey of HLEB DNA sequences from over 90 species of Caribbean anoles has revealed deletions in 3 of 7 short-limbed *Anolis* lineages, with no comparable deletions in long-limbed species. When we generated mouse knockins where the native mouse enhancer has been replaced with the enhancer from a short-limbed or long-limbed species, there are quantifiable alterations in hindlimb bone size in long-limbed vs. short-limbed enhancer knockin mice. Thus, we provide evidence that functional divergence of HLEB is associated with the evolution of limb loss in snakes and the reduction of hindlimb size in *Anolis* lizards.

Program Abstract #105

Identifying the mechanisms responsible for forelimb reduction in flightless birds

Malcolm Logan, Satoko Nishimoto

King's College London, United Kingdom
The evolution of paired appendages in vertebrates was a pivotal event that fuelled the diversification of vertebrate species, enabled colonisation of land and flight ability in birds. Despites the apparent advantages of having limbs, several vertebrate species have independently lost their limbs or reduced either the forelimb or hindlimb programme during their evolution. A striking example of reduction in the forelimb programme is found in ratites, a group of flightless birds. Ratites are found in Africa (Ostrich), South America (Rhea), New Zealand (Kiwi) and Australia (Emu). Initial morphological phylogenies suggested that ratites are monophyletic and evolved from a single flightless ancestor present on the Gondwana supercontinent. Recent studies, however, have indicated that all ratites have evolved from a volant (flighted) ancestor and have lost flight ability independently by convergent evolution. Tbx5 is a T-box transcription factor that plays a key, conserved role in forelimb development and is critical for the establishment of a forelimb bud, acting during the earliest phases of forelimb formation. Previously we have identified the forelimb regulatory element of Tbx5 and we have shown a relative delay in *Tbx5* expression in Emu compared to chicken can explain the adaptions to the forelimb in this species. We present experiments that test two models for how alterations in cis-regulatory elements of *Tbx5* can explain reduction of the forelimb programme in several ratite species.

Program Abstract #107

Foot feathering is caused by a molecular shift in limb identity in the domestic pigeon

Elena Boer[1], Eric Domyan[1], Zev Kronenberg[2], Carlos Infante[3], Anna Vickrey[1], Sydney Stringham[1], Rebecca Bruders[1], Michael Guernsey[1], Sungdae Park[3], Jason Payne[4], Robert Beckstead[4], Gabrielle Kardon[2], Douglas Menke[3], Mark Yandell[2,5], Michael Shapiro[1]

[1]Department of Biology, University of Utah, USA; [2]Department of Human Genetics, University of Utah, USA; [3]Department of Genetics, University of Georgia, USA; [4]Poultry Science Department, University of Georgia, USA; [5]Utah Center for Genetic Discovery, University of Utah, USA

Understanding the molecular mechanisms of morphological variation remains a critical challenge in evolutionary and developmental biology. The domestic pigeon represents an outstanding model to study the genetic and developmental programs that underlie morphological variation, as it displays striking phenotypic variation within a single species and is amenable to genetic crosses, embryonic studies and genomic analyses. Artificial selection by pigeon fanciers has resulted in more than 350 breeds that display variation in a variety of complex traits, including body size, beak morphology, feather color and patterning, and ornamental feathering. Most pigeons have scaled feet; however, in some breeds scaled epidermis is replaced by skin with a variety of feather morphologies. Classical breeding experiments suggest that large feather "muffs" are caused by the synergistic effects of two genetic loci, *grouse* (*gr*) and *Slipper* (*Sl*), which independently produce smaller foot feathers. Recently, our lab demonstrated that *gr* and *Sl* are caused by cis-regulatory mutations in the limb-identity factors *PITX1* and *TBX5*, respectively. To date, the precise molecular mechanisms by which *PITX1* and *TBX5* control limb identity remain poorly understood. To dissect the gene regulatory networks downstream of *PITX1* and *TBX5*, we are currently performing a combination of RNA-seq and ATAC-seq experiments in scale-, grouse-, slipper- and muff-footed embryonic limbs. We also plan to carry out *in vivo* analyses to determine the unique and/or synergistic contributions of *PITX1*, *TBX5* and downstream candidate effectors identified in our studies. In addition, we are utilizing our dataset of 120 resequenced genomes to discover loci that modify foot feathering in the domestic pigeon. By studying the genetic and molecular mechanisms that underlie variation in foot feathering in this unique avian system, we hope to gain insights into the mechanisms that determine epidermal appendage fate.

Program Abstract #108

Airway morphogenesis and morphometric scaling are conserved in chicken, quail, and duck embryos

James Spurlin, Daniel Tzou, Amira Pavlovich, Carolyn Stewart, Jason Gleghorn, Celeste Nelson

Princeton University, USA

The generation of branched tubular networks is a conserved evolutionary process used to facilitate the transport and exchange of materials throughout the body plan of an organism. Many organs with branched networks form in a highly stereotypic manner across individuals within a species. For example, new branches within the embryonic chicken lung form at precise locations along the primary bronchus via apical constriction of the airway epithelial cells. Branching locations in the chicken lung also scale relative to the size of the organ during development. The geometry and branching mechanism observed in the chicken embryonic lung has been well characterized, however it is not known if this branching program is evolutionarily conserved in other species within the avian class. Here, we examined the extent to which this scaling relationship and branching mechanism are conserved in lungs of three bird species representing two taxonomic orders, Galliformes and Anseriformes. Analyzing the development of embryonic lungs of species within the order

Galliformes, which includes the domestic chicken (Gallus gallus var. domesticus) and Japanese quail (Coturnix japonica), revealed that the patterns of branching are spatiotemporally conserved between species within the order. Embryonic Pekin duck (Anas platyrhynchos domestica) lungs were used to represent the airway branching program from the order Anseriformes. In contrast to the species within Galliformes, duck lungs form at slightly different positions and are significantly larger than those of chicken and quail at the same stage of development. Interestingly, confocal analysis revealed that each secondary bronchus forms by apical constriction of the dorsal epithelium of the primary bronchus in all three avian species. Our results suggest that branch positions in the lung are conserved relative to the evolutionary relationship between avian species, and that each species uses apical constriction to initiate branching events.

Program Abstract #109

The revised cell lineage of the larval photoreceptor cells in the ascidian *Ciona intestinalis*

Kouhei Oonuma, Takehiro G. Kusakabe

Dept. Biol., Konan Univ., Japan

The brain of the ascidian *Ciona intestinalis* larva contains two distinct photoreceptor organs, a conventional pigmented ocellus and a nonpigmented ocellus. The photoreceptor cells of these ocelli are ciliary photoreceptor cells resembling visual cells of the vertebrate retina. Precise elucidation of cell lineage of the photoreceptor cells in the ascidian larva is a key to understand developmental mechanisms of these cells as well as evolutionary relationships between photoreceptor organs of ascidians and vertebrates. Previous studies inferred that the photoreceptor cells of the pigmented ocellus are derived from the right a9.33 and a9.37 cells, descendants of a blastomere of the anterior animal hemisphere. The photoreceptor cell lineage is, however, not conclusive because it was only a speculation based on observation of unlabeled embryos without using any photoreceptor markers. Here, we traced the fate of neural plate cells from the late gastrula to larval stages by labeling particular cells of intact (non-dechorionated) embryos at single-cell resolution using the photo-convertible fluorescent protein Kaede. We investigated which cells give rise to the photoreceptor cells by using photoreceptor-specific markers. Our results conclusively indicate that the photoreceptor cells of both pigmented and nonpigmented ocelli develop from two right medial cells (A9.14 and A9.16) of the neural plate, which are derived from the anterior vegetal hemisphere. None of the photoreceptor cells develop from the anterior animal (a-line) blastomeres.

Program Abstract #110

Butterfly color vision: stochastic patterning mechanisms and increased sensory receptor diversity

Michael Perry[1], Michiyo Kinoshita[3], Giuseppe Saldi[2], Lucy Huo[1], Kentaro Arikawa[3], Claude Desplan[1,2]

[1]New York University, USA; [2]NYU Abu Dhabi, UAE; [3]SOKENDAI, The Graduate University for Advanced Studies, Japan

Butterflies use color vision extensively to navigate the natural world. Their retinas are more complex than those found in *Drosophila*, where development and patterning has been heavily studied. Instead of the eight photoreceptors found in flies, butterflies have an additional ninth photoreceptor per ommatidium ("unit eye"). They also have three main types of ommatidia instead of the two distributed stochastically in the fly retina. We set out to determine how butterflies generate increased sensory receptor diversity to provide improved color vision, and how much of the retinal patterning network from *Drosophila* they reuse. We show that the regulatory network that defines photoreceptor subtypes in *Drosophila* is redeployed in butterflies (*Papilio xuthus* and *Vanessa cardui*) to generate additional subtypes. The R7 photoreceptor marker Prospero is expressed in two rather than one photoreceptors per ommatidium. In *Drosophila*, a stochastic decision to express the transcription factor Spineless in R7 determines which of two subtypes of ommatidia is specified. CRISPR/Cas9 knock-out of Spineless in butterflies shows that Spineless also controls stochastic choice in each of the two R7s, suggesting a deep evolutionary conservation of stochastic patterning mechanisms. Having two stochastically distributed types of R7s allows for specification of three ommatidial types instead of two, which in turn allowed for the evolution and deployment of additional opsins, tetrachromacy, and improved color vision. These efforts provide evidence that our extensive knowledge of patterning in the *Drosophila* visual system applies to other groups, and that adaptation for specific visual requirements can occur through modification of this network.

Program Abstract #111

Finding wings in a non-winged arthropod

Courtney Clark[1], David Linz[1], Xavier Bellés[2], Elke Buschbeck[3], Nipam Patel[4], Yoshinori Tomoyasu[1]

[1]Department of Biology, Miami University, Oxford, Ohio, United States; [2]Institut de Biologia Evolutiva, Barcelona, Spain; [3]Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, United States; [4]Department of Integrative Biology, University of California, Berkeley, California, United States

Despite accumulating efforts to unveil the origin of insect wings, it remains one of the principal mysteries in evolution.

Currently, there are two prominent hypotheses regarding insect wing origin: one connecting the origin to a lateral outgrowth of the dorsal body wall (tergum) and the other to ancestral proximal leg structures (pleurites). However, neither hypothesis has been able to surpass the other. To approach this conundrum, we focused our analysis on *vestigial* (*vg*), a critical wing gene initially identified in *Drosophila*. Our investigation in the *Tribolium* beetle led to the identification of two *vg*-dependent tissues in the "wingless" first thoracic segment (T1). Intriguingly, these two tissues may actually be homologous to the two proposed wing origins (tergum and pleurites). This observation, along with our Hox analysis, suggests that insect wings have a dual origin, and that the merger of two unrelated tissues has been a key step in developing this morphologically novel structure during evolution. We are currently testing this hypothesis by (i) analyzing the development of the *Tribolium* T1 wing serial homologs in more detail, (ii) evaluating the presence of the T1 wing homologs in another beetle (a diving beetle, *Thermonectus*) and a hemimetabolous insect (a cockroach, *Blattella*) and (iii) investigating possible wing homologs in a non-winged arthropod (a crustacean, *Parhyale*) in order to gain further insights into insect wing evolution. Intriguingly, through expression analyses and CRISPR/Cas9-based genetic modification, we found that *vg* is important for the development of both tergal and proximal leg tissues in *Parhyale*, suggesting that these tissues may be crustacean wing homologs. Although preliminary, these results provide further support for a dual origin of insect wings and give us a more comprehensive view of insect wing evolution. This work is supported by the National Science Foundation (IOS 1557936 and IOS 0950964 (Y.T.), GRF and EDEN (C.C-H.)).

Program Abstract #112

Changes in insulin signaling underlie the evolution of wing morph specification in populations of the soapberry bug *Jadera haematoloma*

David Angelini, Meghan Fawcett, Alice Grubb Jones, Wenzhen Hou, Mary Parks, Elizabeth Richards, Juan Camilo Vanegas

Colby College, USA

Polyphenic traits develop different final states due to environmental cues. However, it is unclear how developmental processes differ to achieve distinct morphs. Moreover, there are few systems in which developmental changes underlying the evolution of reaction norms for polyphenic traits have been identified. The red-shouldered soapberry bug *Jadera haematoloma* (Heteroptera: Rhopalidae) has been a model of adaptive evolution, but holds great potental as a system for eco/evo-devo studies. This species exhibits polyphenic wing morphs in both sexes. Adults may develop with complete wings and functional flight muscles or brachypterous wings incapable of flight. Wings of each morph differ in size and veination patterns. The specification of wing morphs is determined by juvenile food availability, with increased food access causing an increase in the short-winged morph. Norms of reaction vary by population, with populations adapted to higher nutrient host plants exhibiting less responsive reaction norms and higher frequencies of the short wing morph. These populations also exhibit differences in the expression of insulin signaling components. The reaction norm of bugs from responsive populations can be shifted to resemble those of high nutrient-adapted, low plasticity populations by RNA interference of insulin signaling components, including FoxO and insulin receptor. Knockdown of these genes also produced allometric changes in several appendages. These results suggest that evolution of the polyphenic reaction norm among soapberry bug host races has occurred by changes in the expression of insulin-signaling components. Combining morphometric and transcriptomic comparisons of wing morphs also suggests a limited number of other candidate pathways involved in the specification and differentiation of morphs. This work has been supported by funds from the Colby College Division of Natural Sciences and NSF grant IOS-1350207.

Program Abstract #113

The genetic basis of eyespot size in *Junonia coenia* and development of transparent windows in Saturniid moths

Yuriko Kishi, Rachel Thayer, Nipam H. Patel

University of California Berkeley, USA

Lepidopterans (butterflies and moths) have evolved a vast array of wing patterns; such biodiversity can be ascribed to changes in the developmental pathways that pattern scale distribution, pigmentation, and morphology over the wing surface. Here I present my ongoing work on the development of two striking wing pattern elements: eyespots and transparency. Eyespots are functionally important, and eyespot number, size and prominence vary greatly among species. Previous studies on eyespot development have implicated a number of candidate genes, but their roles remain largely unclear. Here, we take a forward genetics approach to map quantitative trait loci (QTL) that control the size of the anterior eyespots on the dorsal surface of the hindwing in *Junonia coenia*. We started with parents that differ significantly in eyespot size and generated a large F2 family that shows a wide distribution of eyespot size. Our initial results show a broad spread of eyespot size among F2s. We therefore infer that eyespot size is a polygenic trait. We are now engaged in

mapping the loci that control variation in eyespot size. Wing transparency has evolved independently multiple times in Lepidoptera. Despite their separate origins, all transparency involves either an alteration in scale morphology or an absence of scales. Although normal scale development has been characterized, the development of these regions with altered scales or without scales, is unknown. To elucidate the developmental basis for wing transparency, pupal dissections were conducted on two Saturniid moths, *Actias luna and Antheraea polyphemus*, both of which have transparent regions (windows) that lack scales. Early pupal stages showed that scale+socket precursor cells (sensory organ precursor cells) never form.

Program Abstract #114
Revealing evolutionary mechanisms by mapping pigmentation character states and developmental mechanisms onto a resolved fruit fly phylogenetic tree
Jesse Hughes
University of Dayton, United States
Since the origin of the 36 recognized animal phyla over 500 million years ago, subsequent evolution can be largely summarized as the diversification of physiological, behavioral, and morphological characteristics among these original 36 body plans. Diversification continues to this day and can be seen in humans as differences in coloration, lactose metabolism, and energy storage in fat tissue. As all animal characteristics are products of development, a key challenge for contemporary research is to reveal the ways in which development evolves through changes in the uses of genes. To meet this challenge, investigations must prioritize characteristics: that have recently evolved, the direction of evolution is known, and for which the underlying genes can be studied by modern genetic manipulations. One ideal trait is the diverse coloration patterns observed on the abdominal tergites of fruit fly species from the Sophophora subgenus. Prior research has supported a scenario where melanic pigmentation limited to the male abdomen evolved once within this clade through the evolution of a sexually dimorphic pattern of expression for the bric-à-brac transcription factor genes. My research challenges this scenario by looking at the patterns of pigmentation on the abdomens of species representing the diverse Sophophora species groups and interrogating the patterns of bric-à-brac expression during the development of the abdominal tergites. Success in this work will advance the fruit fly pigmentation model as exemplar of how diversity evolves through the re-working of developmental mechanisms.

Program Abstract #115
Resolving the Gene Expression bases for the Convergent Evolution of a Pigmentation Trait
Sumant Grover[1], Victoria Spradling[2], Thomas. M. Williams[3]
[1]University of Dayton, USA; [2]University of Dayton, USA; [3]University of Dayton, USA
The genetic basis by which organisms adapt to an ever changing world remains a topic of great interest to the fields of evolution, development, and conservation biology. It is understood that animal genomes contain over ten thousand genes and distantly related species possess many of the same genes due to common ancestry. What is less well understood is how new traits evolve using these shared genes and whether the genetic basis for evolution favors certain genes over others. At the heart of trait development are genes that encode proteins that regulate the expression of other genes, notably transcription factors and chromatin modifying proteins. Traits can evolve through changes in the expression patterns for these genes or through changes in which target genes they regulate. However, case studies connecting gene expression changes to trait evolution remain few in number. Additionally, it is unclear whether gene expression evolution favors alterations in certain genes over others. In order to understand how a novel trait evolves and to determine whether evolution can prefer certain gene targets for modification, we are studying the convergent evolution of fruit fly pigmentation in the lineages of *Drosophila melanogaster* and *Drosophila funebris*. These two species can be considered biological replicates for the evolution of male-specific pigmentation on the A5 and A6 abdominal segments. To understand the genes involved in the formation and evolution of these similar pigmentation patterns, we are utilizing candidate gene and comparative transcriptomic approaches. Completion of this work will provide novel insights on the genetic changes responsible for a trait's origin, and whether development constrains evolutionary paths to certain genes.

Program Abstract #116
Understanding the evolution of a fruit fly pigmentation gene network from the vantage point of the Ddc gene
Victoria Spradling, Sumant Grover, Lauren Gresham, Thomas Williams
University of Dayton, United States
Understanding the genetic and molecular underpinnings for morphological diversity remains a central goal of evolutionary and developmental biology research. While it is now understood that these traits arise by the orchestrated

expression of numerous genes, comprising a gene regulatory network, what remains poorly understood is how these networks of genes and their expression patterns are initially assembled and subsequently diversify. Gene expression is controlled by DNA sequences that are often referred to as *cis*-regulatory elements (CREs). Each CRE possesses binding sites for transcription factor proteins whose cumulative binding results in a specific pattern of gene expression. It is anticipated that gene expression evolution frequently occurs through the formation, modification, and destruction of CREs, presumably through changes that create or remove binding sites for transcription factor proteins. However the binding site level of CRE evolution has been worked out in very few cases. The fruit fly species *Drosophila melanogaster* has a male-specific pattern of abdominal pigmentation for which the enzyme encoding genes and several of their upstream transcription factor regulators are known. However, the details of how these regulators interact with CREs remain largely uncharacterized. One such enzyme gene that is necessary for this species' pattern of pigmentation is *Dopa decaboxylase* (*Ddc*). Here we share the results of our efforts to uncover the CRE-basis for this gene's expression pattern, and how this regulation and pattern of expression has evolved during the origin and diversification of this male-specific trait. Success here will advance a leading model for the CRE and gene network basis for morphological diversity.

Program Abstract #117

The molecular basis of stripe pattern formation in rodents

Ricardo Mallarino[1], Corneliu Henegar[2,3], Mercedes Mirasierra[4], Marie Manceau[5], Carsten Shradin[6], Mario Vallejo[4], Slobodan Beronja[7], Gregory Barsh[2,3], Hopi Hoekstra[1]

[1]Harvard University, USA; [2]HudsonAlpha Institute for Biotechnology, USA; [3]Stanford University, USA; [4]Instituto de Investigaciones Biomédicas Alberto Sols, Spain; [5]Collège de France, France; [6]Université de Strasbourg, France; [7]Fred Hutchinson Cancer Research Center, USA

Mammalian color patterns, from zebra stripes to leopard spots, are among the most recognizable characters found in nature and can have a profound impact on fitness. However, little is known about the mechanisms underlying their formation and subsequent evolution. Here we take advantage of the naturally occurring color pattern of the African striped mouse (*Rhabdomys pumilio*) to investigate the mechanisms responsible for forming periodic stripes, a common pattern in mammals. We show that stripes result from underlying differences in melanocyte maturation, which give rise to spatial variation in hair color, and we identify the transcription factor *Alx3* as a regulator of this process. In striped mice, *Alx3* is differentially expressed along the dorsal skin prior to the appearance of stripes and localizes to different skin cell types, including melanocytes. *In vivo* functional tests using ultrasound-assisted lentiviral infections in *Mus* revealed that *Alx3* decreases melanocyte maturation and melanin synthesis, recapitulating melanocyte behavior in the light stripes of striped mice. Furthermore, in vitro and biochemical assays show that *Alx3* acts to directly repress *Mitf*, a master regulator of melanocyte differentiation. Finally, we find that *Alx3* is also differentially expressed in the dorsal stripes of chipmunks, which have independently evolved a similar pattern of dorsal stripes. Our results show that differences in the spatial control of *Alx3* expression lead to striped patterns in rodents, revealing both a new factor regulating pigment cells and a previously unappreciated mechanism for modulating hair color, and thereby provide new insight into the ways in which phenotypic novelty evolves.

This work is supported by Howard Hughes Medical Institute

Program Abstract #118

Spatio-temporal expression pattern of neurogenic homologs reveals a possible role in early neurogenesis in *Capitella teleta*

Abhinav Sur

Clark University, USA

How centralized nervous systems (CNSs) evolved remains an unresolved question. Previous studies in vertebrates and arthropods have revealed that similar neurogenic homologs regulate CNS development. In vertebrates and insects, homologs of SoxB1 HMG-box transcription factors regulate cell proliferation whereas proneural genes repress *soxB1* homologs thereby inducing differentiation. Here, we have isolated and studied the spatiotemporal expression patterns of neurogenic homologs in the annelid *Capitella teleta*, which belongs to a separate bilaterian clade (Spiralia) as compared to arthropods (Ecdysozoa) and vertebrates (Deuterosomia). This will help identify which aspects of bilaterian neurogenesis might have been ancestral or were derived within Spiralia. During *C. teleta* brain neurogenesis, neural precursor cells (NPCs) in the surface ectoderm proliferate and generate daughter cells that begin to exit the cell cycle, ingress inward, and generate neural subtypes. Therefore, using whole mount in-situ hybridization, *Ct-SoxB1* was detected in surface cells in the neuroectoderm whereas *Ct-SoxB* was detected in overlapping domains of *Ct-SoxB1* expression, which indicates a possible interaction between the two classes of *soxB* factors similar to vertebrates.

Neural specific gene homologs like *Ct-msi* is expressed in a similar pattern as *Ct-Notch* and *Ct-Delta*, which might indicate involvement with the Notch pathway. Pro-differentiation homologs like *Ct-pros* expression suggests a possible role in specification of neural fate in early development of *C. teleta*. Proneural homologs like *Ct-ngn* is expressed in superficial cells, similar to *Ct-ash1* and *Ct-SoxB1*, whereas, *Ct-neuroD* is expressed in more internalized cells. This study will contribute to better understanding of spatiotemporal CNS development in annelids while also laying groundwork for future functional studies and genome-wide transcriptomic studies.

Program Abstract #119

Ancient origin of the vertebrate enteric nervous system from neural crest-derived Schwann cell precursors

Stephen Green, Benjamin R. Uy, Marianne E. Bronner
California Institute of Technology, USA

The enteric nervous system of jawed vertebrates (gnathostomes) largely arises from vagal neural crest cells that migrate from the hindbrain to the gut and produce neurons throughout the gastrointestinal tract. In order to better understand the evolutionary origins of vertebrate enteric neuron fate and organization, we examined the enteric nervous system in a basally branching vertebrate, the jawless (agnathan) sea lamprey *Petromyzon marinus*. Surprisingly, labeling vagal neural crest cells did not provide evidence of vagal neural crest cell migration to the gut, but instead marked neural crest cells that remain associated with the pharynx. However, DiI labeling showed that migratory cells emerge from the neural tube at later stages to produce cells associated with nerve fibers. A subset of these migratory cells travel to the gut and differentiate into serotonergic cells that remain in close association with the gut epithelium, muscle fibers, and hematopoietic cells. We propose that these migratory cells are homologous to Schwann cell precursors (SCPs), which were recently shown to contribute to a subset of mammalian enteric neurons and parasympathetic ganglia. These data suggest that cells like neural crest-derived SCPs might have had a role in generating the ancient enteric nervous system of early vertebrates and that vagal neural crest cells might have assumed this role during the evolution of jawed vertebrates.

Program Abstract #120

Characterization of Kctd15 function and biological relevance in zebrafish development

Alison Heffer, Graham Marquart, Harry Burgess, Igor Dawid
Section on Developmental Biology, DDB, NICHD, NIH, Bethesda MD, USA

In vertebrates, the neural crest (NC) constitutes a population of embryonic multipotent cells which dissociate from the developing neural tube and migrate throughout the body, giving rise to many diverse cell lineages including pigment cells, craniofacial cartilage, and peripheral nervous system. We previously reported that in zebrafish, the BTB domain-containing protein Kctd15 is an antagonist of NC development, presumed to function by delimiting the NC domain through repression of transcription factor AP-2a. To further study Kctd15 function, we used transcription activator-like effector nuclease (TALEN) technology to introduce frame-shift mutations in the two zebrafish *kctd15* paralogs. Here we report that both *kctd15a* and *kctd15b* homozygous single mutants are viable, with no measurable defects in neural crest development. While fish carrying homozygous mutations in both *kctd15* paralogs are surprisingly also viable and fertile, they are smaller in size and show defects in NC cell lineages. NC markers, including *foxd3* and *sox10,* are expanded in *kctd15* double mutants. Additionally, there is a pronounced increase in the number of melanophores in the mutants, suggesting an upregulation of genes and/or pathways involved in melanophore development. We also report a novel function of *kctd15* in the zebrafish brain: *kctd15* double mutants appear to be missing their torus lateralis, a region implicated in gustatory pathways in other fish. Additionally, double mutant fish are missing barbels, a sensory organ that contains taste buds. We are currently investigating the role of *kctd15* in taste sensing, to test the hypothesis that ineffective feeding behavior may be a cause for the developmental delay and small size of the *kctd15* double mutants.
This research is funded by the National Institute of Child Health and Human Development, NIH

Program Abstract #122

Thyroid hormone integrates craniofacial development and feeding kinematics in zebrafish

Sarah McMenamin[1], Casey Carter[2], Caroline Nazaire[1], Aisha Khalid[1], James Cooper[2]
[1]University of Massachusetts, Lowell, USA; [2]Washington State University, USA

Thyroid hormone is critically important to numerous aspects of vertebrate development, and can affect coordinated suites of traits; thus, the hormone is hypothesized to play an important role in adaptation and diversification. We show that in zebrafish, wild-type levels of thyroid hormone are required for proper craniofacial ossification and development, as well as normal integration of feeding behavior. Zebrafish developing under transgenically-induced hypothyroid conditions show incomplete ossification and altered craniofacial proportions, while genetically hyperthyroid fish develop

hypertrophic lower jaws. We show that thyroid hormone is required for properly integrated suction feeding behavior, and that a lack of thyroid hormone disrupts feeding kinematics in several respects, including changes to jaw protrusion and hyoid depression. Moreover, we find that the hormone coordinates the onset of adult feeding behavior, and that feeding kinematics of hypothyroid adults resemble those of larvae.
To place the traits and integration controlled by thyroid hormone into a broader phylogenetic context, we examined the feeding kinematics of seven other Danionin species. We show that disrupted thyroid hormone metabolism causes some aspects of craniofacial morphometrics and feeding kinematics to resemble those of other species. We conclude that changes in thyroid hormone production or sensitivity may indeed play a role in craniofacial diversification of teleosts and other vertebrates.

Program Abstract #123
Developmental basis of craniofacial evolution in East African cichlids
Thomas Schilling, Pierre Le Pabic, Anthony Long
Univ of California-Irvine, USA
What are the mutations and developmental mechanisms that underlie craniofacial evolution? The spectacular eco-morphological adaptations amongst Cichlidea in lakes Victoria, Malawi and Tanganyika offer an exceptional opportunity to address these questions. Not only have the cichlid radiations in these lakes produced a vast array of skull shapes and sizes, but hybrids of species with different craniofacial morphologies can be generated allowing genome-wide Quantitative Trait Locus (QTL) mapping approaches for identifying genetic variants underlying these differences. We have hybridized two cichlid species from Lake Malawi with vastly different preorbital size and shape: *Dimidiochromis compressiceps* (DC) – a piscivorous species with an elongated and laterally compressed head, and *Copadichromis azureus* (CA) – an omnivorous species with a smaller head. Comparative analysis of the overall DC and CA head skeletons, and of dissected skeletal elements has highlighted a subset of cartilage-derived bones as major modulators of head morphology between these species. In addition, significant but subtle differences in the size and shape of the cartilage precursors of these elements can already be detected at embryonic stages, which are associated with differences in endochondral growth zone width. Our results suggest that modulating embryonic cartilage patterning and growth zone activity in a few skeletal elements provides a simple mechanism for generating large differences in the adult head morphology of DC versus CA, but also between other cichlid species from East African rift lakes. DC/CA hybrids have been generated and genetic mapping in the F2 generation is underway to find the loci underlying these developmental differences.

Program Abstract #124
Role of Lbh during craniofacial development: bringing molecular mechanisms to evo-devo questions.
Helene Cousin, Kara Powder, Dominique Alfandari, Craig Albertson
Univ of Massachusetts, USA
The cranial neural crest (CNC) is a key contributor to the craniofacial development. These cells originate from the border of the future brain during neurulation stages, undergo extensive anterior and ventral migration and differentiate in the many tissues composing the face and the cranium. Changes in migration or differentiation of these CNC are at the origin of the diversity of the neurocranium and viscerocranium among vertebrates. While this plasticity is key to evolution, it is also makes craniofacial structures prone to developmental defects such as cleft lip and palate or mircognathia. We have characterized a SNP in the gene *limb bud and heart homolog* (*lbh*), between the long-jawed pelagic *Maylandia zebra* (MZ)and the short-jawed benthic *Labeotropheus fuelleborni* (LF) that contributes to adaptive variation in the jaw of cichlid fishes. We show that this small transcriptional co-regulator is essential for neural crest migration and craniofacial development in vertebrates such as zebrafish and *Xenopus*. By expressing the LF and MZ version of Lbh in *Xenopus* CNC, we also showed that a single nucleotide change produce discrete shift in the migration of CNC that are consistent the length of jaw of the specie of origin. Combining the power of the *Xenopus* model system and mass spectrophotometry approaches, we have identified promising binding partners of Lbh. We are currently investigating how Lbh and its binding partners affect neural crest migration. Funding: NIH/NIDCR DE016289.

Program Abstract #125
Gene duplication and functional divergence in the APETALA3 lineage of floral organ identity genes
Jesus Martinez-Gomez[1], Kelsey Galimba[1,2]
[1]University of Washington, USA; [2]USDA-ARS Appalachian Fruit Research Station & Department of Cell Biology & Molecular Genetics, USA
The floral organ identity gene *APETALA3 (AP3)*, in the B class of the ABC model of flower development, is a MADS-

box transcription factor involved in stamen and petal identity. The main goal of this study was to conduct a functional analysis of three *AP3* orthologs from the ranunculid *Thalictrum thalictroides*, representing the paleo*AP3* gene lineage, to determine the degree of redundancy versus divergence after gene duplication. Because *Thalictrum* lacks petals, we also asked whether these loci cause ectopic petaloidy of sepals, found in insect-pollinated species. We undertook functional characterization by virus induced gene silencing, protein-protein interaction, and binding site analyses. Our results suggest that, in addition to the conserved role in stamen identity, these genesacquired a new function in ectopic petaloidy of sepals. Moreover, we propose that one paralog coding for a truncated protein acts as a dominant negative that maintains B-class transcript homeostasis. This hypothesis is based on up-regulation of all other B-class transcripts upon targeted silencing of the truncated paralog, on promiscuous protein-protein interactions and on the presence of AP3 binding motifs in all B-class gene promoters..

Program Abstract #126

Suppression of a cyclin-dependent kinase inhibitor disrupts floral organ identity in *Solanum pennellii*

Shuxin Ren[1], David Johnson[1], Laban Rutto[1], Thomas McKnight[2]
[1]*Virginia State University, USA;* [2]*Texas A&M University, USA*

S. pennellii is a wild relative of the cultivated tomato, native to arid regions of Peru. One factor facilitating its survival is the secretion of 2, 3, 4 tri-O-acylated glucose esters (glucolipids) that coat whole plants. These glucolipids contain three fatty acid chains with the lengths of hydrocarbons falling within the range of gasoline (C4-C12). Therefore, *S. pennellii* is a potential source for bio-gasoline production. However, the biomass of *S. pennellii* is relatively less when compared to cultivated tomato. In an effort to increase the biomass of *S. pennellii*, we cloned and knocked out a cyclin-dependent kinase inhibitor, *SpKRP1*, using RNAi technology. The transgenic lines significantly delayed flowering time for at least two months. When flowers initiated, the floral organ identities were completely disrupted. The number and size of sepals was significantly increased and all became leaf-shapes. No petals were observed, but some flowers showed small, light-green-colored, leaf shaped organ at positions where petals should be located. Reproductive parts as the stamen did not produce the anther cone and also failed to produce pollen. All flowers do not have normal shape anthers. Additionally, unlike the wildtype control where the carpels are long and extrude out of anther cones, the transgenic lines presented round-shaped carpels covered with trichomes. Our study demonstrates that SpKRP1 may be a key regulator in the reproductive development of *S. pennellii*

Program Abstract #127

The role of toolkit genes in the evolution of the complex abdominal color pattern of *Drosophila guttifera*

Komal Kumar Bollepogu Raja, Peter Nouhan, Evan Bachman, Alexander McQueeney, Elizabeth Mundell, Amber Peabody, Alexandri Armentrout, Thomas Werner
Michigan Technological University, USA

How gene-regulatory networks drive morphological diversity is an intriguing evo-devo question. Animals share a common genetic toolkit, which orchestrates the building of the basic body plan. Some toolkit genes have been coopted into new developmental pathways, thereby leading to many evolutionary novelties. One example is the formation of color patterns in animals. We developed the fruit fly *Drosophila guttifera* as a model to study complex wing and body color patterns. The coloration of *D. guttifera* is striking, as this species displays spot and stripe patterns on its wings, thorax, and abdomen. Previously, we have shown that the Wingless morphogen is sufficient to induce the pigmentation gene *yellow* (*y*), which is necessary for the production of black melanin spots on the wings of D. guttifera. Our current research focuses on the abdominal pigmentation pattern of the same species, which consists of four distinct sub-patterns: one pair of dorsal, median, and lateral rows of spots, plus a dorsal midline shade. Our *in situ* hybridization and immunohistochemistry data in developing pupae show that the pigmentation gene *y* is expressed as mRNA and protein in the same pattern as the adult melanin pattern. In our search for regulators of *y* expression on the abdomen, we found that the toolkit gene *wingless* foreshadows the entire spotted pattern just before *y* transcription starts, while *abdominal-A*, *decapentaplegic*, *hedgehog*, and *zenknullt* are also expressed at that time, but only in distinct subsets of the abdominal pattern. Using a transgenic reporter assay in *D. guttifera* pupae, we have identified a ~1 kb fragment within the *y* intron that drives DsRed in a pattern closely resembling the adult abdominal spot pattern. We are currently sub-dividing this 1 kb fragment to identify the core enhancer sequence to allow us to narrow down on the putative transcription factor binding sites that may activate *y* in the spotted pattern.

Program Abstract #128

A new toolbox for the fly *Sciara* - a new/old model system that disobeys the rules for chromosome movement on spindles

Susan Gerbi, Yutaka Yamamoto, John Urban, Jacob Bliss
Brown University, USA

The fly *Sciara* offers many unique biological features, and is an outstanding model system to elucidate questions of chromosome mechanics:

- chromosome imprinting;
- a monopolar spindle in male meiosis I;
- non-disjunction of the X chromosome in male meiosis II;
- chromosome elimination in early embryogenesis;
- sex determination; evolution towards parthenogenesis;
- germ line limited (L) chromosomes;
- DNA amplification in salivary gland polytene chromosomes;
- high resistance to radiation.

We have now developed a toolbox to enable *Sciara* research and welcome new investigators [http://brown.edu/go/sciara-stocks]. We are completing the *Sciara* genome with cutting edge approaches for assembly using long reads from the PacBio RSII and Oxford Nanopore MinION, using Illumina reads for polishing, and using BioNano Irys optical maps for scaffolding. Genome annotation used RNA-seq data from the *Sciara* transcriptome interrogating both sexes at multiple stages. We have used the genomic data to identify sequences of "DNA puffs" that represent sites of DNA amplification in salivary gland polytene chromosomes regulated by ecdysone. We have developed methods for transformation of *Sciara* to manipulate its genome. We present here a new method for site-specific integration of large DNA into the *Sciara* genome. Instead of homologous recombination (HR) that is inefficient, we have used the pathway preferred in most cells of non-homologous end-joining (NHEJ) coupled with obligate ligation-gated recombination (ObLiGaRe) for high efficiency precise insertion of large DNA into a unique double-strand break. This approach is easily applicable to a broad range of organisms, including those where a transformation system has not been available. With the new toolbox of the genome assembly and transformation methodology, the time is now ripe to elucidate many canonical processes using the unique biological features of *Sciara*.

Program Abstract #129

Analysis of cell cycle exit and G1 arrest using the live cell cycle reporter FUCCI during embryonic and larval development in the segmented worm *Platynereis dumerilii*

B. Duygu Ozpolat[1], Mette Handberg-Thorsager[2], Michel Vervoort[1], Guillaume Balavoine[1]
[1]Institut Jacques Monod, CNRS, Université Paris Diderot, France; [2]Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Cell cycle regulation plays crucial roles in morphogenesis, maintenance of pluripotency, and cell fate decisions during development. In the frog, normal progression of gastrulation requires cell cycle arrest; in tunicates, a long G2 phase in the epidermis is required for neural tube closure; in mouse embryonic stem cells, alteration of G1/S transition affects self-renewal vs differentiation. To better understand spatio-temporal regulation of cell cycle progression during development in the polychaete annelid (segmented worm) *Platynereis dumerilii* –an emerging model-, we developed a FUCCI (**f**luorescent **u**biquitination-based **c**ell **c**ycle **i**ndicator) live cell cycle reporter. FUCCI relies on the cycling of fluorescent proteins depending on the truncated cell cycle protein they are fused with. We used mVenus-Cdt1 fusion, which accumulates in G1 and is degraded in S/G2 phases. We carried out in vitro-transcribed mRNA injections of fertilized eggs to express the FUCCI construct. Using time-lapse imaging, we analyzed cycling characteristics of different cell populations. Like most polychaetes, *P. dumerilii* reproduces via external fertilization giving rise to swimming larvae that are challenging to live-image due to their ciliary bands. For the first time, we report long-term time-lapse imaging of both embryonic and larval stages of a polychaete. Results show that some terminally differentiated cell types such as multi-ciliated cells exit the cell cycle at G1 phase, indicated by the accumulation of mVenus-Cdt1. In addition, primordial germ cells, which are mitotically quiescent at larval stages, appear arrested at G1 phase, indicated by retention of the mVenus-Cdt1 signal during the first two days of development imaged. The FUCCI tool we are developing will allow us to study cell cycle regulation during several post-larval developmental processes in *P. dumerilii*, such as posterior elongation, segment formation, and regeneration.

Funding:CNRS, Université Paris Diderot, ANR, Labex

Program Abstract #130

***Macrobrachium olfersi*: exploring developmental gene toolkit and associated pathways by transcriptomic analyses of a potential new crustacean model**

Dib Ammar[1,3], Michael Jaramillo[1], Eliane Zeni[1], Heloisa Schramm[1], Frank Guzman[2], Christian Paese[1], Rogerio Margis[2], Yara Müller[1], Evelise Nazari[1]

[1]Universidade Federal de Santa Catarina, BR; [2]Universidade Federal do Rio Grande do Sul, BR; [3]Centro Universitário Católica de Santa Catarina, BR

The crustaceans are one of the largest, most diverse, and most successful groups of invertebrates. The diversity among the crustaceans is also reflected in embryonic development models. However, the molecular genetics that regulate embryonic development are not known in those crustaceans that have a short germ-band development with superficial cleavage, such as *Macrobrachium olfersi.* This species is a freshwater decapod and has great potential to become a model for developmental biology, as well as for evolutionary and environmental studies. To obtain embryonic developmental genes of *M. olfersi*, we performed *de novo* assembly and annotation of the embryonic transcriptome. Using a pooling strategy of RNA, paired-end Illumina sequencing, and assembly with multiple k-mers, a total of 99,751 unigenes were identified, and 20,893 of these returned a Blastx hit. Of these, 2,142 unigenes were assigned to the developmental process category. Developmental genes classically studied was identified by tBlastn, assuch *Even skipped*, *Engrailed*, *Hox* (*Ultrabithorax*, *Antennapedia, Fushi tarazu* e *Sex comb reduced*) and *Distal-less* genes. Different profiles of these genes were expressed in development stages (E3-E10) by semiquantitive RT-PCR. RT-qPCR was conducted, indicating that *Ultrabithorax*, *Antennapedia* and *Sex comb reduced* genes expression was increased, and *Fushi tarazu* was decreased according to the progression of embryonic development. In addition, genes of developmental pathways were found, including TGF-β, Wnt, Notch, MAPK, Hedgehog, Jak-STAT, VEGF, and ecdysteroid-inducible nuclear receptors. To our knowledge, this is the first study of the transcriptome in *M. olfersi*, as well as the first to provide information on a valuable database of development related genes of this species. The results open new possibilities for developmental studies at the molecular level of these prawns, as such *in situ* hybridization, transgenesis and gene knockdown for studies of functional genomic.

Program Abstract #131

Morphogenetic Mechanisms Governing Optic Vesicle Closure in the Cephalopod *Doryteuthis pealeii*

Kristen Koenig

FAS Center for Systems Biology, Harvard University, USA

The eye is a classic case in the narrative of evolved complexity. Visual systems range in structure from single photoreceptor cells, pigmented eyespots and cups, to multi-tissue organs that focus, reflect and absorb light in order to resolve images. Inherently the form of visual organs is important to their functionality and little is known about how these forms develop outside traditional models. Our interest is to better understand the morphogenesis of complex visual systems in a comparative manner with the goal of revealing basic cellular mechanisms involved in elaborating simple organs into more complicated morphologies. We have established the single-chambered eye of the squid *Doryteuthis pealeii* as a model for eye development and our previous work has shown that the cephalopod eye forms through the internalization of two bilateral retina placodes by the future lens and iris tissue. This internalization event generates the optic vesicles, which will ultimately differentiate into all the cell types that compose the eye. We have established *in vivo* imaging protocols that allow us to better understand which morphogenetic processes, such as cell division, cell migration and cell shape change, may contribute to the generation of this complex morphology. Together this work reveals new insights into epithelial morphogenesis and the generation of complexity found across the Bilateria. Support for this work was provided by the Grass Foundation.

Program Abstract #132

Characterization and Staging of Daphnia magna Eye Development

Matthew Beckman, Michelle Grafelman

Augsburg College, USA

At the earliest appearance of eye structures in *Daphnia magna* embryos, two distinct pigmented eye spots are present, which gradually grow and fuse together, leaving adult daphnids with a cyclopean eye. These eye spots are composed of pigmented aggregates of cells that are confined within a single eye field. Though the growth of the 22 individual ommatidia making up the compound eye has been studied and proposed as a model to explain the development of the *Daphnia* eye, the detailed cellular mechanism for this fusion event is unknown. As a first step toward defining events in *Daphnia magna* eye development we have established a methodology to determine stages of eye development, as well as a visual timeline of this process. Here we describe five distinct stages of eye development. In addition, these defined

stages were compared to the overall developmental stages as defined by Stollewerk and colleagues (Mittmann *et al*, Evodevo 5:12 (2014)). Finally we compared the eye development stages to embryo body lengths and a variety of eye spot separation measurements to define morphometric features that correlate with eye development. These data suggest that the *Daphnia magna* eye spots grow larger and closer together throughout development to produce the characteristic cyclopean eye. Funding: Augsburg Biology Department and Dean Sundquist.

Program Abstract #133

***Pomacea canaliculata*: development and regeneration of complex eyes**

Alice Accorsi[1], Eric Ross[1], Davide Malagoli[2], Alejandro Sánchez Alvarado[1]

[1]Howard Hughes Medical Institute, Stowers Institute for Medical Research, Kansas City, MO, USA; [2]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

A systematic molecular and genetic dissection of complex camera-type eye regeneration has been hampered by the paucity of model systems in which to carry out these studies. The freshwater snail *Pomacea canaliculata* has a number of biological attributes that lend this organism ideally suited for studies in development and regeneration. Chiefly among them are ease of culture and reproduction in captivity with abundant and year-round offspring production, direct development and the ability to regenerate complex organs, such as tentacles and eyes after complete amputation. During embryogenesis, the eyes are developed relatively early; the retina, the lens and the general structure of the eyes are already well formed and similar to the adult eyes prior to hatching. Once amputated, the adult complex camera-type eyes, comprised of cornea, lens, retina and optic nerve, can fully regenerate in a relative short time (1 month) and the *de novo* regenerating eye shows a complete reconstruction of all the mentioned layers. To advance our studies, we have generated a high-dimensional transcriptome at high temporal resolution of the regenerating *P. canaliculata* eye. Equivalent work has been done during embryogenesis, providing us with an opportunity to compare embryonic versus regeneration eye ontogeny. Our aim is to determine whether embryonic morphogenetic programs and pathways are reactivated in adult eye regeneration and to identify the stimuli that trigger the reactivation of cell proliferation and morphogenesis of a new complex camera-type eye in adult *P. canaliculata*.

Program Abstract #134

The African killifish *Nothobranchius furzeri*: a new vertebrate model for adult tissue regeneration

Wei Wang, Kirsten Gotting

Stowers Institute for Medical Research, Kansas City, MO 64110, USA

Systematically dissecting molecular and genetic mechanisms underlying tissue regeneration in adult vertebrates has been hampered by lack of good model systems are suitable for large-scale genetic screens. Even in well-established models, such as the zebrafish *Danio rerio* and the mouse *Mus musculus*, large-scale genetic screens in adult animals are still difficult due to the high cost, the relatively long maturation time to adulthood and large space requirements for animal maintenance. In an effort to overcome these difficulties and launch the first comprehensive vertebrate regeneration screen, we propose to exploit two salient biological attributes of the African killifish *Nothobranchius furzeri*: the ability to store diapause embryos for prolonged periods of time, and the remarkable speed of this species to reach adulthood (1.5 months). We have identified, via high-throughput RNA-Seq, about 1000 candidate genes (> 2 log fold changes) involved in various signaling pathways during caudal fin regeneration. Among those candidates, 12 genes are potentially only induced during regeneration, but not activated during embryogenesis, larval development, and/or adult homeostasis. Furthermore, we have developed a highly efficient gene knockout pipeline using the CRISPR/Cas9 system. We are currently inactivating and characterizing the identified candidate genes for their role in regeneration.

Program Abstract #135

Deciphering early neural specifiation in annelids using blastomeres isolations

Allan Carrillo-Baltodano, Néva P. Meyer

Clark University, USA

Early neural fate specification involves a region of ectoderm that receives extrinsic signals to become neural ectoderm. This process has been relatively well-studied in vertebrate and insect model organisms, but not in spiralians, the third clade of Bilateria. By studying the annelid *Capitella teleta*, we can elucidate to what extent extrinsic versus intrinsic signals are involved in early neural fate specification in other metazoans. We first hypothesize that in *C. teleta* the potential to generate brain neural ectoderm is autonomously specified by factors that are asymmetrically segregated to the daughters of the first quartet micromeres (1q), and secondly that the ventral nerve cord (VNC) neural ectoderm is conditionally specified in daughters of the 2d micromere by extrinsic signaling from surrounding blastomeres. Using

mechanical and chemical protocols, we have successfully isolated blastomeres from 2- to 8-cell *C. teleta* embryos. Isolated blastomeres continue dividing for more than 3 days, enough time to assess neural fate via expression of proneural and neuronal genes and proteins. In agreement with our first hypothesis, daughters of isolated 1q cells express the pan-neuronal gene *Ct-elav1*, indicating a possible role for neural determinants in *C. teleta* brain formation. Daughters of isolated D blastomeres (fated to generate the left brain lobe, the VNC, and most of the trunk) generate an almost complete larva including one brain lobe and the VNC. We are currently performing isolations of several blastomeres from 4-cell to 16-cell embryos. Future experiments will examine the transcriptomic profile of isolated blastomeres enabling us to identify the putative genes and signaling pathways involved in early neural specification in spiralians, and therefore provide us with a better understanding of how this specification occurs across Bilateria.

Program Abstract #136

The evolution of planktotrophic indirect development in the polychaete *Hydroides elegans*

Cesar Arenas-Mena

College of Staten Island/City Univ of NY, USA

Planktotrophic larvae of marine invertebrates that are generally different from adults could have evolved from stages already present in the life cycle of the last common ancestor of protostomes and deuterostomes. Current evolutionary scenarios about larval origins overemphasize the discontinuity between larval and adult development and require the prior evolution of undifferentiated and transcriptionally potent cells to account for extreme cases of dramatic metamorphosis. Another possibility is that developmental plasticity of differentiated cells allows the transformation of larvae into adults and the gradual evolution of planktotrophic larvae from juveniles. Undifferentiated imaginal cells would have evolved as secondary developmental short cuts to dedifferentiation and redifferentiation. The expression of histone variant *H2A.Z* is consistent with this scenario. H2A.Zis required for transcriptional potency and it is developmentally regulated during embryogenesis and during larva-to-adult transformation of sea urchins and polychaetes. *Cis*-regulatory analysis in sea urchins is disclosing how *H2A.Z* has fallen under developmental control during the evolution of metazoans in order to stabilize differentiation transcriptional states. Genomic resources and methods are being developed for the experimental characterization of H2A.Z regulation in *Hydroides elegans*, a polychaete with planktotrophic development and equal spiral cleavage. In addition, the expression of transcription factors with endodermal and mesodermal roles in *Hydroides*is generally similar to that of lecithotrophic spiralian orthologs. This suggests that the differences between these developmental modes did not evolve by major upstream regulatory gene expression changes, but possibly rely more on distinct regulation of downstream genes required to form the gut of feeding trochophores. Several other aspects of bilaterian body plan evolution are greatly illuminated by the study of *Hydroides elegans*.

Program Abstract #137

GmSUR2 expression is crucial for soybean root nodule development

Suresh Damodaran

South Dakota State University, USA

Leguminous crops such as soybean form symbiotic nitrogen-fixing root nodules in association with soil-borne rhizobia bacteria. The bacteria reside inside the nodules where they convert atmospheric nitrogen into a plant usable form of nitrogen, and henceforth help reduce the need for nitrogen fertilizer. A better understanding of plant mechanisms that regulate nodule development will enable us to develop biotechnological strategies to optimize nodule formation and nitrogen fixation, or even transfer this trait to non-legume plants. We identified a nodule specific gene cytochrome P450 oxidase enzyme, GmSUR2 based on RNAseq analysis and reciprocal BLAST analysis suggested that this gene is a close ortholog for Arabidopsis SUR2 gene. Tissue specific expression analysis using a promoter: GUS construct revealed that this gene is expressed in root cortex cells in the emerging nodule (EN), and is later confined to nodule parenchyma of mature nodules (MN). Suppression of GmSUR2 using RNA-interference led to a reduction in the number of nodules and resulted in an impaired nodule vasculature branching pattern, suggesting that this gene plays a key role in nodule development. Our primary hypothesis is that potential increase in auxin levels due to reduced GmSUR2 activity resulted in impaired nodule development. Experiments are in progress to validate this and other alternate hypotheses. Understanding the role of GmSUR2 is expected to provide more insights into the role of auxin in nodule development. This research was supported in part by funds from South Dakota Agriculture Experiment Station and Infrastructure support from NSF-EPSCoR cooperative research agreement #IIA-1355423

Program Abstract #138

Retinoic acid receptor regulation of epimorphic and homeostatic regeneration in the axolotl

Matthew Nguyen[1], Pankhuri Singhal[1], Malcolm Maden[3], Randal Voss[2], James Monaghan[1]
[1]Northeastern University, USA; [2]University of Kentucky, USA; [3]University of Florida, USA

Salamanders are capable of regenerating amputated limbs by generating a mass of lineage-restricted cells called a blastema. Blastemas only generate structures distal to their origin unless blastemas are treated with retinoic acid (RA), which results in proximodistal limb duplications due to reprograming of distal cells to proximal fates. Reprogramming is unique to blastemas because RA treatment induces truncation in developing limbs or after redifferentiation has commenced in regenerating limbs. Despite the power of this experimental approach for understanding the role of RA during regeneration and how positional identity is established and maintained, little is known about the transcriptional network that regulates positional information. In this study, we target specific retinoic acid receptors (RARs) to either proximodistally duplicate (*Rarγ* agonist) or truncate (*Rarβ* antagonism) regenerating limbs. RARE-GFP reporter axolotls showed divergent reporter activity in limbs undergoing proximodistal duplication versus truncation suggesting differences in patterning and skeletal regeneration. Microarray analysis identified expression patterns that elucidate proximalization including up-regulation of proximal homeobox gene expression and silencing of distal-associated genes whereas limb truncation was associated with gene expression suggesting precocious skeletal differentiation. In uninjured limbs, *Rarβ* antagonism induced a loss of homeostatic bone regeneration leading to permanent long bone regression. Altogether, our study identifies gene expression programs that regulate RAR's multifaceted roles in the salamander limb including regulation of skeletal patterning during epimorphic regeneration, skeletal tissue differentiation during regeneration, and homeostatic regeneration of intact limbs.

Program Abstract #139

Investigating *Nematostella* Canonical Wnt Signaling and Regeneration via Illumina

Patrick Burton, Adam Togami, Andrew Metzman, Allen Betts, Tyler Cole
Wabash College, USA

Regeneration is a widespread mechanism of animal development yet, because most model systems possess limited regenerative abilities, it remains poorly understood. The cnidarian *Nematostella vectensis* is capable of complete bidirectional regeneration. The Canonical Wnt Signaling pathway plays a conserved role in patterning the primary axis of Metazoa during embryogenesis, including *Nematostella*. While the components of the pathway itself are conserved across animals, the downstream targets of the pathway remain poorly understood in most taxa. As an initial step to investigate the mechanism of regeneration in *Nematostella,* and identify canonical Wnt Signaling targets, we examined gene expression via Illumina sequencing in regenerating polyps. Regenerating *Nematostella* were exposed to alsterpaullone, a GSK3 inhibitor previously shown to promote ectopic oral development via canonical Wnt signaling in *Nematostella*. Alsterpaullone treated samples were compared to control polyps undergoing either oral or aboral regeneration at 24 and 48 hours. We have identified a set of genes whose expression levels are significantly different in alsterpaullone treated samples relative to controls. We have also identified a set of genes whose expression differs in oral versus aboral regeneration. BLAST analysis indicates that many of the genes identified by this research have no known homologs among metazoans. Our results suggest that high throughput sequencing utilizing protein inhibitors is an effective method for identifying conserved signaling pathway targets in non-model systems.

Program Abstract #140

TEAD4 Promotes Proliferation and Self-Renewal of Trophoblast Progenitors: An Implication in Mammalian Placental Homeostasis

Biswarup Saha, Ram P Kumar, Sumedha Gunewardena, Clifford Mason, Soumen Paul
University of Kansas Medical Center, USA

In placental mammals, trophoblast cells are essential for embryo implantation leading to successful progression in pregnancy. In placental development, distinct trophoblast cell types are specified from trophoblast stem cells (TSCs) or TSC-like trophoblast progenitors. However, the molecular mechanisms that regulate self-renewal or differentiation of trophoblast cells are poorly understood. In our study, we show that transcriptional activity of TEAD4, a TEA domain containing transcription factor, plays a crucial role in promoting cell growth and proliferation in the trophoblast progenitors at developing placenta. At the early stage Ectoplacental cone (EPC), TEAD4 is present within the nuclei of a small TSC-like progenitor and can directly regulate expression of TSC-specific genes. Our Global gene expression analysis (RNA-seq) in TEAD4-depleted murine trophoblast stem cells (mTSCs) indicates that TEAD4-mediated gene regulation is important to promote proliferation and self-renewal of mTSCs. Analyses with primary cytotrophoblasts

(CTBs) from first-trimester human placenta confirmed that TEAD4 promotes proliferation by directly regulating expression of several *Cyclins/CDKs* along with other TSC-specific genes. In contrast, differentiated trophoblast cells within matured human placentas harbor only a small number of CTBs with TEAD4 at their nuclei. Laser-capture micro-dissection (LCM) followed by gene expression analyses at those TEAD4 expressing cells in Term placenta have higher expressions of TSC-specific genes along with markers for cell proliferation. Not surprisingly, higher expressions of TEAD4 are associated with proliferating trophoblast cells in human choriocarcinomas, Gestational Diabetes Mellitus (GDM). On the contrary, Intra-uterine Growth Restriction (IUGR) is associated with loss of TEAD4 expressing progenitors. Our overall data clearly indicate that TEAD4, as a marker, plays an essential role in trophoblast cells homeostasis during placental development.

Program Abstract #141

Regulation of Pancreatic Acinar Cell Fate by the Onecut 1 Transcription Factor

Peter Kropp[1], Maureen Gannon[1,2]

[1]Vanderbilt University, United States; [2]Department of Veterans Affairs, United States

The exocrine pancreas, accounting for 98% of pancreas mass, predominantly contains acinar cells that produce digestive enzymes. Injury to acinar cells results in their dedifferentiation and can lead to diseases such as pancreatitis or pancreatic cancer. In spite of this, the factors that regulate acinar cell identity during development and disease remain under-studied. During development, transcription factors, including *Onecut1* (*Oc1*) contribute to differentiation of acinar cells from multipotent pancreatic progenitor cells (MPCs). Oc1 is expressed in MPCs, but becomes restricted to duct and acinar cells after endocrine specification. We have previously shown that loss of *Oc1* from MPCs ($Oc1^{\Delta panc}$) results in ductal cysts during development and a pancreatitis-like phenotype by 3 weeks, but the role of Oc1 in regulation of acinar cell differentiation remains unknown. To determine the impact of *Oc1* loss on developing acinar cells, I performed RNA-Seq on acinar-enriched samples from $Oc1^{\Delta panc}$ and control mice at embryonic day (e)15.5, e18.5 and postnatal day (P)2. These data reveal impairments in expression of transcription factors and signaling pathways essential for acinar cell development including the pro-acinar genes *Mist1* and *Ptf1a*. Histological analysis at these time points demonstrated reduced acinar tissue and increased acinar cell death accompanied by inflammation and acinar-to-ductal metaplasia. Together, these data suggest that *Oc1* is necessary for the proper specification and differentiation of acinar cells. To determine the role of *Oc1* in acinar cell maturation and function I have generated mice with acinar-specific inactivation of *Oc1* ($Oc1^{\Delta acini}$). I am evaluating whether loss of Oc1 in differentiated acinar cells reduces expression of essential acinar-specific genes and whether acinar cells are fully functional in the absence of *Oc1*. These studies will provide greater insight into the complex transcriptional network regulating acinar cell development.

Program Abstract #142

Hippo-Warts pathway regulation of pancreatic epithelial architecture and progenitor cell fate

Caitlin Braitsch, Ondine Cleaver

UT Southwestern Medical Center, USA

Generating new β cells for replacement or regenerative therapies is a major goal for diabetes treatment. This approach depends upon defining the step-wise developmental mechanisms regulating endocrine cell fate from progenitors to differentiated β cells. During embryonic development, multipotent progenitor cells (MPCs) arise from the pancreatic epithelium during a transient period characterized by rapid stratification, microlumen formation, and branching. MPCs give rise to all pancreatic lineages and comprise a heterogeneous but poorly understood population. While the Hippo(Mst1/2) kinases are known to regulate exocrine cells in later stages of pancreas development, the role of Warts(Lats1/2) kinases and transcriptional coactivators YAP/TAZ in the early pancreas bud is unknown. We hypothesize that Lats1/2 signaling is required to regulate pancreatic cell specification, and that YAP/TAZ promote a progenitor program while restricting cell differentiation in the pancreas bud. YAP is expressed in the pancreatic epithelium at embryonic day 9.75 and later, but is absent from endocrine cells, consistent with the hypothesis that YAP is crucial for progenitor cell fate. Deleting Lats1/2 using $Pdx1^{early}Cre$ leads to profound abnormalities in the developing pancreas. At birth, these mice have severe pancreatic morphogenetic defects. Earlier deletion of Lats1/2 from the pre-pancreatic endoderm leads to an expanded MPC pool in the pancreas bud, supporting the idea that YAP/TAZ promote pancreatic progenitors. Data will be presented from current experiments investigating the mechanism by which Lats1/2 control epithelial morphogenesis. Exploiting our understanding of pancreatic developmental mechanisms has implications for future diabetic therapies.

Funding: JDRF (CB), NIH NIDDK (OC)

Program Abstract #143

Role of Sox9 in pancreatic progenitor differentiation

Michael Parsons, Rebecca Beer, Wei Huang, Johhny Wang, Fabien Delaspre
Johns Hopkins University, USA

Zebrafish represents a genetically tractable model system that is ideally suited to studying pancreatic endocrine development. The centroacinar cells (CACs) of the larval pancreas are Notch-responsive and were previously identified as the progenitors of endocrine cells. During larval stages, these duct-associated CACs differentiate to form new islets, which ultimately contribute to the majority of the adult endocrine mass. Uncovering the mechanisms regulating CAC differentiation will facilitate understanding how insulin-producing β cells are formed. It has been known for a while that Notch signaling plays a major role in regulating the differentiation of larval CACs and recently we have reported retinoic acid (RA) signaling is also influential. Indeed, RA- and Notch-signaling pathways cooperatively regulate larval CAC differentiation, suggesting a shared downstream intermediate. Sox9b is a transcription factor important for islet formation whose expression is up-regulated by Notch signaling in larval CACs. Here we report that *sox9b* expression in larval CACs is also up-regulated in response to exogenous RA treatment. Therefore, we hypothesized that Sox9b is an intermediate between both RA- and Notch-signaling pathways.In order to study the role of Sox9b in larval CACs, we generated two cre/lox based transgenic tools, which allowed us to express full-length or truncated Sox9b in larval CACs. In this way we were able to perform both Sox9b gain- and loss-of-function studies and observe the subsequent effect on progenitor differentiation. Our results are consistent with Sox9b regulating CAC differentiation by being a downstream intermediate of both RA- and Notch-signaling pathways. We also demonstrate that adult zebrafish with only one functional allele of *sox9b* undergo accelerated β-cell regeneration, an observation consistent with *sox9b* also regulating CAC differentiation in adults.

Program Abstract #144

Four and a Half LIM domains 1b (Fhl1b) is essential for regulating the liver versus pancreas fate decision and for beta-cell regeneration

Chong Shin[1], Jin Xu[1], Jiaxi Cui[2], Aranzazu Del Campo[2]
[1]Georgia Institute of Technology, USA; [2]Max Planck Institute for Polymer Research, Germany

The liver and pancreas originate from overlapping embryonic regions, and single-cell lineage tracing in zebrafish has shown that Bone morphogenetic protein 2b (Bmp2b) signaling is essential for determining the fate of bipotential hepatopancreatic progenitors towards the liver or pancreas. Despite its pivotal role, the gene regulatory networks functioning downstream of Bmp2b signaling in this process are poorly understood. Through transcriptome profiling of endodermal tissues exposed to increased or decreased Bmp2b signaling, we have discovered the zebrafish gene *four and a half LIM domains 1b* (*fhl1b*) as a novel target of Bmp2b signaling. *fhl1b* is primarily expressed in the prospective liver anlage. Loss- and gain-of-function analyses indicate that Fhl1b suppresses specification of the pancreas and induces the liver. By single-cell lineage tracing, we showed that depletion of *fhl1b* caused a liver-to-pancreas fate switch, while *fhl1b* overexpression redirected pancreatic progenitors to become liver cells. At later stages, Fhl1b regulates regeneration of insulin-secreting beta-cells by directly or indirectly modulating *pdx1* and *neurod* expression in the hepatopancreatic ductal system. Therefore, our work provides a novel paradigm of how Bmp signaling regulates the hepatic versus pancreatic fate decision and beta-cell regeneration through its novel target Fhl1b.

Program Abstract #145

Novel Hox Functions Revealed In The Developing Kidney

Bliss Magella, Mike Adam, Steve Potter
Cincinnati Children's Hospital Medical Center, USA

In mammals both paralogous and flanking *Hox* genes show extensive functional overlap. To better define Hox function in kidney development we generated mice with frameshift mutations in *Hoxa9,10,11* and *Hoxd9,10,11*. Of interest, the nephron progenitors, marked by Six2 expression, showed early depletion in the double homozygous *Hoxa9,10,11-/-;Hoxd9,10,11-/-* mutants. This indicates a key role for Hox genes in regulating renewal/differentiation decisions for progenitors. Therefore the mutant kidneys showed a dramatically reduced size and nephron count. The ureteric bud showed greatly reduced branching in the mutants. We examined development of the interstitium (stroma) using the molecular markers Vim1, Anxa2, Meis1, Slug, p57, and Lef1. Stroma formed and the stromal subcompartments in general appeared normal, although p57, a marker of the medullary region, was nearly absent in the mutants. The developing mutant kidney was disorganized; for example glomeruli appeared at the outermost edge. According to the Hox Code Model the combinatorial set of *Hox* genes expressed defines segment identity. To investigate possible segmentation

defects in the nephrons of mutants we stained for LTA (proximal tubules), Krt8 (collecting duct), Slc12a1 (Loop of Henle). We observed that all segments were present. However, we were surprised to find that some tubules in mutants showed dual identities. We have observed regions of tubules that are positive for Slc12a1 and Krt8 as well as LTA and Krt8. Instead of segment transformation we observed identity ambiguity, with individual cells sometimes expressing multiple markers of distinct nephron segments. Taken together these findings are novel and contrary to the Hox Code model.

Program Abstract #146

Lymphangiogenesis in the craniofacial region of embryonic mice

Yuji Taya, Youichi Shirako, Kaori Sato, Yuuichi Soeno
Dept. of Pathol., Nippon Dental Univ. Tokyo, JP

Lymphatic endothelial cells (LECs) differentiate from blood endothelial cells (BECs) in the common cardinal vein (CCV). Genetic manipulations in mice have identified a transcriptional hub comprised of Prox1, CoupTFII, and Sox18 that is essential for LEC fate specification. We herein focused on differentiation of LECs and development of lymphatic vessels in the craniofacial region of mouse embryos. ICR mouse embryos from E9.5–18.5 were used to collect the tissues in the craniofacial region. Gene expression profiling was performed using DNA microarray and IPA, and was validated by qPCR and immunohistochemistry. For histological analysis, serial sections were multiple-immunolabeled with Prox1/ Vegfr3/ Lyve1/ Ccl21 (LEC markers) and CoupTF2/ Endomucin (VEC markers). The gene expression analysis indicated that the high-level expression of *Sox18, CoupTF2* and *Prox1* showed at E11.5–12.5, E13.5 and E14.5, respectively. *VegfC* was highly expressed at E11.5. *Vegfr3* showed the pattern of constitutive expression. The peak expression levels of *Lyve1, Podoplanin* and *Ccl21* showed at E14.5–16.5. The immunohistochemical analyses demonstrated that when LECs differentiated from VECs and sprouted in CCV of the truncal regions, LECs showed Prox1 (+)/ Vegfr3 (+)/ Lyve1 (+)/ Ccl21 (-) at around E9.5. Their LECs scattered, migrated away forward the craniofacial region, and arrived at the mandibular arches at around E10.5. Their migrating LECs showed Lyve1 (-) until the stages to form the lymph sac and lymphatic vessels. LECs aggregated and formed the small cell masses at E11.5. LECs became to show CCL21 (+) and Endomucin (-). The distribution of their LEC masses gradually extended in the entire craniofacial region. The lymph sacs and lymphatic vessels were beginning to form after around E12.5 and E14.5, respectively. We established the developmental processes of lymphtic vessels that originated from VECs of CCV in the craniofacial region. Supported by JSPS KAKENHI Grant number 26462799.

Program Abstract #147

Temporally regulated differentiation of multipotent mural cells contributes to the growth and maintenance of mouse organs

Urmas Roostalu[1], Alessandra Albertini[1], Neil Humphreys[2], Maj Simonsen-Jackson[2], Giulio Cossu[1]
[1]Division of Extracellular Matrix and Regenerative Medicine, Faculty of Biology, Medicine and Health, University of Manchester, UK; [2]Transgenic Core Research Facility, Faculty of Biology, Medicine and Health, University of Manchester, UK

Mural cells wrap around blood vessels and have well established roles in vascular physiology. Emerging evidence has revealed their broad differentiation potential *in vitro*, suggesting that they may have characteristics of stem cells. Lack of mural cell specific genes and transgenic animal models has prevented to map their fate *in vivo* to prove that they actually do represent tissue resident stem cells and contribute to the growth of various organs. We developed a transgenic mouse model in which sequential activation of CRE and flippase recombinases enables specific fate mapping of mural cells across organs. We show that mural cells give rise to all the cell types of the vascular wall. Early proliferation of mural cells lays the foundation for the smooth muscle layer of the aorta. Remarkably, the majority of endothelial cells in the juvenile growing bone arise from mural cells, in addition to a small fraction of osteoblasts. Mural cell multipotency is not only limited to mesodermal cell lineages since in the maintenance of the exocrine pancreas they also contribute extensively to the homeostasis of acinar epithelial cells. Our data provides the first definitive proof that mural cells are multipotent stem cells in unperturbed healthy tissues *in vivo*.
This work was supported by BBSRC Anniversary Future Leader fellowship to U. R., and British Heart Foundation, Biodesign, Plurimes and Maratò grants to G.C.

Program Abstract #148

Asymmetric Cell Division regulators affect maintenance and differentiation of *Drosophila* blood cell progenitors

Dalmiro Blanco-Obregon[1,2], Carolina Müller[1,2], Lautaro Gandara[1,2], Maximiliano Katz[1,2], Pablo Wappner[1,2]

[1]*Fundacion Instituto Leloir, Argentina;* [2]*Universidad de Buenos Aires, Argentina*
In recent years, the *Drosophila* larval hematopoietic organ, the Lymph Gland, has been established as a useful model to study maintenance and differentiation of blood cell progenitors. Several pathways involved in these processes have been proved to be conserved between *Drosophila* and mammals. However, Asymmetric Cell Division (ACD), one of the defining characteristics of Hematopoietic Stem Cells (HSCs), has not yet been found in the Lymph Gland. We are therefore interested in studying if ACD actually takes place in this organ, by manipulating genes that have been involved in this process, either in other cellular contexts in the fly, or in normal and leukemogenic HSCs. We have found that knock-down or over-expression of some of these genes in Lymph Gland blood progenitors affect their maintenance and differentiation. Concordantly, time-controlled manipulation of ACD genes during immature developmental stages, when early differentiation takes place, leads to similar phenotypes. Progenitors remain unaffected when manipulations are performed after this critical point. Interestingly, the same manipulations produce different outcomes when done in differentiating cells, including premature differentiation in early stages and melanotic tumor formation later, both considered to be leukemia-like phenotypes. We propose that these phenomena are related to time- and context-specific functions of ACD genes in blood cell development in *Drosophila.*
Funding sources: Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (AGENCIA).

Program Abstract #149
NUDC is required for the definitive hematopoiesis in zebrafish
So Hyun Lee
Chonnam National University, KR
NUDC is a member of the nuclear distribution protein family. Human NUDC interacts with thrombopoietin receptor (Mpl) in an N-terminal region and promotes proliferation and differentiation of megakaryocytes. However, a role for NUDC in the hematopoiesis remains elusive. To gain more insights into a mechanism by which NUDC regulates hematopoiesis, we set out to determine a loss-of-function phenotype(s) of NUDC in developing zebrafish embryos. Knockdown of nudc inhibited formation of definitive hematopoietic stem cells (HSCs) in the aorta-gonad-mesonephros (AGM) at 2 days post-fertilization (dpf). In addition, knockdown of nudc decreased number of erythrocytes and myelocytes in the AGM, whereas it did not alter primitive hematopoiesis. These results suggest that NUDC plays an important role in the definitive hematopoiesis. Furthermore, definitive hematopoiesis phenotype in nudc morphants was rescued by nudc mRNA or nudc N-terminal region RNA, but not by nudc c-terminal mRNA. Finally, we observed down-regulation of NUDC in human patients with acute myeloid leukemia (AML), suggesting that our findings in zebrafish might be extended to human. In conclusion, we propose that NUDC plays an important role in the definitive hematopoiesis and that it may be involved in leukemogenesis.

Program Abstract #150
Loss of Ascc2 leads to cardiac defects and embryonic lethality
Nanbing Li-Villarreal, Victor Piazza, Leeyean Wong, Mary E. Dickinson
Baylor College of Medicine, USA
Activating signal cointegrator 1 complex subunit 2 (ASCC2) is a novel protein identified as a part of the TRIP4 transcriptional coactivator complex. The TRIP4 complex was shown to regulate transcription factors such as serum response factor (SRF), c-Jun, p50, and p65 *in vitro* in HeLa cells. Since its identification, the role of ASCC2 in development and disease remains elusive. Using CoIP, we determined that Ascc2 interacts with other Trip4 complex members *in vivo* in mouse embryos. Whole mount *in situ* hybridization showed broad expression of *Ascc2* in the developing mouse embryo from blastocyst stages through gastrulation and somitogenesis stages. Interestingly, *Ascc2* expression is restricted anteriorly with highest expression in the developing heart at embryonic day (E) 8.5 and becomes widely expressed again by E9.5. Recently, utilizing *Ascc2*$^{Tm1b(KOMP)Wtsi}$ mutant mice generated as part of the NIH KOMP2 project, we have determined that a homozygous mutation in *Ascc2* results in embryonic lethality prior to E9.5. Analysis of somite stage matched *Ascc2* homozygotes and littermate control embryos at E8.5 showed variable heart phenotypes in the mutants, some with a heart beat and others without. Further analysis using microCT imaging revealed a small and crescent shaped heart in mutant embryos compared to a looped and inflated heart in stage matched littermate controls. Moreover, expression of Gata4, a transcription factor shown to regulate cardiac development, visceral endoderm differentiation, apoptosis, and cell division, is down regulated in *Ascc2* mutant embryos. These preliminary data suggest an essential role for Ascc2 in murine embryogenesis and cardiac development.

Program Abstract #151

Id1 Is An Evolutionarily Conserved Master Regulator Of Cardiogenic Mesoderm Formation

Michael Yu[1], Thomas Cunningham[1], Wesley McKeithan[1], Sean Spering[1], Matthew Tierney[1], Sonia Albini[1], Mauro Giacca[4], Pier Lorenzo Puri[1], Gregg Duester[1], Jean-Francois Riou[3], Muriel Umbhauer[3], Alessandra Sacco[1], Mark Mercola[2], Alexandre Colas[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, USA; [2]Stanford University, USA; [3]Universite Pierre et Marie Curie, France; [4]International Centre for Genetic Engineering and Biotechnology, Italy

Deciphering the mechanisms of cardiogenic mesoderm formation is fundamental to the application of stem cell biology to cardiovascular regenerative medicine. Here, we report that unbiased functional screening revealed a minimal network governed by the helix-loop-helix protein Id1 that is sufficient to induce multipotent mesoderm from pluripotent stem cells. In this context, Id1 is present in newly forming mesoderm where it represses two inhibitors of mesoderm, Tcf3 and Foxa2, and activates two inducers, Evx1 and Grrp1. Id1 respecifies endodermally fated cells to form mesoderm in both mouse and human pluripotent cells and in *Xenopus* embryos. Id1-programmed progenitors form predominantly cardiac muscle, fibroblasts and vascular endothelial cells. Nonetheless, they are responsive to environmental cues, and retain plasticity to shift fate from cardiac to skeletal muscle upon transplantation into injured adult murine skeletal muscle. This unanticipated role for Id1 sheds light on the evolution of mesoderm induction and enables highly efficient production of human progenitors for regenerative and drug discovery applications.

Program Abstract #152

Defining and visualizing early lateral plate mesoderm emergence in zebrafish

Christopher Hess[1], Karin D. Prummel[1], Susan Nieuwenhuize[1], Anastasia Felker[1], Gopi Shah[2], Sibylle Burger[1], Jan Huisken[2], Christian Mosimann[1]

[1]Institute of Molecular Life Sciences, University of Zurich, Switzerland; [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Cardiovascular and blood cell lineages arise along with kidney, smooth muscle, and limb lineages from uncommitted lateral plate mesoderm (LPM). When and how the LPM is specified, and what program drives its subsequent patterning, remain vaguely defined due to the LPM's dynamic architecture at the embryo periphery. We have isolated the cis-regulatory elements of the zebrafish *draculin* (*drl*) gene that we found by live imaging and lineage tracing to mark the emerging LPM before subsequently refining into cardiovascular lineages. Zebrafish reporter assays uncovered that *drl* expression results from combinatorial activity of three independent elements: an early pan-LPM enhancer and two elements that are active in the later anterior and posterior LPM, respectively. We hypothesize that *drl* pan-LPM enhancer reads out an LPM-defining upstream program. Reporter assays confirmed that *drl* pan-LPM expresses in lamprey and chicken LPM, revealing possible evolutionary conservation of a LPM-defining program. Towards defining this program, we uncovered and confirmed by CRISPR-Cas9 mutagenesis several binding motifs in the *drl* pan-LPM enhancer, including Smad2 sites and binding motifs for the mesendoderm regulators Eomes and FoxH1. Chemical perturbations and misexpression experiments further implicate a graded FGF input on the early *drl* pan-LPM enhancer that restricts the LPM to a restricted position within the emerging embryo. Cre/lox-based genetic lineage tracing and panoramic SPIM imaging with *drl* pan-LPM reporters further revealed that zebrafish LPM forms from a selective mesendoderm territory and gradually becomes restricted to LPM at the end of gastrulation. Imaging combined with other LPM-expressed transgenic reporters revealed the emerging bilateral LPM architecture and allowed us to assign new distinct cell fates to individual LPM stripes. Altogether, our work provides a first structural, developmental and genetic framework to define early LPM emergence.

Program Abstract #153

BMP signaling maintains a mesodermal progenitor state in the presomitic mesoderm.

Richa Sharma[1], Maxwell Shafer[1], Mathieu Tremblay[1], Eric Bareke[2], Jacek Majewski[2]

[1]Department of Biochemistry, McGill University, Canada; [2]Department of Human Genetics, McGill University, Canada

The presomitic mesoderm (PSM) is a mesenchymal tissue made of progenitor cells that are gradually segmented, differentiating into somites under the influence of the segmentation clock. PSM specification is modulated by signals from the Wnts, Fgfs and Retinoic acid (RA) pathways, which exhibit a graded distribution along the A-P axis during development of the PSM and the somites. The presomitic mesoderm progenitors mature as they move rostrally (anteriorly) through the different morphogenetic gradients during somitogenesis. The maintenance of the undifferentiated PSM progenitors is vital during embryogenesis and axis elongation. However, it is unclear how the molecular network is maintained in the PSM progenitor cells. We developed a primary culture system, wherein we isolate PSM cells from E9.5

mouse embryos. We found that these cells differentiate in culture and lose the expression of PSM markers. Using a candidate approach, we found that treatment with BMP4 can cause them to restore their original PSM fate. Conversely, sustained BMP4 in newly sorted PSM progenitor cells is able to maintain their undifferentiated state. Expression profiling by RNA sequencing suggests that treatment with Bmp4 modulates most of the known PSM and paraxial mesoderm molecular network. Also, deep-sequencing data analysis indicates that the ectopic cell differentiation (without BMP4 treatment) make lineages of the vasculature, blood, bone, muscle and heart. Further, when live embryo cultures were treated with Noggin (inhibitor of BMP4) we observed a loss of PSM markers by quantitative PCR analysis. These results suggest that BMP4 sustains the PSM progenitor fate during development. We plan to confirm the loss of PSM markers in-vivo by in-situ hybridization and Immunohistochemistry in embryos treated with Noggin. In conclusion, we want to establish that BMP4 regulates the maintenance of PSM network in tailbud progenitors during somitogenesis.

Program Abstract #154

Genomic analysis of Hox protein binding sites

Nishal Patel, Bony de Kumar, Ariel Paulson, Brian Slaughter, Mark Parrish, Robb Krumlauf
Stowers Institute for Medical Rsearch, USA

Hox genes encode a highly conserved family of transcription factors that are important in controlling the basic body plan along the anterior-posterior axis in bilaterians. In mouse they are organised into 4 clusters (HoxA, HoxB, HoxC and HoxD) that arose from duplication and divergence of an ancestral cluster.

The aim of my research is to identify Hox binding sites on a genome-wide basis to understand the sequence specificity, binding partners, downstream target genes and mechanisms of transcriptional regulation essential for their function. We have been using ChIP-Seq to identify binding sites for group 1 Hox proteins (Hoxa1 and Hoxb1) and co-factors in mouse ES cells differentiated into pre-somitic mesoderm (PSM) cells (Chal et al 2015) and comparing and contrasting this data with similar analysis in ES cells differentiated into neural progenitors (NP) cells. The goal is to understand how Hox genes are able to elicit distinct transcriptional responses in different tissue contexts. Our analyses have found that Hoxa1 binds to more sites in mesodermal cells than in neural cells. Many of these mesodermal sites are also associated with Hoxb1 binding in PMS cells suggesting an important role for Hoxa1 and Hoxb1 in mesoderm differentiation. Motif analysis of the binding sites also suggests that the two Hox proteins employ different binding partners in control of their gene regulatory networks. These data provide insight into the role of Hox proteins in PSM tissue and how highly similar transcription factors are able to elicit differing transcriptional responses.

The work presented here is funded by the Stowers Institute for Medical Research.

Chal J et al. (2015). Differentiation of pluripotent stem cells to muscle fiber to model Duchenne muscular dystrophy. Nature Biotechnology. 33 (9):962-9

Program Abstract #155

Opposite roles for receptor tyrosine kinase in somatic versus visceral muscle founder cell fate specification in *Drosophila*

Sarah Popadowski[1,2], Yiyun Zhou[1,2], Marc S Halfon[1,2,3]
[1]Univerisity at Buffalo-SUNY, USA; [2]NYS Center of Excellence in Bioinformatics and Life Sciences, USA; [3]Roswell Park Cancer Institute, USA

Drosophila muscle development requires two cell types, founder cells (FCs) and fusion competent myoblasts (FCMs). While both cell types are necessary, it is the founder cells which confer specific identity upon the developing muscle fibers. We have observed key differences in the mechanism of founder cell specification between embryonic somatic and visceral musculature. In both types, FC specification of Ras throughout the mesoderm leads to an increase in FCs at the expense of FCMs. In the somatic mesoderm, this is a direct effect of downstream effectors, such as the ETS-domain factor Pointed (Pnt), binding to and activating FC specific enhancers; loss of Pnt leads to loss of FC fates. However, mutation of ETS consensus binding sites in a visceral mesoderm FC-specific *mib2* enhancer leads to broadened rather than lost FC gene expression, similar to what is seen with Ras activation. This suggests that the role of RTK signaling in visceral mesoderm is to relieve repression of FC fate, rather than to induce it as in the somatic mesoderm. Consistent with this, the mutant *mib2* enhancer shows broadened FC expression even in the absence of RTK signaling. Preliminary RNAi data suggest that the responsible repressor is the Pnt-related Ets21c, and we are currently assessing *Ets21c* mutant alleles to confirm this. Interestingly, we find that the need for RTK signaling in FC specification can be circumvented in the absence of the transcription factor Lameduck (Lmd), unlike the situation in somatic mesoderm, where the loss of Lmd affects FCM development without affecting FC specification. We are continuing to define the relationships between RTK/Ras signaling, Lmd, and other known or suspected members of the muscle development pathway to better

understand the novel ways in which visceral mesoderm FC fates are specified versus the better-understood mechanisms in the somatic musculature.

Program Abstract #156

Modeling Skeletal Myogenesis in vitro with Pluripotent Stem cells and its Applications

Jerome Chal[1,2,3], Ziad Al Tanoury[4], Masayuki Oginuma[1,4], Marie Hestin[1], Bénédicte Gobert[4], Suvi Aivio[1], Getzabel Guevara[1], Alexander P. Nesmith[5], Kevin K. Parker[5], Olivier Pourquié[1,2,3,4]

[1]Brigham and Women's Hospital, USA; [2]Harvard Medical School, USA; [3]HSCI, USA; [4]IGBMC, France; [5]Wyss Institute, USA

Skeletal muscles constitute the most abundant tissue in our body. During development, myogenic progenitors originate principally from the paraxial mesoderm tissue. Using the key signaling pathways controlling paraxial mesoderm specification and differentiation as a guide, we recently developed protocols to differentiate efficiently mouse and human pluripotent stem cells (PSCs) into skeletal muscles derivatives *in vitro* (Chal et al, 2015). Strikingly, the differentiating cultures recapitulate key stages of presomitic mesoderm (PSM) differentiation and myogenic commitment. Within a month, the progenitors can give rise, through several myogenic phases, to contractile millimeter-long muscle fibers and their associated (satellite-like) Pax7-positive progenitors. Such self-organization is reminiscent of spontaneous organogenesis described in other PSC-derived *in vitro* systems such as optic cup and gut. This model-in-a-dish offers the unique opportunity to study muscle development at high resolution in an unprecedented quantitative manner. This is particularly valuable for difficult to access developmental stages *in vivo*, gain and loss of function studies or to study the human model system. Finally, there is currently no efficient treatment for muscle dystrophies, where patients suffer of muscle wasting that can lead to permanent immobilization and death. Our differentiated PSC cultures offer an attractive system to develop treatments for muscle diseases, notably through the creation of *in vitro* models of muscle diseases and also as a possible source of cells for regenerative medecine approaches. With this in mind, we have optimized PSC-derived myogenic cultures to enable optimal phenotype characterizations and developed novel bioengineering approaches (Chal, Al Tanoury et al, 2016). This work was partly supported by the French Muscular Dystrophy Association (AFM), the European Research Council, the FP7 EU grant Plurimes and by the INGESTEM project (ANR).

Program Abstract #157

Structural and functional conservation of MyoD from three electric fish: Induction of muscle gene expression in non-muscle cells

Iliana Hernandez, Michael McDowell, Robert Güth, Graciela A. Unguez

New Mexico State University, USA

Electrocytes are the electrogenic cells that make up the electric organ (EO) of the teleost *Sternopygus macrurus*. In *S. macrurus*, electrocytes are modified striated muscle cells that disassemble their contractile apparatus and downregulate most of their myogenic phenotype. Mature electrocytes are not contractile yet they retain some myogenic characteristics: they are multinucleated, receive cholinergic innervation, and express some muscle genes including titin, desmin, and the myogenic regulatory factors (MRFs) MyoD, myogenin, Myf5, and MRF4 (Cuellar et al., 1996; Kim et al., 2008). Further, *S. macrurus* MyoD and myogenin can induce non-muscle cells into multinucleated myotubes with a similar conversion efficiency to their mammalian homologs (Kim et al., 2009). Recent study showed that EO in other electric fish species – *E. virescens* and *E. electricus* - also express MRFs (Gallant et al., 2013). The first aim of this study was to determine the level of amino acid sequence conservation in the functional domains of *E. virescens* (EvMyoD) and *E. electricus* (EeMyoD) MyoD, i.e., the N-terminal, basic domain, helix-loop-helix, and the C-terminal (Tapscott, 2005). The second aim tested the extent to which MyoD from SmMyoD), EvMyoD and EeMyoD could transcriptionally activate the skeletal muscle program in mouse non-muscle cells. Our bioinformatics analysis showed that MyoD from each electric fish contained all four functional domains. Our functional *in vitro* studies followed the same protocol that testedthe induction capacity of SmMyoD (Kim et al., 2008) using Lipofectamine 2000 (Invitrogen) and Fugene HD (Promega). Immunolabeling of transfected mouse C3H/10T1/2 cells showed that EeMyoD was most effective in converting this cell line into multinucleated myotubes with differentiated muscle markers. These data are consistent with a functional conservation of an MRF-dependent control of the skeletal muscle program in all three species of electric fish studied.

Program Abstract #158

Identification of new regulators of tendon cell induction using the zebrafish

Jessica Chen[1,2], Matthew King[1], Cliff Tabin[2], Jenna Galloway[1]

[1]Massachusetts General Hospital, USA; [2]Department of Genetics, Harvard Medical School, USA

Tendons and ligaments are necessary for force transmission and stability in the musculoskeleton. Despite their importance for our movements, the pathways regulating their specification are not well understood. In order to identify new pathways involved in tendon cell induction, we turned to the zebrafish model as it is amenable to forward screen-based approaches. We have previously shown that zebrafish and mammalian tendons have similar molecular, morphological, and ultrastructural properties, making them excellent models to study vertebrate tendon development. Using this system, we performed a chemical screen using a known bioactive library and discovered that statin compounds promote a dose-dependent expansion of the craniofacial and pectoral fin tendon program. Chemical rescue and genetic loss of function experiments indicate that the statin-mediated expansion of tendon progenitors is specific to mevalonate pathway. Further pathway dissection shows that this effect is caused by inhibition of the prenylation branch and specifically geranylgeranyltransferase type I. Analysis of the other musculoskeletal lineages reveals that Hmgcr inhibition specifically expands the tendon lineage and negatively impacts cartilage and bone formation. Surprisingly, the expansion is not a consequence of increased cell proliferation. Instead, the expanded populations of craniofacial tendon cells are descendents of the cranial neural crest that we believe have been recruited from a chondrogenic and osteogenic fate. Taken together, we show a specific branch of the mevalonate pathway has a critical role in regulating tendon cell specification in the developing embryo.

Program Abstract #159
Screening for regulators of proteoglycan synthesis
Brian Eames
University of Saskatchewan, CA
Proteoglycans (PGs) are sugar-coated molecules that perform both structural and biological functions. For example, a variety of cell types impart mechanical properties to their extracellular matrix by secreting abundant PGs, and PGs affect growth factor signalling. Our lab works on identifying novel members in the PG synthesis pathway through genetic screens in larval zebrafish and in 3D culture systems. We target skeletal cells, such as chondrocytes and osteoblasts, given the sheer abundance of PGs secreted by these cells. A genetic screen for skeletal defects in larval zebrafish identified old and new members of the PG synthesis pathway, including *xylt1* and *fam20b*, respectively. Surprisingly these mutants had accelerated patterns of gene expression and bone formation, indicating that, normally, cartilage PGs inhibit skeletal development. Currently, we are using transgenics and mutant analyses to determine whether skeletal defects in PG mutants are caused by aberrant growth factor signalling. Also, we are setting up a high-throughput imaging platform to screen 3D-cultured chondrocytes using an shRNA library. Specifically, we are looking at interactions of the PG synthesis and cell secretory pathways, since we characterized some secretion defects in skeletal cells of our PG mutant zebrafish. We hope that our approaches can reveal novel therapeutics for such diseases as osteoarthritis, which is characterized by defects in PGs at articulating surfaces of the skeleton.

Program Abstract #160
Functional roles for Irx3 and Irx5 in the hypertrophic chondrocyte to osteoblast lineage program
Songjia Wen
The University of Hong Kong, HK
The *Iroquois* (*Irx*) homeodomain transcription factors have been found essential for morphogenesis of brain, inner ear, retina and heart. In developing limb, *Irx3/5* are essential for establishment of early anterior posterior polarity. However, the role of *Irx3/5* in endochondral bone formation is not defined. Here we show that *Irx3/5* compound null (*Irx3/5* DKO) mouse display a severe skeletal phenotype including loss of scapula, tibia and digit 1, as well as hypoplastic pelvis and femur at E16.5. We found that *Irx3* and *Irx5* single null mutants display osteopenia and dwarfism. Overlapping pattern in the osteoblasts, osteocytes and at the chondro-osseous junctionof *Irx3* and *Irx5*, together with the skeletal dysplasia of both compound and single mutants suggest that *Irx3/5* are required in hypertrophic chondrocyte to osteoblast lineage. Thus, we have analyzed the $Col10a1^{cre/+}$; $Irx3^{LacZ/LacZ}$; $R26^{tom/+}$ and $Col10a1^{cre/+}$; $Irx3^{f/f}$ $Irx5^{egfp/egfp}$; $R26^{tom/+}$ to specifically tracing the descendants of hypertrophic chondrocytes. At neonatal stage, fewer hypertrophic chondrocyte descendants are found in both *Irx3* single KO lineage tracing and *Irx3* conditional KO lineage tracing cases, implying a defective hypertrophic chondrocyte to osteoblast transition. Subsequent double labeling study consolidate that proportion of *Col1a1* and *tdtomato* double labeled hypertrophic chondrocyte descendants become smaller in *Irx3* conditional KO mutants. Our results strongly suggest that *Irx3* and *Irx5* are critical factors regulating hypertrophic chondrocyte to osteoblast transition.

Program Abstract #161

Post-mitotic progenitors contribute to cell type diversity in planarians

Omri Wurtzel[1,2], Isaac Oderberg[1,2], Peter Reddien[1,2]

[1]Whitehead Institute for Biomedical Research, USA; [2]Howard Hughes Medical Institute, USA

Diverse animals maintain and renew their tissues through adult stem cells (ASC). In planarians, highly regenerative flatworms, multiple classes of specialized ASCs replenish all tissues, yet whether individual specialized ASCs can generate one or multiple cell types is unknown. We addressed this question by single cell RNA sequencing on 303 planarian cells at different stages of maturation. We identified 32 transcription factors associated with differentiation or type specification and systematically inhibited their expression by RNAi. Phenotypic analysis associated genes with 7 different mature cell types that are distinct in spatial expression and/or function. Our results indicate that the heterogeneous stem cell population generates a diversity of cell types but that specification events, which greatly expand cellular diversity, occur post-mitotically. Ablation of the stem cell compartment coupled with RNAi-mediated perturbation of the body axes revealed lack of cell type plasticity in terminally differentiated cells, opposite to results obtained from analysis of the stem cell compartment. Our results identified transcription factors associated with cell-type specification, functions in regenerative and homeostatic contexts, and established a role for post-mitotic cells in generating a diversity of cell types.

Funding: OW is The Howard Hughes Medical Institute Fellow of The Helen Hay Whitney Foundation
PWR is a Howard Hughes Medical Institute Investigator

Program Abstract #162

A tissue-size control mechanism in the mouse blastocyst

Nestor Saiz, Kiah M Williams, Venkatraman E Seshan, Anna-Katerina Hadjantonakis

Sloan Kettering Institute, USA

Precise control of tissue size is critical for organismal development and tissue homeostasis. During the preimplantation stages of mammalian development, three cell lineages – the extra-embryonic trophectoderm (TE) and primitive endoderm (PrE) and the pluripotent epiblast (EPI) – need to be generated by the time the blastocyst-stage embryo implants into the uterus. The blastocyst represents a paradigm of regulative development, capable of accommodating experimental perturbations in cell composition while developing to term. However, the cellular basis for these regulative abilities has not been established. We have applied a high-resolution, single-cell image analysis pipeline to address the cellular and molecular mechanisms that give the blastocyst its regulative abilities. We have found that the inner cell mass (ICM) of the mouse blastocyst shows an invariant composition of PrE and EPI cells, which is maintained despite alterations in ICM size or overall embryo size. Furthermore, timed modulation of the FGF-MAPK signaling pathway showed that ICM cells commit to the PrE or EPI lineage in an asynchronous manner. These data indicate that a consistent ICM composition is achieved through incremental allocation of cells to each lineage from a common progenitor pool, thus providing a means to coordinate lineage specification with population size. These results lead us to propose the existence of a previously unidentified mechanism for tissue size control operating in the ICM, which underlies the regulative abilities of the mammalian blastocyst.

[This work is supported by a fellowship from the Tri-Insitutional Stem Cell Initiative, funded by the Starr Foundation, awarded to NS, as well as a NYSTEM grant #N13G-236 and an NIH P30 grant.]

Program Abstract #163

OCT4 promotes pluripotency and differentiation to primitive endoderm

Alyson Lokken, Amy Ralston

Michigan State University, USA

OCT4 is a critical mediator of pluripotency in the embryo and is required for reprogramming of somatic cells to induced pluripotent stem cells. Our lab has identified an additional novel role for OCT4 in the specification of the primitive endoderm (PE) in the mouse blastocyst. Loss of OCT4 results in a loss of expression of essential PE genes, indicating a requirement for OCT4 not only in maintaining pluripotency but also in promoting PE differentiation. The mechanisms underlying the requirement for OCT4 in PE development are not understood. We hypothesize that OCT4 exerts dual functions in promoting pluripotency and differentiation in the preimplantation embryo through cell type-specific protein-protein interactions. Alternatively, modulation of OCT4 levels in a cell-type or embryonic-stage specific manner may act to maintain pluripotency or specify cells to differentiate to PE. To test these hypotheses, we have performed chromatin immunoprecipitation experiments to examine OCT4 and putative cofactor localization and redistribution during pluripotent-to-PE cell differentiation using a cell line model. Further ChIP-Seq analysis will provide insight into the direct

targets of OCT4 during differentiation as well as identify surrounding transcription factor binding sites that may indicate novel transcription factor cooperation with OCT4 in directing cell fate specification. Additionally, we have engineered a tamoxifen-inducible OCT4 to modulate OCT4 activity in cell lines as well as in vivo through CRISPR genome editing to examine the effect of OCT4 levels on pluripotency and differentiation. Understanding how OCT4 maintains pluripotency and promotes PE differentiation will allow us to exploit the pluripotency-driving function of OCT4 for use in reprogramming with applications to regenerative medicine, as well as inform our understanding of preimplantation development and potential causes of early pregnancy loss. NIH R01 awarded to A. Ralston, 5R01GM104009

Program Abstract #164
Metabolic shift is required for extraembryonic endoderm differentiation
Mohamed Gatie, Gregory M. Kelly
Western University, Canada, CA
Mouse extraembryonic endoderm (XEN) is comprised of cells from the inner cell mass that have differentiated first into primitive endoderm (PrE), and later into parietal (PE) and visceral endoderm (VE). F9 teratocarcinoma cells treated with retinoic acid (RA) mimic the transition to PrE, and to PE when they are subsequently treated with dibutyryl cyclic AMP (db-cAMP). During this differentiation we have identified a change in metabolic profile whereby undifferentiated F9 cells move out of a glycolytic state to one involving oxidative phosphorylation to supply the energy demand. We have shown that F9 cells treated with RA and RA/db-cAMP have decreased mRNA and protein levels of the glycolytic markers PKM1/2, LDHA, PDK1, and phospho-PDH. We have also shown that while chemical inhibitors of PDK1 and LDHA promotes F9 cell differentiation in the absence of RA, cells stably over-expressing mPDK1 did not form PrE. In comparison to undifferentiated F9 cells, mitochondrial membrane potential and mitochondrial ROS production (mROS) are also significantly higher in PrE and PE. Although the data points to a metabolic shift during differentiation, it is not known whether or not this shift is sufficient to induce cells to form XEN. To address this, we plan to expand our studies by stably expressing mLDHA, mPKM2, or an oxygen-insensitive mHIF-1α to see if the over-expression of these glycolytic proteins blocks differentiation. If our predictions are correct then we should also see a reduction in mitochondrial membrane potential and mROS production. These studies will be corroborated using shRNAs against Ldha and Pdk1. Together, these results will shed new light on how F9 cell differentiation is intricately linked to the metabolic profile, which has implications on how XEN forms in vivo. The authors acknowledge funding from NSERC Canada and CHRI support for this research.

Program Abstract #165
Hedgehog Signaling and Metabolic Status in F9 Cells
Nicole Cuthbert, Gregory Kelly
University of Western Ontario, Canada
Hedgehog (Hh) proteins play a role in patterning of the vertebrate embryo. In mouse, Indian Hedgehog (IHH) is linked to eXtraembryonic ENdoderm (XEN) formation, but details on the signaling pathway and how this occurs is unknown. By the late blastocyst stage the ICM gives rise to the epiblast and the primitive endoderm (PrE), the latter contributing to parietal (PE) and visceral endoderm (VE). Collectively, PrE, PE, and VE comprise the XEN. F9 teratocarcinoma cells treated with retinoic acid (RA) mimic the differentiation to PrE, while subsequent treatment with db-cAMP commits the cells to PE. Differentiation in vitro involves several signaling pathways including canonical Wnt/b-catenin, which is activated by GATA6. This master regulator of endoderm and XEN formation also up-regulates the gene encoding IHH, which when translated serves as a ligand that is required, but not sufficient to differentiate F9 cells to PrE. Interestingly, the overexpression of GLI3A, an active downstream mediator of the Hh pathway, is sufficient to differentiate F9 cells into a XEN lineage. This evidence points to Hh signaling interacting on another pathway, possibly one involving canonical Wnt signaling involving b-catenin, which is known to interact with the repressor form of Gli3 (Ulloa et al., 2007). A decrease in glycolytic markers LDHA and PDK1, and a concomitant increase in mitochondrial membrane potential and mitochondrial ROS production were also noted to accompany differentiation. While changes in metabolic state accompanying differentiation are well known, it is not known whether they impact on Hh signaling or are influenced by the Hh pathway itself. If the latter, these results would place IHH in a hierarchy, upstream of the metabolic changes required for XEN formation. The authors acknowledge funding from NSERC Canada and CHRI support for this research.

Program Abstract #166
Cell Plasticity During *Xenopus* laevis embryogenesis
Talia Hart, Krissie Tellez, Brigette Jong, Carmen Domingo

San Francisco State University, USA
The fertilized egg undergoes many divisions to give rise to an adult organism comprised of many specialized cell types. Our lab is interested in understanding how *in-vivo* dynamics influence cell plasticity. Through cell transplantation experiments, we showed that neural ectoderm cells remain responsive to muscle-inducing signals during gastrulation. To better understand this response we examined four confounding factors: (1) the range of plasticity, (2) age of the host embryo, (3) different locations in the host embryo, and (4) the timing of commitment to a specific cell type. Our results show that cells from the prospective anterior neural region lose their ability to change their fate by the end of gastrulation. In contrast, cells from the posterior neural ectoderm lose their ability to change their fate much later at mid neurulation. By varying the age of the host embryo, we show that prospective neural cells are likely to change their fate to form muscle when grafted to the dorsal blastopore region at the end of gastrulation. In addition, by grafting prospective neural cells to different muscle fated regions, we further determined that the region lateral to the dorsal blastopore lip is the most conducive for inducing a muscle fate. Finally, we transplanted both rhodamine-labeled prospective neural cells along with fluorescein-labeled prospective muscle cells to the same muscle inducing region of host embryos to determine whether the timing and behavior of the prospective neural cells was similar to prospective muscle cells. Our results show that prospective neural ectoderm cells from the posterior region form muscle cells at the same spatial and temporal rate as prospective mesoderm cells. However, cells from the anterior neural ectoderm region showed a delay in their ability to form muscle. Together these results suggest that the posterior prospective neural ectoderm has a surprising ability to retain its plasticity during development and adopt a muscle fate.

Program Abstract #167

Regulation of temporal fate determinants in *Drosophila* type II neuroblast lineages

Qingzhong Ren, Ching-Po Yang, Zhiyong Liu, Sugino Ken, Kent Mok, Yisheng He, Tzumin Lee
Janelia Farm Research Campus, HHMI, USA

In *Drosophila* type I neuroblast (NB) lineages, temporal gradients of Imp and Syp RNA binding proteins have been shown to direct the orderly generation of distinct neuronal fates across development. However, it is unclear whether Imp/Syp and/or other factors direct the development of the more complex, type II NB lineages. The type II NBs are similar to mammalian neural stem cells, in that they utilize intermediate progenitor (INP) cells. Here we characterize temporal fate changes in *Drosophila* type II NB lineages, examine previously described temporal factors, and search for additional genes that regulate temporal fate. Using cell lineage mapping, we show that INPs are conferred with distinct temporal fates across development. To examine the genetic basis of these changes, we performed whole transcriptome profiling of pure type II NBs over developmental time. Previously described temporal factors Imp and Syp are also temporally regulated in type II NBs. The high-to-low Imp gradient restricts early fates to early-born INPs, while the low-to-high Syp gradient boosts late fates in later-derived INPs. Further, we examined additional genes that are dynamically expressed in type II NBs and identified an upstream initiator of the Imp and Syp gradients. Thus, Imp/Syp gradients and their initiator control INP temporal fates in mammalian-like type II NB lineages. It would be interesting to examine whether the same or similar temporal gradients act as temporal cues in mammals.

Program Abstract #168

Evidence supports in silico prediction of sarcomere gene regulation by microRNAs in the electric organ of a weakly electric fish.

Alexander Chaidez[1], Matthew Pinch[1], Robert Güth[1], Manoj P. Samanta[2], Graciela A. Unguez[1]
[1]New Mexico State University, USA; [2]Systemix Institute, USA

Skeletal muscle is comprised of cells with malleable phenotypic properties. One example of the extreme plasticity of the contractile muscle phenotype is observed in the vertebrate teleost *Sternopygus macrurus*, wherein a select population of muscle cells transdifferentiate into the non-contractile, current-producing cells of the electric organ (EO) called electrocytes. Mature electrocytes lack sarcomeres yet continue to express some muscle proteins. This partial muscle phenotype is manifested despite findings showing that the EO transcribes all contractile genes at levels similar to those found in muscle (Pinch *et al.*, 2016). We hypothesize that the electrocyte phenotype is maintained by microRNA-dependent post-transcriptional regulation similar to that reported in other vertebrates. To test this hypothesis, we performed deep RNA sequencing (n=1) of miRNAs in muscle and EO tissues from adult *S. macrurus*. We identified three miRNAs, miR-30, miR-193b and miR-365, which are upregulated in EO compared to muscle and reported to be involved in muscle determination (miR-30a) and gene regulation (miR-193b, miR-365) in mammals (O'Brien *et al.*, 2014, Sun *et al.*, 2011). Using computational tools (TargetScanFish v6.2 and RNAhybrid), we have also identified eight sarcomeric genes in *S. macrurus* with 3'-untranslated regions containing conserved predicted target sequences for these 3 miRNAs in

zebrafish and *S. macrurus*. Whole transcriptome data show all eight transcripts are similarly expressed in both muscle and EO tissues. However, our initial expression analysis of these gene products using immunolabeling reveals an absence of the sarcomeric protein myomesin - a target of miR-30 - in EO but not in muscle of adult fish. Protein detection analysis of other sarcomeric genes targeted by these miRNAs will be completed. These data will be used to inform future experiments to test functional relationships between these miRNAs and their predicted targets in muscle and EO of *S. macrurus*

Program Abstract #169

Dynamic lineage marker expression and fate analysis reveals plasticity of individual neural plate border cells

Daniela Roellig

California Institute of Technology, USA

The neural plate border of gastrula stage embryos contains precursors to neural crest cells, ectodermal placodes and the neural tube. To examine how these fates become segregated, we performed a fine-scale, quantitative analysis of transcription factor expression in individual neural plate border cells as a function of time. The results reveal significant overlap of neural, neural crest, placodal and ectodermal markers in individual border cells from early gastrula stages until the time of neural tube closure. Using a Sox2 (neural) enhancer, we tested the prospective fate of neural plate border cells. The results reveal Sox2-derived cells contributing to both the neural tube and neural crest. Moreover, reducing Sox2 levels results in expansion in numbers of neural crest cells at expense of neural tube cells. Taken together, these results suggest that neural plate border cells have the ability to contribute to multiple ectodermal fates and the segregation of individual lineages does not occur until around the time of neural tube closure.

Program Abstract #170

Small molecule screening in zebrafish reveals a role for Akt in neural crest specification

Christie Ciarlo[1,2], Charles K. Kaufman[1,3], Christopher D'Amato[1], Beste Kinikoglu[4], Song Yang[1], Yi Zhou[1], Eric C. Liao[4,5], Leonard I. Zon[1,5]

[1]Stem Cell Program and Hematology/Oncology, Children's Hospital Boston, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts, USA; [2]Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts, USA; [3]Dana Farber Cancer Institute, Dept. of Medical Oncology, Boston, Massachusetts, USA; [4]Center for Regenerative Medicine and Division of Plastic and Reconstructive Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA; [5]Harvard Stem Cell Institute, Cambridge, Massachusetts, USA

The neural crest provides an excellent model for the study of cellular behavior during development. Arising at the border of neural and non-neural ectoderm, this highly dynamic cell population migrates throughout the early embryo and differentiates into a variety of tissue types. The inductive roles of FGF, Wnt, and BMP at the neural plate border are well established, but the signals required to stimulate and maintain expression of neural crest specifiers remain incompletely characterized. To better understand essential pathways in neural crest development, we have conducted a screen in primary zebrafish embryo cultures to find chemicals that decrease expression of *crestin:GFP*, a neural crest marker. We focused on the natural product caffeic acid phenethyl ester (CAPE), which decreases *crestin* expression in zebrafish embryos within two hours of treatment. Without affecting cell number, CAPE disrupts the neural crest gene regulatory network, leading to a failure of the neural crest to migrate and differentiate. As demonstrated by both whole mount in situ hybridization and RNA-sequencing of *sox10:GFP*+ cells, CAPE treatment decreases expression of *sox10*, *foxd3*, *snai1b*, *pax7a* and *dlx2a*, while leaving *tfap2a* and *tfap2c* expression unchanged. CAPE-treated embryos show a reduced number of pigmented melanocytes preceded by reduced *mitfa:GFP* expression. Time lapse imaging of *sox10:GFP* transgenic embryos demonstrates defective neural crest migration in CAPE-treated embryos. Using our primary cell culture system, we found that CAPE inhibits Akt activation selectively in the context of FGF stimulation. Furthermore, microinjection of constitutively active Akt1 mRNA rescues *crestin* expression and pigmentation in CAPE-treated embryos. Our work indicates that Akt activation is required for neural crest specification. Given the role of neural crest specifiers in melanoma tumorigenesis, this work provides insight into both normal development and disease processes. Funding: F31CA180313

Program Abstract #171

P0-Cre transgene labels cranial neural crest and notochord in early mouse embryos

Guiqian Chen[1], Yoshihiro Komatsu[2,3,4], Shigeto Miura[4], Satoshi Kishigami[4], Tomokazu Fukuda[4], Greg Scott[4], Manas K Ray[4], Hongxiang Liu[1], Yuji Mishina[2,3,4]

[1]University of Georgia, USA; [2]University of Michigan, USA; [3]University of Texas Health Medical School, USA; [4]National

Institute of Environmental Health Sciences, USA
P0-Cre mouse line has been widely used to label and genetically modify the neural crest (NC) derivatives combining with the loxP-flanked mice. It has been reported that *P0-Cre* labels NC *derived* cells from E9.5 and the transgene is restricted to the NC lineage tracing in mid to late gestation using a *CAAG-CAT-Z* (*CAAG-lacZ*) Cre reporter mouse line. However, it is not clear about how specific *P0-Cre* transgene is in labeling NC cells at earlier stage when NC cells are specified and delaminated. To better understand and interpret the data from P0-Cre line about NC contributions to the development of organs and tissues, we analyzed the *P0-Cre* model in labeling NC cells in early mouse embryos focusing on the cranial NC. First we compared two *Cre* reporter mouse lines, i.e., *CAAG-lacZ* and *ROSA26R* (*R26-lacZ*), to validate their reporter activity during early embryogenesis using an epiblast-specific Cre driver (*Meox2-Cre*). We found that *R26-lacZ* reporter expression emerge earlier (E6.5 vs E9.5) and respond to the Cre activity more reliably than *CAAG-lacZ*. Further, the *R26-lacZ* reporter strain was selected to analyze the expression of *P0-Cre* in early embryos. *P0-Cre/R26-lacZ* was first seen in the notochord at early embryos (E8.5, 4-somite). At later stages (E8.5, 7-19 somites), *P0-Cre* transgene expression was observed in the migrating NC cells. The *Cre* immunoreactivity was highly co-localized with the NC cell markers (p75, Sox9) with the greatest extent at 14-somite stage. In summary, our data demonstrated that *P0-Cre* transgene is specific in labeling cranial NC cells and notochord in early mouse embryos and is a useful tool for studies on NC and notochord lineages.

Program Abstract #172
Investigating Myosin Heavy Chain 9 as a target of the antiphosphatase Paladin during chick neural crest development
Julaine Roffers-Agarwal, Edward J. Stronge, Laura S. Gammill
Department of Genetics, Cell Biology and Development, University of Minnesota, USA
The neural crest is a multipotent, migratory, embryonic cell type that forms much of the vertebrate craniofacial skeleton and peripheral nervous system. While a transcriptional network that regulates neural crest cell fate has been defined, post-translational modifications that spatiotemporally modulate the activity of neural crest proteins, including proteins in this network, are less clear. We are studying the antiphosphatase Paladin (Pald) in order to understand the importance and function of phosphorylation during neural crest development. Based upon mutational analyses, we previously concluded that Paladin is an antiphosphatase that influences the expression of a subset of neural crest transcription factors and is required to achieve timely neural crest migration. Given that antiphosphatases bind and protect phosphorylated residues, we are now using Pald to identify phosphorylation-modulated factors in the neural crest using both in vitro and in vivo assays. We identified one candidate, Myosin Heavy Chain 9 (MYH9), as a phosphorylation-dependent Pald interactor in a yeast two-hybrid assay. MYH9 mRNA and protein are enriched in premigratory and migratory neural crest cells, and we confirmed that Pald and MYH9 interact in chick cells. Currently, we are determining the residues of MYH9 with which Pald interacts and their phosphorylation status in neural crest cells. Additionally, we are beginning to query Pald-interacting proteins using immunoprecipitation from neural crest cells followed by mass spectrometry. Together these data will help us identify proteins that modulate neural crest development in a phosphorylation-dependent manner, thus expanding our understanding of an underappreciated level of control within the neural crest developmental program.
Funding: NIH F32DE019973; K22DE015309; R03DE023368; American Association of Anatomists; Minnesota Medical Foundation.

Program Abstract #173
Probing the signals that regulate the transition from proliferation to differentiation in the zebrafish retina.
Kara Cerveny[1], Audrey Williams[1], Terra Vleeshouwer-Neumann[1], Amanuel Tafessu[1], McKenzie Givens[1], Wilson Horner[1], Dayna Lamb[1], Tess Tumarkin[1], Alison Bryant[1], Leonardo Valdivia[2], Steve Wilson[2]
[1]Reed College, USA; [2]University College London, UK
Neurogenesis in growing tissues requires a tight balance between progenitor cell proliferation and differentiation. In the zebrafish retina, neuronal differentiation proceeds in two stages with embryonic retinal progenitor cells (RPCs) of the central retina enabling the first rounds of neuronal differentiation, and stem cells in a peripheral germinal zone, called the ciliary marginal zone (CMZ), supporting later neurogenesis. To probe the mechanisms that control how RPCs transition from proliferation to differentiation, we recently analyzed two new alleles of *gdf6a*. Although Gdf6a had been linked to dorsal-ventral patterning, our data show that Gdf6a is also required during both early and late phases of retinal neurogenesis. Decreased Gdf6a is correlated with increased retinoic acid (RA) pathway activity, and pharmacological perturbations of RA signaling suggest that RA regulates the timing of RPC cell cycle exit and neuronal differentiation. Preliminary analyses of CMZ gene expression during eye morphogenesis combined with recent fate-mapping experiments

raise the possibility that dorsally expressed *gdf6a* limits RA pathway activity and maintains a population of RPCs that will eventually give rise to the CMZ.

Program Abstract #174
AKAP200 modulates Notch signaling
Neeta Bala, Ursula Weber, Ekaterina Serysheva
Icahn School of Medicine at Mount Sinai, USA
A Kinase Anchoring Protein 200 (AKAP200), a scaffolding protein invovled in Protein Kinase A (PKA) localization, was identified as a novel regulator of Planar Cell Polarity (PCP), the polarisation of cells across the plane of an epithelium.Using *D. melanogaster* as a model system, we observed that AKAP200 modified the gain of function (GOF) adult eye phenotype of the core PCP genes *diego* and *prickle*. AKAP200 overexpression caused deltas in the wing and loss of macrochaete, and the AKAP200 mutants we generated caused supernumerary macrochaete and loss of photoreceptors, all Notch related phenotypes. Importantly, Notch signaling is downstream of the PCP pathway in the eye, the context, where AKAP200 was identified. To investigate the link between AKAP200 and Notch signaling, we performed co-immunoprecipitation assays and demonstrated a physical interaction between AKAP200 and Notch. Furthermore, genetic interaction experiments showed a strong dominant interaction with Notch pathway components in a PKA independent manner. Loss of AKAP200 suppressed the Notch GOF phenotype in the eye and thorax and western blot analyses revealed reduced Notch protein levels, likely leading to the suppression of the Notch overexpression phenotype. AKAP200 has previously been linked to the E3 ubiquitin ligase Cbl that targets proteins for lysosomal degradation (Sannang et al, 2012). We found that AKAP200 is unable to modify the Notch GOF eye phenotype in the absence of *cbl*, suggesting that AKAP200 requires Cbl to act on Notch. Similarly, AKAP200 could no longer modulate Notch signaling in the presence of a lysosomal inhibitor. We are currently investigating further the mechanism by which AKAP200 exerts its effects on Notch protein levels. Nonetheless, the identification of AKAP200 as a novel modulator of Notch signaling has advanced our understanding of the tight regulation of Notch signaling, which is aberrant in a wide array of developmental defects and diseases.
NIH RO1 EY013256

Program Abstract #175
Genetic control of Peripodial Epithelium identity in the eye disc of *Drosophila*
Qingxiang Zhou, Tianyi Zhang, Dana DeSantis, Philip Smith, Brady Nesbitt, Francesca Pignoni
SUNY Upstate Medical University, USA
We describe here the identification of a class of genes required for proper development of the *Drosophila* eye disc epithelium. Through an RNAi-based screen of 2800 genes, we have identified a set of genes whose loss-of-function leads to transformation of the peripodial epithelium (PE)—a non-neuronal tissue juxtaposed to the developing retina—into a second, mirror-image retina. This novel phenotype suggests that the presumptive PE has retinal potential that is suppressed by PE determinants. We will present detailed analyses of these loci and a first outline of the eye PE determining network.
Funding Sources: R01EY013167 (FP), Steinbach Award (FP), Fight for Sight Award (TZ), Research to Prevent Blindness Unrestricted Grant; Lions Club of Central New York District 20-Y1 (SUNY-UMU, Dept. of Ophthalmology)

Program Abstract #176
Dissection of the mechanisms restricting specific retinal progenitor cells to the production of cones and horizontal cells
Nicolas Lonfat, Connie Cepko
Harvard Medical School, USA
The development of the retina into a highly organized structure occurs via the production of over 60 cell types from a pool of retinal progenitor cells (RPCs). While RPCs are generally multipotent, recent studies have shown that some terminally dividing progenitors are restricted to the production of specific types of daughter cells. How specific RPCs make any specific type of neuron is currently not understood. To address this question, we investigated the molecular mechanisms that restrict a subset of RPCs to the production of only cones and horizontal subtypes, dissecting the transcriptional regulation of Otx2 and Onecut transcription factors that have been shown to promote the cone and horizontal cell fates. We used a combination of ChIP-seq and ATAC-seq analyses on chick retinal cells to identify putative regulatory elements of Otx2 and Onecut. Candidate regions were tested for enhancer activity using ex vivo electroporation, along with morphological and immunohistochemical identification of cells that express such constructs. Using bioinformatics

analyses of the positive sequences combined with RNA-seq transcriptomes, we identified putative upstream transcription factors that may play roles in promoting the fate of cones and horizontal cells during development. Furthermore, the identification of Otx2 and Onecut enhancers allows for FACS-sorting of RPCs, as well as cones and horizontal cells that express these reporters, to assess the homogeneity of the RPCs and better characterize them. Progress on understanding how to generate cones will contribute to the development of therapies for retinal diseases.
Funding sources: SNSF Early Postdoc.Mobility, HFSP Long-Term fellowship

Program Abstract #177
Coordination of neural patterning and morphogenesis in the frog by repression of oct4 family pluripotency factors
Cameron Exner
UC Berkeley, USA
The embryonic precursor of the vertebrate central nervous system, the neural plate, is patterned along the anterior-posterior axis and shaped by morphogenetic movements early in development. Recent work in our lab has identified the genes *sall1* and *sall4*, known regulators of pluripotency in other contexts, as important for both the patterning and morphogenesis of the neural plate. My work has demonstrated that these two genes are required for induction of posterior neural fates, cell shape changes that contribute to neural tube closure, and later neurogenesis. Defects in these processes that occur upon *sall* knockdown appear to be a consequence of a failure of the neural plate to differentiate. Consistent with this idea, *sall*-deficient neural tissue exhibits an aberrant upregulation of *pou5f3* family genes, the *Xenopus* homologs of the well-known mammalian stem cell maintenance factor, *oct4*. Furthermore, overexpression of *pou5f3* genes in *Xenopus* causes defects in neural patterning, morphogenesis, and differentiation that phenocopy those observed in *sall* morphants. In all, this work shows that *sall1* and *sall4* act to repress *pou5f3* family gene expression in the neural plate, thereby allowing vertebrate neural development to proceed. More broadly, this example demonstrates how precisely timed differentiation of a tissue, regulated in this case by repression of pluripotency factors, can be critical for subsequent patterning and morphogenesis.

Program Abstract #178
Characterizing Sox21-protein interactions and their functions in neurogenesis
Dillon Damuth, Elena Silva, PhD
Georgetown University, USA
The large Sox family of transcription factors (TFs) recognize the same 8 bp consensus binding sequence and require a partner protein(s) to specifically affect the transcription of target genes. However, the interactomes of only a few of the 30 Sox proteins invertebrates are known, and even less known is known about the functional significance of these interactions. Here, we investigate how these interactions contribute to cell lineage coordination and focus on Sox21, a SoxB2 protein,to not only study how function is influenced by partner proteins, but also how those interactions contribute to cell fate within the process of neurogenesis. This project is focused toward two interactions in effort to bridge the knowledge gap of how protein-protein interactions may serve to coordinate the specification and differentiation of neurons. Previous data from our work of *Xenopus laevis* neurogenesis suggests Sox21 is necessary to maintain neural progenitor cells in a $Sox2^{+}$ proliferative state, while low levels are also necessary to promote neural maturation. It is hypothesized that Sox21 is capable of having seemingly contradictory functions due to differential interactions with partner proteins across stages of development. In studying these interactions, we aim to test how these partners may differentially guide transcriptional specificity of Sox21.

Program Abstract #179
Early Molecular Events During Retinoic Acid Induced Differentiation of Neuromesodermal Progenitors
Thomas Cunningham, Sean Spiering, Alexandre Colas, Gregg Duester
Sanford Burnham Prebys Medical Discovery Institute, USA
During late gastrulation, bipotent neuromesodermal progenitors (NMPs) residing in the caudal lateral epiblast drive coordinated body axis extension by generating both posterior neuroectoderm that forms the spinal cord, and presomitic mesoderm that forms somites. Retinoic acid (RA) is a known proneural cue in differentiating embryonic stem cells (ESCs) and is required for neurogenesis in vivo; however, the early molecular response to RA signaling during posterior neuroectoderm formation is poorly defined. We previously reported that RA signaling is needed for the normal balance of NMP progeny, with a lack of RA activity favoring mesodermal over neuroectodermal differentiation due at least in part to a failure of caudal *Fgf8* repression. Here, we designed and implemented a platform to enable robust study of RA function during NMP differentiation in a physiologically relevant, cell-type specific context using an unbiased, genome-wide ESC-

derived entry point with accompanying genetic and in vivo corroboration. We took advantage of recent protocols to derive *Sox2+/T+* NMPs by treating ESCs with WNT and FGF signals that mimic the signals present in the late gastrulation caudal lateral epiblast. We then treated ESC-derived NMPs with a short 2-hour pulse (to limit detection of secondary targets) of 25 nM RA (the normal endogenous concentration as opposed to 1 μM which is often used) or vehicle control and undertook whole genome transcriptome analysis using RNA-seq. Differential expression analysis of this dataset yielded a cohort of novel putative RA signaling targets in the NMP niche (either activated or repressed), several of which are pertinent to NMP biology. Importantly, we validated the top ranked genes in vivo by examining expression in E8.5 *Raldh2-/-* embryos (that lack RA activity). Understanding the role of RA in NMP differentiation is critical for developing cell-based therapies to treatment motor neuron disease or spinal cord injury. Supported by NIH R01 GM062848.

Program Abstract #180

A temporal barrier by a transcriptional repressor is required for key transcription factors to be used repeatedly in the ascidian embryo

Tatsuro Ikeda, Yutaka Satou
Department of Zoology, Graduate School of Science, Kyoto University, Japan

Cell fates are specified by sequential and combinatorial inputs of transcription factors and signaling molecules. Many regulatory factors are used repeatedly to specify different cell fates, and hence expression of regulatory factors needs to be controlled spatially and temporally in a precise manner. In the ascidian, *Ciona intestinalis*, two transcription factors, Foxa.a and ZicL (a Zic ortholog), and FGF signaling are required for inducing developmental fates of the brain and notochord. In the presumptive notochord cells, Foxa.a and ZicL are expressed at the same time, and these two factors and Ets activated by FGF signaling activate *Brachyury*, which specifies the notochord fate. On the other hand, in the brain lineage, *Foxa.a* and *ZicL* are not expressed simultaneously; *Foxa.a* is expressed first, and *ZicL* begins to be expressed after *Foxa.a* expression is diminished. In the present study, we found that a transcriptional repressor, Bz1 (Blimp1-related Zinc Finger protein), plays an important role in temporal regulation of *Foxa.a* and *ZicL* expression in the brain lineage. In *BZ1* morphant embryos, *Foxa.a* expression did not stop at the time when it stopped in normal embryos. As we have previously shown (Ikeda et al., *Development* 2013, 140, 4703-4708), Bz1 also represses precocious expression of *ZicL* in the presumptive brain cells. As a result, *Foxa.a* and *ZicL* were expressed at the same time in the presumptive brain cells of such morphants. Because presumptive brain cells receive FGF signal, this overlapping expression of *Foxa.a* and *ZicL* led to ectopic activation of *Brachyury* and notochord marker genes in the brain lineage. Thus, Bz1 temporally controls expression of *Foxa.a* and *ZicL* in the brain lineage, so that the notochord fate specifier *Brachyury* is not activated in this lineage. The temporal regulation of *Foxa.a* and *ZicL* is essential for fate choice between the notochord and the brain.
(a Grant-in-Aid from the Japan Society for the Promotion of Science; 15J01153)

Program Abstract #181

The transcriptional factor Ap2e defines the terminal differentiation of the basal vomeronasal sensory neurons.

Paolo Emanuele Forni
University at Albany (SUNY), USA

The vomeronasal organ (VNO) is a specialized olfactory subsystem responsible for the detection of pheromones. Pheromone signals play a pivotal role in social interaction in a large number of mammals. The VNO of mice is composed of two types of sensory neurons that selectively express receptors encoded by one of the two vomeronasal receptor (Vr) gene super families: V1r and V2r. The V1r and V2r expressing neurons respectively localize in the apical and basal areas of the vomeronasal sensory epithelium, express different G-protein subunits, and project to spatially distinct areas of the accessory olfactory bulb. Both apical and basal sensory neurons originate from common progenitor cells but the mechanism underlying the differentiation into one of the two neuronal cell types is still unknown. We found that, in the nasal area of mice, the transcription factor Ap2ε (tfap2e) is only expressed in the VNO. By exploiting a knock-in/knock-out Ap2ε:Cre mouse line we analyzed Ap2ε expression, genetic lineage and function. Our data indicates that, in the VNO Ap2ε is only expressed by basal V2r+ vomeronasal neurons and not by the V1r+ apical neurons. Analyzing Ap2ε:Cre null mice we found that Ap2ε loss of function leads to an almost complete loss of V2r+ cells positive for basal vomeronasal cells markers and to an inverse and proportional increase in cell positive for apical neuronal markers. As no differences were detected in total cell number, proliferation or cell death, we propose that Ap2e is the master regulator for the basal vomeronasal terminal differentiation.
SUNY startup funds

Program Abstract #182

Embryonic taste bud precursor cells are progressively assembled

Erin Golden, Linda Barlow

University of Colorado Anschutz Medical Campus, USA

Taste buds, the primary receptor organs of the gustatory system, are the first step in relaying taste information to the brain. These sensory structures comprise a heterogeneous collection of 50-100 specialized epithelial cells (taste receptor cells, TRCs) encompassing three morphological classes that together transduce taste information (i.e. sweet, bitter, sour, salt, umami) into neural code. This information directly influences dietary choice and is intimately linked to human health and disease, yet despite its importance, our understanding of how the taste system is developed and maintained remains limited. Development of murine taste buds begins during mid-gestation (~E12.5), when bilateral rows of epithelial thickenings, termed taste placodes, first appear on the surface of the anterior tongue. Previously, our lab has shown that these taste placode cells, which express the morphogen Sonic hedgehog (Shh), are exclusive taste bud precursor cells (TBPCs) that differentiate into the first postnatal TRCs (Thirumangalathu *et al.* 2009). Recently, we have identified that taste placode formation is actually a dynamic process and that newly specified Shh+ TBPCs continue to be added to placodes for several days following induction (~E12.5-E15.5), through a process that we have termed placode assembly. Furthermore, we have found that TBPCs begin differentiating into TRCs before birth, much earlier than was previously appreciated, and that this process is already underway at E17.5. Here we will discuss the cellular mechanisms underlying placode assembly and explore the lineage capacity of early versus later specified TBPCs. In the future, we will address the molecular mechanisms that govern the onset of TRC differentiation and the shifting role of these signaling factors throughout the course of taste bud development.

Funding Sources: NIDCD R01 DC012675

Program Abstract #183

GSK3 regulates hair cell fate in the sensory epithelium of the cochlea

Kathryn Ellis[1], Takayuki Okano[2], Elizabeth C. Driver[1], Matthew W. Kelley[1]

[1]NIDCD/NIH, USA; [2]Dept. of Otolaryngology, Head and Neck Surgery, Kyoto University, Japan

The sensory epithelium of the mammalian cochlea (the organ of Corti, OC) is composed of two types of innervated mechanosensory hair cells – the medial inner hair cells (IHCs) and the lateral outer hair cells (OHCs) and six types of supporting cells. IHCs and OHCs differ structurally and functionally and are separated by a single row of boundary supporting cells. The overall structure comprises an exquisitely patterned mosaic of cells whose proper differentiation and organization is critical for hearing. While many of the molecular mechanisms responsible for specification of the sensory epithelium and the hair cells are well understood, little is known about how an otherwise homogeneous prosensory domain develops into two functionally distinct medial and lateral domains. Glycogen synthase kinase 3 (GSK3), a serine/threonine protein kinase, has been shown to play an important role in many signaling pathways including Hh, Notch, GPCRs, and most commonly, in canonical Wnt. To determine whether GSK3 plays a role in medio-lateral patterning within the OC, multiple GSK3 antagonists were tested in vitro in cochlear explants. Inhibition of GSK3 leads to a dramatic increase in the size of the medial domain and a proportional decrease in the size of the lateral domain. Lineage tracing reveals that this shift occurs as a result of lateral cells adopting a medial cell fate. This is significantly different from the changes observed in response to activation of canonical Wnt signaling; an increase in the number of overall hair cells. Based on these results, we are working to identify the Wnt-independent roles of GSK3 in cochlear sensory epithelium patterning. We have demonstrated that BMP4, an inducer of the lateral OC domain, is reduced following GSK3 inhibition and that ectopic BMP4 treatment rescues the shift in lateral to medial cell fate. This work will help to elucidate the molecular mechanisms that are necessary for patterning the OC along the medio-lateral axis.

Program Abstract #184

Alternative splicing by Esrp1 regulates cochlear development and function in mammals

Alex Rohacek[1], Thomas Bebee[1], Ricky Tilton[2], John Germiller[2], Yoseph Barash[1], Ian Krantz[2], Russ Carstens[1], Douglas Epstein[1]

[1]University of Pennsylvania, USA; [2]Children's Hospital of Philadelphia, USA

Alternative splicing allows for individual genes to encode several mRNA transcripts and, therefore, multiple proteins. This process grants greater functional diversity to a relatively limited genome. Despite this, few studies have addressed the importance of alternative splicing during organ formation. Epithelial Splice Regulatory Protein 1 (Esrp1) belongs to a family of RNA binding proteins that affect alternative splicing of transcripts within most epithelial tissues of the developing embryo, including the inner ear. Whole exome sequencing identified ESRP1 mutations in individuals with

profound sensorineural deafness. We found that *Esrp1* deficient mice display defects in inner ear morphology, including a significant shortening and widening of the cochlear duct. We performed RNA-seq comparing wild type and *Esrp1*$^{-/-}$ cochleae and uncovered a significant reduction of mRNA transcripts expressed in the stria vascularis (SV), a nonsensory structure essential for ion homeostasis and proper hearing function. Further analysis reveals that *Esrp1*$^{-/-}$ ears are missing all essential proteins of the SV. Interestingly these cells display inappropriate activation of markers of the neighboring Reissner's membrane, which is dramatically expanded in these mutants. Fgf signaling has previously been implicated in the development of Reissner's membrane and loss of Esrp1 results in aberrant splicing of Fgfr2, altering its ligand binding specificity. We show that this splicing switch in Fgfr2 leads to ectopic expression of Fgf target genes within the SV. Removal of an allele of Fgf9 in an Esrp1 mutant leads to a complete rescue of the SV gene expression program and a restoration of these cells. These results demonstrate the importance of Esrp1 dependent alternative splicing in controlling the cellular identity and morphogenesis of the cochlear duct and implicate mutations in ESRP1 as a novel cause of deafness in humans.
Supported by R01DC006254 (DJE) and T32GM008216 and F31DC014647 (AMR).

Program Abstract #185
Breaking the Atoh1 autoregulatory loop: the repression of Atoh1 by Ngn1 during inner ear development
Héctor Gálvez García[1], Juan Tena[2], Fernando Giraldez[1], Gina Abelló Sumpsi[1]
[1]Universitat Pompeu Fabra, ES; [2]Centro Andaluz de Biología del Desarrollo, ES
The functional unit of the inner ear consists of hair cells (HC), supporting cells and neurons. Atoh1 and Neurog1 are bHLH factors crucial for HC and neuronal development, respectively. Both genes are induced early in inner ear development, but the expression of Atoh1 is delayed with respect to Neurog1, resulting in HCs developing after neurons. Atoh1 activates its own transcription through a class A E-box located in a 3'enhancer, and this is probably its major mechanism of activation. This work was aimed at understanding how Neurog1 interferes with Atoh1 regulation and HC formation. Reporter assays on chick embryos and P19 cells showed that Neurog1 repression is accounted for by its interaction with the 3'Atoh1 enhancer. The effect is cell autonomous and independent on Notch signaling. The 3'Atoh1 enhancer consists of two regions, A and B that behaved differently in the developing otic vesicle. Enhancer B showed higher and broader activity than the complete 3'enhancer, whilst enhancer A was silent. The differential activity of A and B enhancers correlated well with their different accessibility as revealed by ATAC-seq analysis of mouse otic vesicles. Neurog1 was able to activate multimers of the class A E-box present in the 3'Atoh1 enhancer, however, it turned into a repressor when this region was flanked by class C and N boxes. Surprisingly, the deletion of the DNA binding domain of Neurog1 did not abolished the ability of Neurog1 to repress Atoh1 or HC fate. Repression did require, however, the integrity of dimerization domain Helix1. Together, the results suggest that the repression of Atoh1 by Neurog1 relies on an indirect mechanism that prevents the binding of Atoh1 to the class A E-box of enhancer B, rather than on active repression.
Supported by MINECO and Fundació La MaratóTV3, Spain

Program Abstract #186
The Lin-41 (Trim71)/let-7 axis controls sensory progenitor cell proliferation and hair cell differentiation in the developing chicken auditory organ
Lale Evsen, Angelika Doetzlhofer
Johns Hopkins School of Medicine, USA
Lin-41, as well as its negative regulator *let-7* was initially identified in a screen for heterochronic genes in *C. elegans.* Lin-41, an ubiquitin ligase and RNA-binding protein, plays a critical role in stem cell maintenance and proliferation, and negatively controls differentiation events. In contrast, let-7 microRNAs inhibit proliferation. We recently discovered that *Lin-41* is highly expressed in neural-sensory progenitors in the murine and avian auditory organs. *Lin-41* is expressed within the otic vesicle neural-sensory competent domain and later in auditory progenitors, but rapidly declines in expression with increasing hair cell (HC) differentiation in both the murine and avian inner ear, whereas mature *let-7* levels increase in expression with increasing HC differentiation. To address the role of *Lin-41* and *let-7* miRNAs in auditory sensory development we manipulated their levels in the developing chicken auditory organ using *in ovo* micro-electroporation together with *in ovo* cell proliferation assays. We found that over-expression of *Lin-41* in the chick auditory organ lead to excess progenitor cell proliferation and an inhibition of HC differentiation. *Let-7* miRNA levels are reduced when over-expressing *Lin-41*. Over-expression of a *let-7* sponge, which inhibits the activity of all let-7 miRNAs, resulted in an increase in progenitor proliferation and inhibition of HC differentiation similar to the *Lin-41* over-expression phenotype. Over-expression of *let-7b* resulted in a decrease in progenitor proliferation; however, surprisingly

we did not observe premature HC differentiation, instead we observed a severe reduction in *Atoh1* transcript expression. These findings suggest that Lin-41 is critical for maintaining auditory sensory progenitors in a proliferative and undifferentiated state. Moreover, it indicates that *let-7b* negatively regulates *Atoh1* mRNA expression in the developing auditory organ and too high *let-7b* levels block auditory HC differentiation.

Program Abstract #187

Ordered architecture of skin hair follicle epithelium is sustained by a dynamic cellular flux

Tianchi Xin[1], Panteleimon Rompolas[2], Valentina Greco[1]

[1]Yale University, USA; [2]University of Pennsylvania, USA

Tissue architecture is crucial for tissue function. Yet we still don't understand the cellular and signaling mechanisms that stem cells and their differentiated progeny adopt to establish a proper tissue architecture. The hair follicle provides a unique model system to interrogate such mechanisms because it periodically rebuilds its architecture during stereotypic hair cycles. Previous work showed that hair progenitors are spatially organized at the bottom of the follicle onto a dome-like cellular platform from which the several differentiated cell layers are generated and centrically converge to form the hair shaft. How this ordered architecture is built from a handful of stem cells (SCs) remains unclear. By tracking the same cells in the same live mice over time, we show that SCs are amplified and initially organized in a stereotypic manner. Specifically, the position of each SC at the onset of hair follicle growth restricts the fate to a specific group of hair progenitors. Unexpectedly, as the progenitor platform is established at the bottom of the follicles, the hair progenitors do not appear to be restricted to specific fates any longer but rather directionally and dynamically change their position suggesting flexibility in cell fate choices. This cellular flux appear to follow a gradient of Wnt activation which is continuously active as shown by FRAP analysis of a live Wnt reporter. Current gain- and loss-of-function single cell manipulation studies are addressing the causality of Wnt relationship to the observed flexibility in hair progenitor cell fate choices. This study underscores the dynamic cellular and signaling mechanisms employed to establish a highly ordered tissue architecture.

Program Abstract #188

A previously uncharacterized cell population in the zebrafish presents a potential connection between the lymphatic and the immune systems

Marina Venero Galanternik[1], Daniel Castranova[1], Matthew Swift[2], Brant M. Weinstein[1]

[1]NICHD, NIH, USA; [2]Georgetown University, USA

Macrophages carry out a variety of critical immune system-related functions during development and adult life. A specialized population of macrophages termed perivascular macrophages (PVMs) are found exclusively next to blood vessels, but their origin and function remains unclear compared to other tissue-resident macrophage populations. We are characterizing these cells using a number of specific transgenic lines previuosly used to distinguish between blood and lymphatic vessels. Our preliminary analysis suggests that PVMs may share a lineage relationship with lymphatic endothelium. In adults, PVMs cover the entire zebrafish optic tectum, allowing us to readily isolate these cells for RNAseq profiling. The fact that PVMs are closely associated with vessels hints at potential roles during angiogenesis and lymphangiogenesis, under homeostatic or pathological conditions. We are currently carrying out additional studies to better understand the origins and function of this novel cell type.

Program Abstract #189

Numb regulates somatic cell lineage commitment during early gonadogenesis

Yi-Tzu Lin[1], Lindsey Barske[2], Tony Defalco[3], Blanche Capel[1]

[1]Cell Biology Department, Duke University, United States; [2]University of Southern California, United States; [3]Division of Reproductive Sciences at Cincinnati Children's Hospital Medical Center , United States

During early gonadogenesis, the proliferation of the Coelomic Epithelium (CE) contributes to supporting cells (*Sertoli* cells in the male) and other cell types in the gonad. In dye-labeling experiments, it appeared that a single CE cell could give rise to more than one cell type suggesting an asymmetric division responsible for distinguishing *Sertoli* progenitor cells from other cell types in the gonad. We found in E11.5 gonads, NUMB and NOTCH displayed reciprocal patterns in the CE. The well-established role of NUMB and NOTCH in asymmetric division led us to investigate whether this pathway is responsible for acquisition of different cell fates from CE precursor cells. To address this question, we used the conditional $Numb^{flox/flox}/Numb\text{-}like^{-/-}$ mouse driven by ROSA-CreER, a tamoxifen inducible and ubiquitously expressed Cre line. Tamoxifen was administrated at E8.75 and embryos were analyzed at E11.5-E13.5. Mutant gonads had a bumpy surface at E13.5, suggesting mis-regulation of cell proliferation at the CE. Although LHX9 is normally restricted to the

undifferentiated cells in the CE domain, we found large patches of undifferentiated LHX9$^+$ cells in the mutant gonads. Using mitotracker, we confirmed the LHX9$^+$ cells were derived from the CE. We then found that the mutant phenotype could be rescued by DAPT treatment, a Notch antagonist, in a dosage dependent manner suggesting that the phenotype was due to over-activation of Notch signaling resulting from loss of *Numb/Numbl*. Surprisingly, neither SRY expression nor the number of supporting cell progenitors was affected in the mutants. These results indicate that NUMB is required for differentiation of interstitial cells, but not *Sertoli* progenitors, and are difficult to reconcile with the idea that multiple cell types arise from a common progenitor in the CE. We are using the Confetti mouse model combined with the adenovirus Cre system to revisit this question.
This work was supported by NIH-HD039963 to BC.

Program Abstract #190
STAT signaling regulation and its effects on border cell specification in *Drosophila*
Afsoon Saadin, Lindsay Mercer, Bradford Peercy, Michelle Starz-Gaiano
University of Maryland, Baltimore County, USA
Morphogens are signaling molecules that can act across tissue domains and evoke concentration-dependent responses. In *Drosophila*, oogenesis provides a tractable way to characterize morphogen signaling because of the array of available genetic tools and the small transparent tissues that permit direct imaging. In the fly ovary epithelium, Unpaired (Upd) can act as a morphogen to activate the well-conserved Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) signaling pathway. Localized release of Upd from specialized epithelial cells, called polar cells, induces nearby cells to adopt specialized fates. Cells with the highest levels of STAT signaling become the motile border cells, and this appears to be affected by tissue architecture. Through genetic screening, we have identified multiple new regulators of STAT activity that influence border cell specification. We are using a combination of mathematical modeling, cell biology, and genetics to understand how regulators are integrated signaling cascades. Specifically, we have uncovered a specific role for a vesicle trafficking protein, alpha-Snap, that is required in the polar cells for exocytosis of Upd. This gene is required for border cell specification. In addition, we found that the SWI/SNF chromatin remodeling factor Brahma modulates STAT signaling, as well as signaling from other pathways, to influence cell specification and migration. Our work shows that multiple, overlapping levels of regulation are needed to control STAT activity. This work was funded in part by an NSF-Undergraduate Biology Mathematics Training Grant to Drs. Leips and Neerchal, and an NSF CAREER Award to MSG.

Program Abstract #191
Maternally loaded transcripts in the sea urchin egg are asymmetrically localized and reorganized after fertilization
Allison Edgar, David McClay
Duke University, USA
We combined experimental embryology with RNA sequencing of microdissected eggs and zygotes in the sea urchin *Paracentrotus lividus* to characterize differentially localized maternal mRNAs. We found RNAs asymmetrically distributed along the animal-vegetal axis in the egg; identified massive reorganization of some of these transcripts between fertilization and first cleavage; and used classical embryological techniques to show that their functions extend temporally beyond early cell lineage specification to affect post-gastrulation cell fate decisions. Among these transcripts are signaling receptors, cytoskeletal organizers, and transcriptional repressors, whose role may be to maintain pluripotency and hence competence to respond to later signaling events in the cells that inherit them. We are using these data to characterize the initial state of the sea urchin developmental gene regulatory network, which integrates interactions at the molecular and cellular level across the embryo to ask how transcriptional regulatory states and signaling interactions interact to establish cell identities during development.

Program Abstract #192
Local translation on the spindle is critical for cell fate determination and development
Jessica Poon, Gary Wessel, Mamiko Yajima
Brown University, USA
Localized mRNA translation on the mitotic apparatus is hypothesized to be an essential biological strategy that enhances new functional protein on site, with strong spatial and temporal control. This mechanism may be important particularly in embryonic cells that are large (over 80 um in diameter, large diffusion volume) and that undergo rapid cell divisions (repetitive M-phase in 30 minutes with no intervening G-phase) requiring immediate input of specific protein without relying on diffusion kinetics from translation elsewhere in the cell. Evidence to test such a hypothesis on the spindle has

been, however, limited. Here we take advantage of the asymmetry of Vasa protein, a conserved RNA-helicase, on the spindle during embryogenesis while using multiple live imaging techniques to learn that local protein synthesis within a single spindle can be unequal and help drive asymmetric cell divisions. Further, we found that more than one third of general translation in the cell occurs specifically on the spindle, serving as a major site of local translation at M-phase during sea urchin embryogenesis. Surprisingly, tethering Vasa to the plasma membrane caused ectopic local translation on the membrane and developmental defects in the embryo. These results suggest that local translation on the spindle is an essential mechanism for asymmetric protein distribution and Vasa plays a critical role in the process, providing another level of regulation for quality control in cell division.

Program Abstract #193

The effects of Bisphenol A and Bisphenol S regarding the Transgenerational Aspects of the Reproductive System of *Caenorhabditis elegans*

Sophia Touri, Alexandra Marques, Michael Ionta, Nisharg Parikh, Taisha Mathieu, Maria Agapito
Saint Peter's University, USA

Bisphenol A is also known as BPA, which is a well known compound used in the production of polycarbonate plastics and epoxy resins. BPS is another bisphenol analog that acts as a plasticizing agent to replace BPA. It can be found in "BPA-free" plastics and similarly in BPA, it is a cause for infertility. BPA can be found on the covering of metal cans, plastic bottles and containers, and even dental caries. BPA has been found in breast milk and studies are showing that early exposure to babies can cause further infertility complications. BPA and BPS differ in their chemical compositions slightly. BPS is composed of a sulphur atom attached to two oxygen atoms; while BPA is composed of a carbon atom attached to two methyl groups. In this study, BPA and BPS are separately and synergistically tested to determine if BPA and BPS cause any effect on the reproductive system functioning in *C. elegans*. The nematodes were treated with bisphenol analogs under acute (2 hour) and chronic (24 hour) exposures. Quantitatively, this study measured egg laying, hatching, fertilization and reproductive rates. The parental generation and the three subsequent offspring generations were analyzed in this study. The data demonstrated that both BPA and BPS had the same toxicity effects, however the levels of toxicity varied within the bisphenol analogs. Both bisphenol analogs were significantly different to the control. In summary, BPA and BPS evoked long-term negative effects on the reproductive system of *C. elegans* and this effect may be similar to humans.

Sponsored by the following Grants: ICFNJ Grant, TriBeta National Honor Society

Program Abstract #194

The effects of bisphenol A and bisphenol F on the reproductive system using *Caenorhabditis elegans*

Suyapa Penalva-Lopez, Merna Sawaged, Leonard J. Sciorra, Maria Agapito
Saint Peter's University, USA

Bisphenol A is a chemical compound that has been used for over 40 years to harden plastics. This compound is present in plastics such as water and baby bottles. Recent research has determined that such chemicals can have a negative effect on the endocrine system, as well as enhancing PCOS. Concerns of the toxicity of these chemical compound led companies to replace bisphenol A to other compounds. These replacing agents are also part of the bisphenol family. One replacements agent that is currently being used to replace BPA is Bisphenol F which is known as BPF. Limited literature research gives no certainty that BPF is less harmful than BPA. The main focus of this study is to determine the effects of acute and chronic exposure of bisphenol A and bisphenol F on the reproductive function over several generations using *C. elegans* as a model system. The following techniques (egg-laying, hatching rate, reproductive rate and the fertilization assay) were used to determine the toxicity effects of the bisphenol family. The data suggested that both BPA and BPF disrupts the functions of the reproductive function and both bisphenol analogs may have similar toxicity effects. It is yet to be elucidated whether both bisphenol analogs target the same mechanism of action and whether this is similar to human.

Program Abstract #195

An investigation of odd-skipped homologs in *C. elegans*

Elizabeth Del Buono, Gabriella Scoca, Elizabeth Schoell, Amy C. Groth
Eastern CT State University, USA

The *odd-skipped* (*odd*) gene family consists of highly conserved transcription factors that have been frequently associated with developmental disease across taxa. ODD transcription factors have been associated with kidney disease and craniofacial abnormalities in mice, humans, chicks, and other organisms. The model nematode, *Caenorhabditis elegans*, has two homologs of mammalian *odd* genes, *odd-1* and *odd-2*, which have been shown to be expressed in the gut of

developing worms. *Caenorhabditis elegans* has been identified as a robust model in the study of kidney disease and other developmental anomalies. Homologs of mammalian cystic kidney disease and cleft palate-related genes, such as the Bardet-Biedl syndrome (*BBS*) and paired-box (*PAX*) gene families, contain putative ODD binding sites in their promoters. These binding sites suggest that ODD transcription factors are involved in similar pathways in *C. elegans* and mammals. In addition, a previous study of *odd* homologs in *C. elegans* classified RNAi-mediated knockdown of *odd-1* as lethal. We report that neither enhanced RNAi-mediated knockdown of *odd-1*, nor a mutant of *odd-1* that removes most of the gene (*odd-1*(*tm848*)), cause any obvious lethality. Furthermore, we have utilized GFP reporters (JR2004 and JR2005, gifts of the Rothman lab) to further define ODD expression patterns in adult *C. elegans*. ODD-2 appears to be expressed in the posterior intestinal nuclei and in rectal gland cells, while ODD-1 appears to be strongly localized in the nucleoli of the posterior intestinal cells. With this knowledge, we aim to further elucidate the roles of *odd-1* and *odd-2* in the development of *C. elegans* and gain insight into their roles in human diseases. Financial support was provided by the Eastern Connecticut State University Biology Department, a CSU-AAUP Faculty Research Grant, and an ECSU-AAUP Jean H. Thoresen Scholarship.

Program Abstract #196

Redundancy of pax family genes in *Caenorhabditis elegans* development

Elizabeth A. Schoell, Elizabeth J. Del Buono, Dr. Amy C. Groth

Eastern Connecticut State University, USA

The *paired box* (*pax*) family genes code for transcription factors found in a variety of tissues in humans, mice, *C. elegans*, and more. There are nine *PAX* family genes in humans that are involved in embryonic development. Mutations to several members of the human *PAX* gene family result in developmental abnormalities. For example, a loss-of-function mutation in human *PAX-3* causes Waardenburg syndrome, a congenital disease causing reduced pigmentation and/or loss of hearing. Human *PAX-2* and *PAX-8* have been directly associated with congenital anomalies of the kidney and urinary tract (CAKUT) and renal cancers in humans. *C. elegans* have five *pax* gene homologs: *pax-1*, *pax-2*, *pax-3*, *vab-3*, and *egl-38*. In *C. elegans*, *pax* genes play a role in embryonic and larval development, vulval development, head and tail development, germline function, and more. PAX-2 and EGL-38 proteins have been shown to influence both somatic and germline cell death in *C. elegans*, indicating a conserved function of these transcription factors in coordinating organogenesis. However, combinations of the other *pax* family genes in *C. elegans* have not yet been studied. RNA interference (RNAi) can be used in combination with mutant strains of *C. elegans* to study the possibility of gene redundancy and phenotype enhancement of certain *pax* genes. This method has been utilized to examine the effects of multiple *pax* genes on levels of embryonic and larval lethality, slow growth, and sterility. Data indicates that *pax-3* RNAi increases lethality in a *vab-3(e1062)* mutant strain. Preliminary data suggests that *pax-3* RNAi does not increase sterility in an *egl-38(e1490)* mutant strain. We hope to elucidate interactions between all *pax* family members of *C. elegans* to uncover potential roles of *PAX* genes in human developmental pathways. This work was supported by the Eastern Connecticut State University Biology Department, a CSU-AAUP Faculty Research Grant, and an ECSU-AAUP Jean H. Thoresen Scholarship.

Program Abstract #197

Teratogens & Developmental Origins of Obesity in *C. elegans*

Caralina Marin de Evsikova, Alexei Evsikov

University of South Florida, USA

Obesity is a pandemic in the US afflicting over 78.6 million adults and 12.7 million children. One possible cause for the rise in obesity is the widespread presence of environmental teratogens acting as obesogens. Obesogens are toxicants acting in early development to shift metabolism towards positive energy balance that escalates into obesity in later life. Putative obesogens span a variety of chemical classes, such as estrogen mimics (bisphenol-A BPA, diethylstilbestrol DES), metals and metalloids (cadmium CdCl, arsenic As_2O_3), biocides (triclosan, tributyltin TBT), pesticides (fenthion FEN), and combustion pollutants (cigarette smoke CSE, nicotine NIC, benzo-α-pyrene BAP). To test the hypothesis that toxicants act in early development to alter energy efficiency at later adult stages, *C. elegans* were exposed to putative obesogens or controlthroughout the larval stages and the resultant offspring were assessed for energy balance. After parental exposure to obesogens, the resultant offspring were assessed for lipid content (area of Oil Red O staining), energy intake (pharyngeal pumping rates) and energy expenditure (thrashing assay). At sub-lethal doses, most putative obesogens shifted energy balance toward lipid storage in the F1 generation (10μM BPA, 10μM DES, 0.1μM Triclosan, 0.1μM TBT, 0.1μM FEN, 1.0μM CSE, 0.5μM BAP, 0.5μM CdCl, 0.5μM As_2O_3). In addition, many obesogens in other model organisms have been proposed to act via nuclear hormone receptors, specifically peroxisome proliferator-activated

gamma (PPARγ), to increase lipid content, albeit published studies report the nematode genome lacks PPARγ homologs. We used a "double-bait" comparative bioinformatics approach to identify potential homologs to PPARγ and identified at least 12 putative PPAR homologs between worms and humans. The results of this study establish *C. elegans* a model system for screening putative obesogens acting in early development.

Program Abstract #198

***Drosophila melanogaster* as a Model for Obesity-Induced Metabolic Syndrome and Associated Developmental Aberration**

Alice Pieplow, Melody Maniex, Karla Soto Sauza
California State University, Long Beach, USA

Obesity is the leading cause of heart disease, diabetes and stroke; as of 2010, over 60% of adults were overweight or obese in the United States alone. In *Drosophila melanogaster*, major metabolic pathways are highly conserved and well-studied, making the fruit fly an ideal model system for elucidating the molecular basis for metabolic syndrome. With recent focus on nuclear receptors as a means of understanding metabolism and nutrient sensing in vertebrates, two *Drosophila* orthologs of mammalian nuclear factors were identified for study. *Drosophila* Hormone Receptor 96 (DHR-96) and Hepatic Nuclear Factor Four (HNF4) are directly involved in the hepatic response to dietary signaling. Both DHR-96 and HNF4, as well as the transcription factor Krüppel (Kr), are expressed in the fat body of the third larval instar. The fat body is the major organ of nutrient storage and the mediator of the immune response. Using triglyceride assays we confirm that high fat diet leads to obesity in the fruit fly. We compare fertility, life span, and developmental timing in studies on the multi-generational effects of obesity. Utilizing digoxygenin-labeled antisense RNA probes and qPCR, molecular markers of development will be quantified. These markers include EIP-74EF, urate oxidase, SGS-3, and LSP-2, which will be assayed through third larval instar development to clarify the obesity-associated developmental changes observed in *Drosophila;* relative to an alpha-tubulin control. Studies utilizing a *Kr-lacZ* reporter demonstrate that expression levels of *Kr* are significantly increased in the third larval instar in response to obesity.
This project was supported by the Louis Stoke's Alliance for Minority Participation NSF grant HRD-1402873; and by the Chancellor's office, College of Natural Sciences and Mathematics, CSULB.

Program Abstract #199

***Drosophila* Clueless: a protein involved in mitochondrial biogenesis and quality control**

Rachel Cox, Aditya Sen
Uniformed Services University of the Health Sciences, USA

Mitochondria are responsible for providing ATP and other important metabolites to the cell. These organelles can manufacture excessive amounts of damaging free radicals. Most proteins used for mitochondrial function are encoded in the nucleus; mitochondrial DNA (mtDNA) encodes only thirteen proteins, 22 tRNAs and 2 rRNAs. If mtDNA accumulates mutations, this can lead to a decrease in ATP production and human disease. Over 1000 nucleus-encoded transcripts are required for the myriad of biochemical reaction carried out by mitochondria. Many mitochondria-destined nuclear transcripts are translated in the cytoplasm and imported into the organelle. However, recently, there is evidence that co-translational import also occurs, however the proteins and mechanisms involved are poorly understood. *Drosophila* lacking Clueless (Clu) have mitochondrial defects. Null mutants are sterile, have mislocalized swollen mitochondria, low ATP levels and increased mitochondrial oxidative stress. These mitochondrial defects are direct as we have shown that Clu peripherally associates with the mitochondrial outer membrane. Clu physically and genetically interacts with the Parkin-PINK1 complex, implicating it in mitochondrial quality control. Furthermore, Clu associates with Translocase of the Outer Membrane 20, a receptor for the outer membrane protein import complex. We show that *Drosophila* Clu is able to bind mRNAs, in agreement with the yeast ortholog Clu1 and human ortholog Cluh. We also find that Clu binds the ribosome and can do so at the outer mitochondrial membrane. These data support a model by which Clu localizes mitochondria-destined mRNAs for co- or site-specific translation. Furthermore, since Clu appears to also play a role in the mitophagy pathway, we believe Clu may act as a link between mitochondrial function (through protein import) and mitochondrial quality (mitophagy). Funding: R21NS085730-01A1 and USUHS BIO-71-3019 to R.T.C.

Program Abstract #200

A novel transgenic rat harboring Phox2b BAC useful for analysis ontogeny of and function of respiratory rhythm generator neuronal complex

Keiko Ikeda[1,3], Hiroshi Onimaru[2], Kiyoshi Kawakami[3]

[1]*Hyogo College of Medicine, JP;* [2]*Showa University School of Medicine, JP;* [3]*Jichi Medical University, JP*
The pivotal role of the respiratory neural center is automatic respiratory rhythm generation to maintain homeostasis. The neuronal network responsible for respiratory rhythm generation of neonatal rodent resides in the ventral side of the medulla and is composed of two groups; the parafacial respiratory group (pFRG)/ the retrotrapezoid nucleus (RTN) and the pre-Bötzinger complex group (preBötC). In the RTN/pFRG, part of the pre-inspiratory (Pre-I) neurons work as central chemoreceptor neurons, i.e., CO_2 sensitive and those neurons express homeobox gene *Phox2b*. *Phox2b* encodes a transcription factor and is essential for the development of the sensory-motor visceral circuits. Mutations in human *PHOX2B* cause congenital hypoventilation syndrome, which is characterized by blunted ventilatory response to hypercapnia. Here we describe the generation of a novel transgenic (Tg) rat harboring fluorescently labeled Pre-I neurons in the RTN/pFRG. As expected, the expression of the *Phox2b*-EYFP reporter occured in sites of endogenous PHOX2B in developing central nervous system of rat embryo. The Tg ra also showed fluorescent signals in autonomic enteric neurons and carotid bodies. It is a potentially powerful tool for dissecting the entire picture of the respiratory neural network during development and for identifying the CO_2/O_2 sensor molecules in the adult central and peripheral nervous systems.

Program Abstract #201

Using human and mouse genetics to understand congenital forebrain malformations

Andrew DiStasio, Ashley Driver, Chelsea Menke, Laura Runck, Marshall Lukacs, Kristen Sund, Elizabeth Schorry, Robert Hopkin, Donald Gilbert, Rolf Stottmann
Cincinnati Children's Hospital Med Center, USA
The mammalian neocortex is an enormous network of cells, each making thousands of connections. Many congenital structural brain defects have a genetic origin but we still lack a full understanding of the genes and mechanisms involved. We have continued to use ENU mutagenesis in the mouse to efficiently generate and capture genetic mutations in loci important for cortical development. We have also used next-generation sequencing approaches in human patients to identify mutations leading to human malformations of cortical development. We will describe our latest findings in both mouse models and human sequencing. In particular, we highlight an ongoing study in which we have performed exome sequencing on a family with two children with congenital microcephaly. An initial microarray analysis indicated a region of homozygosity which contains a recessive mutation in *COPB2, Coatomer protein complex, subunit Beta-2*. The missense mutation is in a highly conserved portion of the *COPB2* gene which is highly expressed in the proliferative cells of the mouse cortex. We have shown that reduced function of *Copb2 in vitro* through RNAi leads to reduced proliferation and abortive autophagy. We have gone on to generate an allelic series of *Copb2* in the mouse using genome-editing technologies. We conclude from these that complete loss of mouse *Copb2* is lethal prior to E8.5 whereas homozygosity for the human missense mutation is largely tolerated. Interestingly, doubly heterozygous mice carrying both a null allele of *Copb2* and an allele mimicking the human mutation do not survive to weaning and have several CNS deficits similar to the human *COPB2* patients. We are complementing these *in vitro* and *in vivo* studies with modeling *COPB2* mutations (loss of function and hypomorphic function) in human embryonic stem cells differentiated into neural progenitors and neural rosettes to recapitulate the cortical architecture.

Program Abstract #202

Generation of a mouse model for Fibrous Dysplasia caused by McCune-Albright Syndrome causing GasR201H mutation

Sanjoy Khan[1], Wantae Kim[1], Gene Elliot[2], Yingzi Yang[1]
[1]*Harvard School of Dental Medicine, USA;* [2]*NHGRI, NIH, USA*
Bone Fibrous dysplasia (FD, OMIM#174800) is a crippling skeletal disease in human caused by missense activating mutations of the *GNAS* gene, encoding the stimulatory G-protein $G\alpha_s$ that transduces signals from G protein coupled receptors. FD could result in severe adverse conditions such as bone deformity, fracture, severe pain in the bone, and ultimately lead to wheelchair confinement. In human FD lesions, bone trabeculae are abnormal in architecture, structure and mineral content. Also, the bone marrow space is devoid of both hematopoietic tissue and adipocytes and replaced with fibrotic tissue. FD is often characterized by impaired osteoblast differentiation, woven bone formation and lytic bone lesions. All these result in a mechanically incompetent and brittle bone. So far there is no cure as the molecular and cellular bases of this devastating skeletal disease remain to be elucidated. The lack of appropriate animal models for FD in McCune-Albright Syndrome has severely hampered the advancement of molecular and cellular understanding of FD. Currently available transgenic mouse models could not precisely model the disease as the activated $G\alpha_s$ is expressed from a different genomic locus driven by an artificial promoter. To this end, we have successfully generated a knock-in mouse line in which the mouse *Gnas* mutation corresponding to a human FD mutation (R201H) has been conditionally knocked

into the mouse *Gnas* locus. When crossed with early osteoprogenitor specific Cre (Prx1-Cre) line, mutant mice developed FD phenotype similar to human FD. We observed deformed bone with abnormal trabeculae and the marrow space is replaced with fibrotic tissue at 8 weeks of age. We also observed increased osteoclasts number in the bone. Our data suggest that our new knock-in model will be a very useful tool to study the molecular mechanisms underlying FD in human.
Funding sources: NHGRI intramural research, Harvard School of Dental Medicine startup

Program Abstract #203
Unidirectional Eph/ephrin signaling drives pathological cell sorting by generation of a cortical actin differential.
Jeff Bush, Audrey O'Neill, Terren Niethamer, Andrew Larson
University of California At San Francisco, USA
Boundary formation is a key developmental process controlled by Eph/ephrin signaling, and its disruption can result in congenital anomalies. Craniofrontonasal syndrome (CFNS) is a condition comprising multiple structural anomalies affecting neurological, craniofacial, and axial skeletal development. CFNS is caused by mutations in the *EFNB1* gene and subsequent perturbation of cell segregation and boundary formation. Ephrin-B1 has both ligand and receptor signaling capabilities and this bidirectional signaling has been proposed to mediate cell segregation by driving changes in cell migratory and adhesive properties. Cell segregation can also occur by changes in cortical tension driven by the actin cytoskeleton, contributing to a change in differential interfacial tension between cells, but whether Eph/ephrin signaling regulates this process is unknown. Here, we utilize mouse models and a new hiPSC model of CFNS to interrogate the cellular and molecular mechanisms of Eph/ephrin developmental boundary formation in the mammalian embryo. We find that Ephrin-B1-driven cell segregation does not depend on bidirectional signaling, but instead unidirectional kinase-dependent forward signaling is both necessary and sufficient for cell segregation. In cell culture, unidirectional signaling results in a cortical actin differential between Ephrin-B1 expressing and non-expressing cells and generation of this differential and cell segregation is dependent on Rho-kinase (ROCK). Finally, consistent with our cell culture work, we find that cell segregation *in vivo* also depends on ROCK activity, but not the activity of Rho family GTPases, Rac1 or Cdc42. Together our data suggest a new model for Eph/ephrin mediated cell segregation in which unidirectional signaling results in a cortical actin differential that drives cell segregation by modulating differential interfacial tension.

Program Abstract #204
Enpp1 regulates joint ossification through the Gas and Hedgehog signaling axis
Yunyun Jin[1], Jelena Gvozdenovic-Jeremic[2], Yingzi Yang[1]
[1]Department of Developmental Biology, Harvard School of Dental Medicine, USA; [2]National Human Genome Research Institute, National Institute of Health, USA
The cartilage in the synovial joint has to be maintained throughout life. Calcification of joint leads to osteoarthritis and digit stiffness. We have shown previously that $G\alpha_s$ inhibits Hedgehog (Hh) signaling which spatially restricts bone formation by controlling osteoblast cell fate choice of mesenchyme progenitors. The ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (Enpp1), which converts ATP to AMP and pyrophosphate outside of the cells, is essential for phosphate homeostasis. *Enpp1* loss of function results in mineralization of articular cartilage. Here we report that in the *Enpp1*$^{-/-}$ joints, Hh signaling is ectopically activated. In addition, we found that Enpp1 may regulate $G\alpha_s$-mediated GPCR signaling as loss of $G\alpha_s$ enhanced joint calcification in *Enpp1*$^{-/-}$ mutants. Attenuating Hh signaling by removing Gli2 with Prx1 Cre partially rescued the joint ossification of *Enpp1*$^{-/-}$. These findings suggest that Adenosine may signal through GPCR to regulate $G\alpha_s$-Hh signaling and this pathway plays an important regulatory role in maintaining joint integrity.
Funding sources: NIH/ NHGRI intramural, Harvard School of Dental Medicine start up funding

Program Abstract #205
Systematic dissection of the role of ABC transporters in Stem Cell Multidrug Resistance
Michele Markstein, Hannah Dayton, Jon DiRusso, Elefteria Puka, Anna Yeaton, Matthew Johnson, Edridge D'Souza
University of Massachusetts Amherst, USA
A widely observed, yet poorly understood, feature of mammalian stem cells is that they tend to express high levels of transmembrane proteins called ABC transporters that pump or “efflux” hydrophobic molecules out of the cell. This feature is shared with cancer stem-like cells, endowing them with the ability to efflux virtually every chemotherapeutic out of the cell. This stem cell associated feature renders 50% of recurring tumors in humans multidrug resistant. Understanding the roles and regulation of efflux pumps in both normal and cancer stem cells therefore holds great

therapeutic promise. However, to date, no systematic studies have been performed to understand why stem cells express high levels of ABC transporters. Here we report the discovery that *Drosophila* intestinal stem cells, like their mammalian counterparts, efflux chemotherapeutics in an ABC dependent fashion. This finding suggests that ABC mediated efflux is an evolutionarily conserved property of stem cells. We show that efflux can be visualized with fluorescent dye assays and that the efflux property alone can distinguish stem cells from their daughter cells. These assays can also distinguish younger daughter cells from older daughters. Our results explain why some chemotherapeutics are more damaging to daughter cells than neighboring stem cells, and in some instances even stimulate stem cells to over-proliferate. To expand our understanding of multidrug resistance in stem cells, we performed an RNAi screen against each of the 55 ABC transporters encoded in the *Drosophila* genome and found that individual pumps can affect stem cell survival, stem cell proliferation, and drug efflux. Based on these findings, we are now poised to conduct chemical screens using adult *Drosophila* for inhibitors of stem cell multidrug resistance.

Program Abstract #206

Combined Sry-Related HMG Box-4 (SOX4) and SOX11 signaling is required for normal renal development in vivo

Sushant Gavhale, Michel Arsenault, Blanca Esparza-Nino, Ashley Patriquen, Glenda M Wright, Sunny Hartwig
University of Prince Edward Island, CA

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of childhood end-stage renal disease. Members of the Sry-Related HMG Box-C (SOX-C) subfamily of nuclear transcription factors, including Sox4 and the closely-related Sox11, play overlapping and essential roles in controlling progenitor cell fate during organogenesis *in vivo*. We have previously demonstrated that both *Sox4* and *Sox11* are expressed in multiple progenitor cell populations during murine kidney development. Genetic ablation of *Sox4* in the nephrogenic lineage ($Sox4^{nephron-}$ mice) results in CAKUT and end stage renal failure by 5 months in vivo, demonstrating an essential and non-overlapping role for *Sox4* in kidney development *in vivo*. Here we present our preliminary findings following conditional ablation of both *Sox4* and *Sox11* in the nephrogenic lineage ($Sox4/11^{nephron-}$). In contrast to $Sox4^{nephron-}$ mice, $Sox4/11^{nephron-}$ animals die neonatally by postnatal day 1 (P1); lethality is associated with renal insufficiency (n=30) of variable presentation including bilateral renal agenesis (n=4), unilateral renal agenesis (n=2), severe hypoglomerularopathy (n=1), and widespread, cystic renal dysplasia (n=2). Characterization of adult compound *Sox4/Sox11* heterozygotes is currently underway, along with quantitative assessment of the embryonic phenotypes of $Sox4/Sox11^{nephron-}$ kidneys, including quantitative assessment of renal morphogenesis, nephron progenitor proliferation and apoptosis, nephrogenesis and marker analysis. Collectively, our work will define a novel *Sox4/11* signaling pathway that controls normal kidney development in vivo.

Acknowledgment: Funding from the Kidney Research Scientist Core Education and National Training (KRESCENT) Program and the Kidney Foundation of Canada (Sunny Hartwig's grant) are gratefully acknowledged.

Program Abstract #207

Hoxa13 in development and disease of the digestive and urogenital tracts

Marine Roux[1,2], Marie Kmita[1,2]
[1]Institut de Recherches Cliniques de Montréal, CA; [2]Université de Montréal, CA

Establishment of the body's architecture is closely linked to the activity of the Hox gene family. In mammals, they are organized in four groups or "clusters". Within each cluster, genes are arranged on the chromosome in a sequence reflecting their order of expression during development and their expression domains along the anterior-posterior axis. Hox genes of the "group 13" are expressed the most posteriorly. Accordingly, expression studies show expression of Hoxa13 in the limb, the developing gut and urogenital system. Genetic lineage analysis confirmed the contribution of Hoxa13 expressing cells and their progeny to the distal portion of the digestive tract as well as to the bladder and the ureters, in particular to the smooth muscle layers. Complete inactivation of Hoxa13 is lethal at mid-gestation due to placental defects, which has limited our understanding of Hoxa13 function. Nonetheless, observation of the Hoxa13+/-;Hoxd13-/- mutants showed a genetic interaction between the two genes in the formation of the posterior regions of the digestive and urogenital systems, suggesting an important function for Hoxa13 in the development of these organs. Using conditional inactivation, we were able to obtain viable animals to study the digestive and urogenital tracts in absence of Hoxa13. Using classical histology, immunofluorescence and in situ hybridization methods as well as genome-wide analysis of gene expression by high throughput sequencing we provide new insights into the function of Hoxa13. Finally, our data suggest that the conditional Hoxa13 mutant mouse could represent a useful model for the study of digestive and urinary tract pathologies.

Funding source: CIHR

Program Abstract #208

Wnt5a regulates apoptosis in the common nephric duct by controlling SHH signaling

Kangsun Yun

Cancer and Developmental Biology Laboratory, National Cancer Institute, National Institutes of Health, United States

Previously we reported that loss of *Wnt5a* from mouse mesoderm results in the duplication of the nephric duct (ND) and ureter and duplex kidney formation. In addition, ablation of *Wnt5a* from the ND and surrounding mesenchyme induced hydronephrosis and hydroureter following ureterocele formation but without ureter duplication. This phenotype was precipitated by the abnormal insertion of the ureter into the bladder. Here, we further investigate the cause of aberrant ureter insertion by analyzing *Wnt5a* deletion using *Dll1Cre* and tamoxifen-inducible *Osr1CreER^{T2}* mouse lines. Mutants were characterized by retarded ureter maturation and a persistent common nephric duct (CND), which normally degenerates during the maturation process. This phenotype occurred only with tamoxifen injection using *Osr1CreER^{T2}* at or before E9.5; whereas it was never observed with tamoxifen injection at E10.5. Apoptosis of the CND in mutants was dramatically decreased at E10.5, despite ND insertion into the cloacal epithelium, suggesting that regulation of apoptosis is critical for a proper ureter-bladder connection. *Shh* expression in the cloacal epithelium was increased in mutants, and loss of a single copy of *Shh* rescued CND apoptosis and the hydronephrotic phenotype, suggesting that WNT5a regulates apoptosis in the CND by controlling SHH signaling. Our work provides novel insight into the regulation of apoptosis during ureter insertion and a possible mechanism to explain the role of *Wnt5a* mutations in Congenital Anomalies of the Kidney and Urinary Tract.

Program Abstract #209

Identification of a new RNA binding protein Rbm24 that controls early eye development and is linked to the ocular defects anophthalmia and microphthalmia

Soma Dash, Salil A. Lachke

Department of Biological Sciences, University of Delaware, USA

Eye development begins in late-gastrulation and involves the activity of several transcription and signaling factors. Disruption of these developmental processes causes eye disorders such as anophthalmia (no eye) and microphthalmia (small eye) - affecting 1-3.2 in 10,000 human births, as well as cataracts (opaque lens). Indeed, several transcription and signaling genes are linked to these disorders. However, the significance of post-transcriptional gene expression regulators in eye development or their relevance to ocular birth defects is not established. Here we use a bioinformatics resource called *iSyTE* (integrated Systems Tool for Eye gene discovery) to identify a new RNA binding protein Rbm24 that functions in mammalian eye development. Immunostaining shows that Rbm24 protein is expressed early in eye development, in the optic vesicle and the lens placode. To investigate its function in the eye, we developed constitutive *Rbm24* targeted deletion mouse mutants (*Rbm24$^{Gm/Gm}$*) and undertook their phenotypic and molecular characterization. *Rbm24$^{Gm/Gm}$* embryos are small in size compared to controls, and exhibit perinatal lethality between embryonic day (E) 12.5 and E14.5 due to circulation defects. All *Rbm24$^{Gm/Gm}$* mouse embryos exhibit microphthalmia, while a subset exhibit anophthalmia. Interestingly, Sox2, a transcription factor linked to anophthalmia in humans, is severely down-regulated in *Rbm24$^{Gm/Gm}$* embryos, giving insights into the molecular basis of these defects. Further, *Rbm24$^{Gm/Gm}$*microphthalmic embryos exhibit a severe reduction in the lens epithelial and fiber cell markers E-cadherin and g-Crystallin. These data demonstrate that *Rbm24$^{Gm/Gm}$* mice exhibit defective eye and lens cell fate. Together, these findings suggest that Rbm24 deficiency impairs mammalian eye development and represent the first report of an RNA binding protein post-transcriptional regulator that is involved in the control of Sox2 expression and is linked to microphthalmia and anophthalmia.

Program Abstract #210

Characterization of Slc52a3, a riboflavin transporter expressed in hair cells and linked to human hearing loss

Elizabeth Driver[1], Stephen McInturff[1], Amy Northrop[1], Tracy Fitzgerald[2], Rani Elkon[3], Ronna Hertzano[4], Matthew Kelley[1]

[1]Laboratory of Cochlear Development, NIDCD, NIH, USA; [2]Mouse Auditory Testing Core Facility, NIDCD, NIH, USA; [3]Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Israel; [4]Department of Otorhinolaryngology, School of Medicine, University of Maryland Baltimore, USA

Differentiation and survival of the mechanosensory hair cells (HCs) of the inner ear are dependent on the transcription factor *Atoh1*. Although the importance of *Atoh1* in the development of HCs is well-established, only some of its downstream targets involved in subsequent HC differentiation have been characterized. To identify more genes involved in early HC development, as well as potential *Atoh1* targets, we compared the transcriptomes of cochlear epithelia from

Atoh1 wild-type and *Atoh1* null mouse embryos at embryonic day 15. We have identified several previously unreported HC-specific genes and determined their expression patterns in the developing inner ear. One such gene is *Slc52a3*, a recently identified riboflavin transporter. Mutations in *Slc52a3* in humans are linked to riboflavin transporter deficiency syndrome (formerly Brown-Vialetto-Van Laere syndrome), which includes juvenile onset cranial nerve degeneration and sensorineural hearing loss. We find that *Slc52a3* is expressed in all HCs of the inner ear during HC development. To examine the role of *Slc52a3* in HC function, we obtained *Slc52a3* mutant mice, and while the homozygous null mutation results in perinatal lethality, we observed no obvious defects in cochlear or vestibular HCs at that stage. To avoid the early mortality caused by the null allele, we also generated tissue-specific mutants using *Emx2-cre*, expressed throughout the cochlear epithelium. *Emx2-cre; Slc52a3*$^{fx/del}$ animals are viable, and their auditory function was assessed by ABR and DPOAE testing. Auditory testing of conditional mutants indicated that thresholds in *Emx2-cre; Slc52a3*$^{fx/del}$ animals were not significantly different from those of non-mutant litter mates. Although *Slc52a3* is clearly necessary for overall viability, it may be dispensable for HC function in mice. We will discuss potential reasons for the difference in phenotypes between mice and humans. Supported by funds to the NIDCD Division of Intramural Research.

Program Abstract #211
Mice carrying Mek1Y130C mutation present Cardio-Facio-Cutaneous phenotype
Rifdat Aoidi
Université Laval, CA
Ras-MAPK signaling pathway is one of the most investigated pathway due to it implication in various cellular processes such as proliferation, differentiation, survival and cell death. The somatic deregulation of the Ras-MAPK pathway is one of the first causes of cancer, leading this pathway to be studied in the context of oncogenesis. Germline mutation of Ras-MAPK pathway can also cause developmental syndrome named RASopathies. The RASopathies share overlapping characteristics such as craniofacial dysmorphology, cardiac malformations, cutaneous abnormalities and developmental delay. During the past decade, several RASopathies has been characterized with mutations in gene that encode members of Ras-MAPK pathway. Among them, Noonan syndrome (NS), LEOPARD syndrome, Costello syndrome (CS), Langius syndrome (LS) and cardio-facio-cutaneous syndrome (CFC). CFC syndrome is a rare syndrome which prevalence is unknown; around 300 cases have been identified. Four genes are associated with CFC: *B-RAF*, *KRAS*, *MEK1* and *MEK2*. *MEK1* and *MEK2* mutation are rarely found in CFC patients (25%), thus, few are known about the mechanistic and the origins of the observed phenotypes. Thus far, no mouse model with a mutation in *MEK1* or *MEK2* gene has been reported. Of *MEK1* and *MEK2* mutations, *MEK1*^{Y130C} is the most common. In order to investigate the molecular and developmental effect of *Mek1*^{Y130C} mutation we generated mice carrying this mutation. *Mek1*$^{+/Y130C}$ mice only present cardiac phenotype consisting in a pulmonary stenosis. *Mek1*^{Y130C} allele is suggested as a dominant mutation. In order to investigate this allele we generated *Mek1*$^{Y130C/-}$ mice. These mice present a pulmonary stenosis and they also present skull dysmorphology. We are currently investigating the *Mek1*$^{Y130C/-}$ functions using MEFs. (Supported by CIHR)

Program Abstract #212
Mechanisms of alcohol-induced holoprosencephaly in Cdon mutant mice
Mingi Hong, Robert Krauss
Icahn School of Medicine at Mount Sinai, USA
Holoprosencephaly (HPE) is a common birth defect in which bilateral symmetry of the forebrain and/or midface fails to form. HPE is associated with heterozygous mutations in the Nodal and Sonic hedgehog (SHH) pathways, but clinical presentation is highly variable, and many mutation carriers are unaffected. This scenario appears to be explained by a "mutation-plus-modifier" model. CDON is SHH coreceptor. *CDON* mutations are found in HPE patients. *Cdon* mutant mice develop HPE in a strain-dependent manner. 129.*Cdon*$^{-/-}$ mice have a sub-threshold defect of *Shh* signaling and are sensitized to HPE-modifying factors. Previously we found that, while individual loss of *Cdon* or fetal ethanol exposure does not cause HPE in 129S6 mice, the two together produce defects in early midline patterning, inhibition of *Shh* signaling in the developing forebrain, and a broad spectrum of HPE phenotypes later in development. We report here that the window of sensitivity to ethanol-induced HPE in *Cdon* mutants is very narrow and over by E7.5, prior to initial expression of *Shh*. Expression of the Nodal pathway target genes *FoxA2*, *Gsc* and *Lefty2* were decreased specifically in ethanol-treated *Cdon*$^{-/-}$ embryos. Additionally, CDON binds to Cripto, an essential Nodal coreceptor critical for gastrulation. We hypothesize that transient disruption of Nodal signaling during gastrulation by loss of *Cdon* and ethanol exposure results in subsequent defective *Shh* signaling and HPE. Much of ethanol's toxicity is ascribed to its metabolism and associated oxidative stress. We find that treatment of *Cdon* mutant mice with *tert*-butanol, which is not subject to oxidative metabolism, induces HPE and defects in Shh signaling in *Cdon*$^{-/-}$ embryos. Furthermore, antioxidant treatment

does not rescue ethanol- or *t*-butanol-induced HPE. We propose a model wherein alcohol itself, not its metabolism, is the HPE-inducing teratogenic insult and that it acts to modify Nodal signaling in *Cdon*$^{-/-}$ embryos.

Program Abstract #213
Determination of developmental phosphate transport mechanisms.
Mary Wallingford, Cecilia Giachelli
University of Washington, USA
Phosphorus is an essential nutrient required in adults and developing embryos for many functions, from bone development to cell signaling pathways. Dysregulated phosphate handling is causative of several disorders, including basal ganglia calcification, which is linked to mutations in the phosphate symporter, SLC20A2, and the phosphate exporter, XPR1. High extracellular levels of phosphate can lead to the deposition of hydroxyapatite in blood vessels, negatively impacting circulatory system function and increasing risk of cardiovascular morbidity and mortality. Overall, we aim to better understand the roles of phosphate in development, health, and disease. The study presented herein focuses specifically on phosphate handling in the placenta and embryo. We screened candidate sodium-dependent placental phosphate transporters, and identified the type III family, including Slc20a1 and Slc20a2. Importantly, clinical research has shown that placental Slc20a1 and Slc20a2 levels are reduced in severe preeclampsia. We tested the hypothesis that Slc20a1 and Slc20a2 regulate maternal-fetal phosphate transport through the use of mouse models, tissue explants, and *in vitro* systems. Knock out mice revealed specific phenotypes in vascular development. *Slc20a1* null mice were embryonic lethal, with impaired yolk sac vascular development and nutrient uptake. *Slc20a2* null mice were subviable with restricted fetal growth. *Slc20a2* null mice also had neurovascular calcification, abnormal placental vascular development, abundant placental calcification, and phenotypes reminiscent of preeclampsia symptoms. Finally, we characterized human placental calcification and identified three novel types, two of which are increased in preeclampsia with fetal growth restriction. Future work will test the hypothesis that Slc20a1 and Slc20a2 scavenge phosphate, deliver it to the developing embryo, and protect the placenta against high phosphate-induced vascular calcification during pregnancy.

Program Abstract #214
Fam172a: a new Ago2 interacting partner important for neural crest cell development.
Felix-Antoine B Simard, Catherine Bélanger, Nicolas Pilon
University of Quebec at Montreal, CA
Aberrant development of neural crest cells (NCCs) leads to multiple genetic syndromes and cancers known as neurocristopathies. However, the genetic cause and underlying pathogenic mechanism of many neurocristopathies are currently unknown. To address this, we carried out a genetic screen in the mouse based on random insertional mutagenesis. In one of the mouse lines obtained, named *Toupee*, NCC-related defects are caused by transgene insertion in the poorly characterized gene *Fam172a*, resulting in a loss-of-function allele. Since Fam172a was predicted to contain an Arb2 (Argonaute binding protein 2) domain, we first verified whether Fam172a physically interacts with Argonaute-1 (Ago1) and/or Argonaute-2 (Ago2) in different cell lines and mouse tissues using immunofluorescence and co-immunoprecipitation. These analyses revealed that Fam172a can only bind Ago2, and that this interaction is restricted to the nucleus compartment. RT-PCR analyses further revealed that some alternative splicing events known to be regulated by Ago2 are also affected in e12.5 *Toupee*$^{tg/tg}$ embryos, thereby demonstrating the functional relevance of the Fam172a-Ago2 interaction. To verify the global impact of *Fam172a* loss on the NCC transcriptome, we subsequently performed a comparative RNAseq analysis of FACS-recovered e10.5 NCCs (*Toupee*$^{tg/tg}$ vs WT; with NCCs being labeled by the G4-RFP transgene) and found that ~5000 genes are dysregulated in the mutants – at both the transcriptional and splicing levels. Among these, 145 genes known to play key roles in the NCC gene regulatory network were notably found to be downregulated. Using immunofluorescence and live cell imaging, we finally observed that these transcriptional changes negatively impact NCC survival, proliferation and migration in e10.5 embryos. In summary, we found that Fam172a is an Ago2 binding protein with a key role in the regulation of the NCC transcriptome, suggesting that *FAM172A* mutation might be a cause of neurocristopathy.

Program Abstract #215
Investigating the roles of ribosomal genes in craniofacial development and disease: Tcof1, Polr1c and Polr1d
Karla Terrazas, Annita Achilleos, George Bugarinovic, Paul Trainor
Stowers Institute, USA
Neural crest cells (NCC) are a transient, multipotent, migratory population of cells that are unique to vertebrates and give rise to an array of tissues including craniofacial cartilage and bone, and the peripheral nervous system. Many birth defects,

termed neurocristopathies, are attributed to deficiencies in NCC induction, migration, proliferation, and differentiation. To better treat and prevent neurocristopathies it is critical to understand the processes that regulate each phase of NCC development. One such process that is critical for cell survival, through its regulation of cell growth and proliferation, is ribosome biogenesis. Although this process is believed to function globally, tissue specific congenital anomalies can occur as a result of perturbations in ribosome biogenesis. These conditions are termed ribosomopathies. One such ribosomopathy is Treacher Collins syndrome (TCS), which is characterized by craniofacial anomalies including hypoplasia of the facial bones and cleft palate. Mutations in 3 genes have been identified in TCS patients: *Tcof1, Polr1c* and *Polr1d*, all of which are involved in ribosomal DNA (rDNA) transcription. TCS is also a neurocristopathy because it arises through defects in NCC development thus we are investigating the roles that *Tcof1, Polr1c* and *Polr1d* play in NCC development and survival, in ribosome biogenesis and in the pathogenesis of TCS and other ribosomopathies. More specifically, we are characterizing the genetic interactions between *Tcof1, Polr1c* and *Polr1d* and the tissue specific requirements for ribosome biogenesis and ribosomal genes during embryogenesis. Our results will aid in understanding the roles that ribosome biogenesis plays in NCC development and in the pathogenesis of congenital anomalies. This work is supported by the Stowers Institute for Medical Research, and the National Institute for Dental and Craniofacial Research.

Program Abstract #216

Modelization of Waardenburg-Hirschsprung syndrome in mice via neural crest-specific upregulation of the Nr2f1-A830082K12Rik gene pair

Karl-F. Bergeron, Baptiste Charrier, Tatiana Cardinal, Nicolas Pilon
University of Quebec At Montreal, CA

Waardenburg syndrome is a human neurocristopathy characterized by a combination of skin/hair depigmentation and inner ear defects. Waardenburg syndrome is in some cases comorbid with Hirschsprung disease, a disorder marked by an absence of neural ganglia in the distal colon triggering functional intestinal obstruction. Here, we report that the *Spot* mouse line – obtained through an insertional mutagenesis screen for genes involved in neural crest cell (NCC) development – is a model for Waardenburg syndrome cases incorporating Hirschsprung disease. Homozygous *Spot* mice at weaning age are depigmented and display spatial orientation defects and intestinal blockage, resulting respectively from lack of NCC-derived melanocytes (in the skin and vestibule) and myenteric ganglia (in the colon). Detailed examination of embryonic guts during their colonization by NCCs (via time-lapse imaging and marker analysis) revealed that the *Spot* mutation negatively impacts migration and proliferation of NCC-derived enteric neural progenitors due to their premature differentiation towards the glial lineage. Via whole genome sequencing, we localized the *Spot* insertional mutation between an overlapping gene pair – consisting of the known gliogenesis-promoting gene *Nr2f1* and the uncharacterized lncRNA gene *A830082K12Rik* – and a block of conserved non-coding sequences found to possess silencer activity in luciferase assays. RNAseq and 3C data showed that the *Spot* transgenic insertion disrupts a long-range interaction between the silencer element and the *Nr2f1-A830082K12Rik* pair, thereby leading to robust upregulation of both genes in NCCs. Other data suggest that *A830082K12Rik* is involved in the activation of *Nr2f1* transcription in *cis* and that NCC-directed overexpression of *Nr2f1* alone is enough to phenocopy the *Spot* phenotype. Altogether, our work thus points to silencer elements of *Nr2f1-A830082K12Rik* as novel candidate loci for Waardenburg syndrome and/or Hirschsprung disease.

Program Abstract #217

A Zebrafish Model of Hirschsprung Disease Identifies mapk10 as a Modifier of Enteric Nervous System Phenotype Severity

Tiffany Heanue[1], Werend Boesmans[2], Donald Bell[1], Koichi Kawakami[3], Pieter Vanden Berghe[2], Vassilis Pachnis[1]
[1]The Francis Crick Institute, United Kingdom; [2]KU Leuven, Belgium; [3]National Institute of Genetics, Japan

Hirschsprung disease (HSCR) is a common developmental abnormality of the enteric nervous system (ENS) characterized by absence of ENS ganglia in the distal colon and severe intestinal dysmotility. The genetics of HSCR is complex: while mutations in the *RET* tyrosine kinase are associated with all HSCR cases, mutations in other interacting susceptibility loci play important roles in disease presentation. Despite recent progress, the heritability and pathogenesis of HSCR and the pathophysiology of the associated gut dysmotility remain unclear. We have developed a zebrafish model for combined genetic, developmental and physiological studies. We show that zebrafish heterozygous for a *ret* null mutation are characterised by absence of ENS neurons in distal gut segments, a defining feature of HSCR pathology. Furthermore, video recording of gut motility *in vivo* showed that absence of ENS neurons induces defective intestinal peristalsis, analogous to the colonic dysmotility in HSCR patients. Additionally, individual $ret^{+/-}$ larvae show varying expressivity of

the neuronal defects, a well-established feature in the complex genetics of HSCR. To explore the developmental basis of the ENS deficits, we used novel transgenic tools and live imaging methods to show that ENS progenitors migrate at reduced speed in *ret*$^{+/-}$ animals, without changes in proliferation or survival. Finally, using gene-knock down and gene-editing techniques we show that *mapk10* activity is required for normal ENS development in vertebrates. Significantly, introduction of a *mapk10* mutation into the *ret*$^{+/-}$ background enhanced the severity of the ENS phenotype, identifying *mapk10* as a candidate HSCR susceptibility loci. Our studies identify *ret* mutant zebrafish as a powerful model for HSCR, exhibiting genetic, cellular and physiological features of the disease, providing mechanistic insight into HSCR presentation and serving as a sensitised genetic model to identify sought-after HSCR susceptibility genes.

Program Abstract #218

Characterization of Zebrafish Models of filamin C Related Cardiomyopathy

Rasha Alnefaie[1], Rene Begay[2], Luisa Mestroni[2], Matt Taylor[2], Deborah Garrity[1]

[1]Colorado State University, USA; [2]University of Colorado Denver, USA

Dilated cardiomyopathy (DCM) is a group of heart muscle diseases which leads to arrhythmia and eventually heart failure. Familial DCM is caused by mutations in about 30 known genes however, the genetic basis remains unknown for approximately 50% of DCM cases. Recently, we reported novel splicing variants in the filamin c (FLNC) gene in two cardiomyopathy-afflicted Italian families, supporting the hypothesis that that FLNC mutation is linked to DCM in humans (Begay et. al, in press). To better understand how mutations in FLNC contribute to cardiac phenotypes, we created a zebrafish loss-of-function model for two FLNC paralogous genes. Knockdown of zebrafish *flnca* or *flncb* using translation-blocking morpholinos led to dysmorphic or dilated cardiac chambers, and abnormal looping of the heart tube suggestive of systolic dysfunction. Ultrastructural analysis by transmission electronic microscopy for *flncb* morphant embryos at 48hpf indicated disorganized myofibrils with fewer consecutive sarcomeres. Particularly, z-discs were irregular or apparently absent, and numerous small vacuoles and potentially autophagous vesicles were observed. Analysis of a new *flncb* mutant identified a nonsense mutation in exon 1 (of 48 total) predicted to encode little or no FLNC protein. Homozygous mutation of *flncb* leads to arrhythmia and severely reduced contraction of the heart, as well as increasing loss of touch response in skeletal muscles, and lethality. Although many human FLNC mutations are missense alleles that encode proteins with the potential for dominant negative activity, these loss of function studies in zebrafish indicate that FLNC haploinsufficiency is sufficient for severe cardiac and skeletal muscle phenotypes that resemble MFM.

Acknowledgement**:** Colorado Clinical and Translational Sciences Institute

Program Abstract #219

Alagille Syndrome factor Jagged directly specifies duct lineage in the liver and pancreas

Keith Gates[1], Danhua Zhang[1], Michael Parsons[2], Gage Crump[3], P. Duc Si Dong[1]

[1]Sanford Burnham Prebys Medical Discovery Institute, USA; [2]Johns Hopkins University, USA; [3]University of Southern California, USA

The hepatopancreatic ductal system functions to transport hepatic bile and pancreatic enzymes. However, its role as a source of multipotent progenitors is controversial. Also unclear is the mechanism of ductal paucity in Alagille Syndrome, a disease associated with JAGGED1 and NOTCH2 mutations. Using the zebrafish model to generate viable embryos with compound homozygous mutations in two of the Notch ligand genes, we demonstrate that Jagged1b (Jag1b) and Jagged2b (Jag2b) are the ligands required for induction of all detectable canonical Notch signaling and for the specification of all intra hepatic ducts, intra pancreatic ducts, and neogenic pancreas endocrine cells during organongenesis. The acinar and hepatocyte compartments are surprisingly not lost in these double mutants, and together with results from extensive lineage tracing of Notch active cells, we conclude that duct cells do not function as a significant source of progenitors for the developing acinar and hepatocyte lineages. Further, genetic mosaic analysis suggests that insufficient Jagged signaling from endoderm derived cells can lead to failure in duct specification, a contrast to the prevailing model for duct paucity in Alagille Syndrome. This study sheds light on longstanding questions regarding the specification of the intra hepatopancreatic duct cells and their role as multipotent progenitors, and suggests an alternative mechanism for Alagille Syndrome ductal paucity.

Program Abstract #220

It's a HARS Knock Life: Elucidating the role of Histidyl-tRNA Synthetase (HARS) in auditory and visual system development

Ashley Waldron[1], Jamie Abbott[2], Susan Robey-Bond[2], Christopher Francklyn[2], Alicia Ebert[1]

[1]*Department of Biology, University of Vermont, USA;* [2]*Department of Biochemistry, University of Vermont, USA*
Enzymes called Aminoacyl tRNA Synthetases are responsible for the ligation of an amino acid to its cognate tRNA molecule. Of particular interest to us is Histidyl tRNA Synthetase (HARS), which attaches the amino acid histidine to its associated tRNA molecules. Mutations in this protein have been associated with two different neurological disorders: one being a deafness-blindness disorder and the other a peripheral neuropathy. These associations raise the interesting question of why mutations in an enzyme required for protein synthesis throughout the body would cause such tissue specific disorders. To address this question we are establishing zebrafish models of these two disorders with the intent of analyzing differences in HARS function between affected and unaffected tissues. In order to utilize zebrafish as a model for these HARS related disorders most effectively, we first needed a better general understanding of HARS in this species and so we have begun characterizing the genetics of zebrafish HARS. We have also begun preliminary studies on the function of zebrafish HARS, and have shown through gene knock-down studies that HARS may be particularly important for development and maintenance of the auditory and visual systems.

Program Abstract #221

One fish, two fish…old fish, new fish: characterization of a progeria mutant as a model of aging in the zebrafish

Chunmei Li[1,2], Christian Lawrence[1], Carrie Barton[3], Robert Tanguay[3], Matthew Harris[1,2]
[1]*Boston Children's Hospital, USA;* [2]*Harvard Medical School, USA;* [3]*Oregon State University, USA*
Aging can be defined as the progressive decline of tissue function over time and normally occurs after the organism reaches sexual maturity. While many aspects of the cellular regulation of aging have been defined, we still do not understand the global regulation of aging such as mechanisms that restrict the onset and expression of aging phenotypes. Zebrafish is wildly used as a model to understand the genetic basis of vertebrate early development and disease, however, little attention has been paid to late phenotypes including aging. Here, we asked if we could use the zebrafish as a tool to interrogate the timing of senescence and the molecular basis of aging. In large-scale screens for adult skeletal phenotypes, we isolated a progeric mutant, *freurentner* (*frnt*), that exhibits precociously aging phenotypes arising during juvenile stages. Homozygous *frnt* has severe defects in several tissues, many having high proliferative or metabolic demands. Histological analysis shows effects of *frnt* on stem cell maintenance, suggesting stem cell deprivation might be the cause for the aging phenotype. We have defined calorie restriction and NAD+ modifying regimens in the zebrafish that are sufficient to attenuate longevity and behavior defects associated with *frnt*. Molecular analyses suggest that rescue may act through alteration of Sirt1 activity and feedback regulation of redundant pathways. Through genetic mapping and generation of allele series of *frnt*, we have identified potential candidates for altered gene function underlying *frnt*. We are functionally verifying activity of these gene candidates in aging zebrafish and association in human aging as well. The identification of the *frnt* model of aging in the zebrafish broadens our knowledge on how aging is genetically and developmentally regulated. Further this model provides unique tools to capitalize on the power of genetic screens in the zebrafish to interrogate the molecular and physiological regulation of aging.

Program Abstract #222

Zebrafish pih1d3 mutants display a primary ciliary dyskinesia-like phenotype

Santeri Kiviluoto, Shiaulou Yuan, Zhaoxia Sun
Yale School of Medicine, United States of America
Primary ciliary dyskinesia (PCD) is a genetically heterogenous congenital disorder that affects motile cilia. PCD manifests as chronic infections of the respiratory tract and is often accompanied with infertility and abnormal situs of heart, liver, intestines or spleen. Motile cilia typically have a 9+2 architecture with nine outer and two inner microtubule doublets. The anchoring dynein arms that move along the microtubules to produce ciliary movement are frequently affected in PCD. However, many of the genes that cause PCD remain elusive. Zebrafish offer attractive advantages over many other models for studying PCD. The relatively quick development, transparency, and genetic tractability of embryos allow us to extract genes that are involved in the development of the disorder. In a large viral insertional mutagenesis screen in zebrafish, we identified a mutant, pih1d3$^{hi1392Tg/hi1392Tg}$ that exhibits several phenotypes reminiscent of PCD. Within three days post-fertilization the homozygous mutants display randomized cardiac looping, cystogenesis within the glomerular-tubular region of the pronephric kidneys, and ventral body curvature, a typical marker for ciliary dysfunction in zebrafish. High-speed differential interference contrast microscopy revealed that motile cilia of the olfactory bulb and pronephric ducts are essentially immotile. Interestingly, these phenotypes could be partially rescued by injecting embryos with human PIH1D3 mRNA, indicating that the function of the protein is conserved. This further suggests that PIH1D3 could be involved in PCD in humans.
This research was funded by the NIH (R01HL125885-01A1).

Program Abstract #223

The roles of RNA Polymerase I and III subunits Polr1c and Polr1d in zebrafish models of Treacher Collins syndrome

Kristin Watt[1,2], Annita Achilleos[1], Cynthia Neben[3], Amy Merrill[3], Paul Trainor[1,2]

[1]Stowers Institute for Medical Research, USA; [2]University of Kansas Medical Center, USA; [3]University of Southern California, USA

Ribosome biogenesis is a global process required for growth and proliferation in all cells, and disruptions in this process result in tissue-specific disorders termed ribosomopathies. Ribosome biogenesis begins with the transcription of ribosomal RNA (rRNA) by RNA Polymerases (Pol) I and III, however little is known about the specific functions of individual RNA Pol subunits during embryonic development. Mutations in *POLR1C* and *POLR1D*, which are subunits of RNA Pol I and III, cause Treacher Collins syndrome in humans, a ribosomopathy characterized by malformation of the facial bones. We discovered that *polr1c* and *polr1d* are dynamically expressed during zebrafish embryonic development and play important roles specifically in craniofacial development. *polr1c* and *polr1d* loss-of-function mutants exhibit diminished 45S rRNA transcription and reduced ribosome production. This leads to Tp53-dependent neuroepithelial apoptosis and a diminished population of neural crest cells, which are the precursors of the craniofacial skeleton. Interestingly, genetic inhibition of *tp53* can ameliorate the skeletal deficiencies in *polr1c* and *polr1d* mutant zebrafish. Our studies therefore provide new information and a deeper understanding of the role of Pol I and Pol III subunits during craniofacial development and in the pathogenesis of Treacher Collins syndrome. Furthermore, these zebrafish models will be useful for understanding the importance of ribosome biogenesis in specific tissues during embryogenesis.

Funding for this research was provided by the Stowers Institue for Medical Research.

Program Abstract #224

Histidyl-tRNA synthetase mutation linked to peripheral neuropathy has multiple conformations

Jamie Abbott, Christopher Francklyn

University of Vermont, USA

Damaged nerves of the peripheral nervous system can cause distal muscle weakness and sensory loss, peripheral neuropathy. There are over 40 identified loci that link genetic defects to peripheral neuropathy, six of which encode for aminoacyl-tRNA synthetase (aaRS) enzymes. Many studies have revealed these aaRS mutations result in reduced cell viability and loss of primary aminoacylation function. Histidyl-tRNA synthetase (hHARS) is a ubiquitously expressed aaRS required for protein synthesis. hHARS catalyzes the addition of histidine to histidyl-tRNA in a two-step reaction that consists of amino acid activation followed by aminoacylation. Recent case studies have linked heterozygous mutations in the hHARS gene to peripheral neuropathy. One such mutation encodes an R137Q substitution, which alters a conserved salt bridge interaction in the dimer interface. R137Q hHARS exerts dominant lethality in yeast and leads to aberrant commissural axonal processes and locomotor defects when expressed in *C. elegans*. While R137Q hHARS is severely reduced in amino acid activation, this defect and aminoacylation overall are partially rescued by tRNA. Based on differential scanning fluorimetry experiments, the T_m for R137Q is 5 ºC lower than WT hHARS. Surprisingly, analytical ultracentrifugation data indicate that WT and R137Q hHARS possess similar Svedberg values but different frictional coefficients. Thus, R137Q hHARS can retain dimeric structure while exhibiting different shape conformers. We are currently using PC12 cells as a neuronal model to determine how R137Q elicits a toxic gain of function phenotype after differentiation. Proteomic approaches will be used to identify protein-protein interactions of WT and R137Q hHARS in a neuronal cell context. With these approaches, we seek to understand the biochemical reasoning behind how R137Q specifically damages the peripheral nervous system.

Funding Source: NIHGM54899

Program Abstract #225

The impact of metabolic intermediates on the crosstalk between glycosylation and acetylation of histones in glioblastoma

Maria Cecília Nunes[1], Ana Luiza Barbeitas[2], Mariana Cabanel[1], Wagner Dias[1], Katia Carneiro[1]

[1]Federal University of Rio de Janeiro, BR; [2]Federal University of Rio de Janeiro State, Brazil

The epigenetic profile of genes involved in embryonic development has emerged as a potential mean to better understand events of cellular fate decision and behavior. In this context, tumor development characterizes an interesting field of study, since it could recapitulate embryonic development, except for the lacking of an extremely instructive environment such as the developing embryo. Our group recently demonstrated that cancer stem cells development recapitulates some specific embryonic pathways towards tumor differentiation. Metabolic intermediates from diet constitute histone post

translational modifications (PTM), through different enzymes and its catabolic reactions such as acetyl and O-linked N-acetylglycosamine (O-GlcNAc). Since cancer is a disease with disrupted metabolism, the intersection between different PTM in histones emerges as a tractable readout on the metabolic status of cancer cells and its impact on cancer genetics. Interestingly, O-GlcNAc modification aroused as a new component of the histone code and has been already shown to display a cross-talk with other PTM. However there is a lack of information focusing on the relevance of this PTM on glioblastoma (GBM) development. For this reason, we started to characterize the pattern of protein glycosylation found in GBM cells under hyper-acetylation and hyper-glycosylation conditions. Interestingly, hyper-glycosylated cells presented cell cycle arrest associated to an increased viability. On the other hand, hyper-acetylation blocks this protective role of glycosylation in tumor cells leading to cell cycle arrest and apoptosis. In accordance, protein and histone glycosylation profile is disrupted in the hyper-acetylated background, indicating a crosstalk between these two PTM in histone in cancer cells. Thus, our data suggest that the balance between glycosylation and acetylation plays a key role in GBM cells metabolism and physiology.
Financial support: FAPERJ/CAPES/CNPq

Program Abstract #226
Hnf4a is a master gene that can generate columnar metaplasia in oesophageal epithelium
Jonathan Slack, Benjamin Colleypriest, Zoe Burke, Leonard Griffiths, Mark Farrant, David Tosh
University of Bath, GB
Barrett's metaplasia is the only known precursor to oesophageal adenocarcinoma and is characterized by replacement of stratified squamous epithelium by columnar epithelium with intestinal characteristics. The underlying molecular mechanisms responsible for the change in cellular phenotype are poorly understood. We have therefore explored the role of two transcription factors, CDX2 and HNF4α in the conversion. First, we demonstrate the expression of CDX2 and HNF4α in human biopsy samples of Barrett's metaplasia. Second, a new organ culture system for adult murine oesophagus is described which expresses markers characteristic of the normal stratified squamous epithelium: p63, K14, K4 and loricrin. These cultures were transduced with adenovirus expressing *HNF4α* and *Cdx2*, and the phenotype following infection was determined by a combination of PCR, immunohistochemical and morphological analyses. We find that ectopic expression of *HNF4α*, but not of *Cdx2,* induce a columnar morphology with expression of the intestinal markers *Tff3*, *villin,* K8 and E-cadherin. Since *HNF4α* is present in the human condition and is sufficient to induce a columnar phenotype in adult mouse oesophageal epithelium, these data suggest that upregulation of *HNF4α* is a key early step in the formation of Barrett's metaplasia.

Program Abstract #227
Deletion of platelet derived growth factor receptor alpha in urorectal mesenchyme caused anorectal malformations
C Qian, RCL Ng, ZL Wu, PKH Tam, VCH Lui
Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong
In mammals, urorectal development starts at early embryonic stage, defective urorectal development results in anorectal malformations (ARMs), which are common congenital developmental defects of the anus and the urethra in newborns. Although multiple genes and signaling pathways have been implicated in the pathogenesis of the ARMs, the etiology and embryology of ARMs are still largely unknown. Platelet derived growth factor receptor alpha (*Pdgfra*) is a cell surface receptor tyrosine kinase, upon binding to its ligands (*Pdgfa-d*), mediates intracellular signaling and regulates embryonic development. We have characterized that the expression of Pdgfra is tightly regulated in the developing urorectal mesenchyme, and its dysregulation is associated with urorectal defects in animals with ARMs. Knockout of *Pdgfra* induces early embryo lethality which precludes investigation of *Pdgfra* in urorectal development. We conditionally deleted *Pdgfra* in urorectal mesenchyme in mice at different embryonic stages, and investigated the development of the anus and the urethra of these mutants. Both female and male mutant embryos exhibited ARMs-like phenotypes including the incomplete cloaca septation, anteriorly displaced anus, defective development of the anal folds and the urethra. We also detected elevated apoptosis of the urorectal mesenchyme in mutants, which could explain the urorectal defects of the mutants. In conclusion, we demonstrated a temporal and spatial requirement for *Pdgfra* mediated signaling in the urorectal development, and its dysregulation could cause urorectal developmental defects in resemblance of ARMs in human.
Funding: This work is supported by HK RGC HMRF (Project No.: 02132306) to VCH Lui

Program Abstract #228

HSF1 critically attunes proteotoxic stress sensing by mTORC1 to combat stress and promote growth

Kuo-Hui Su[1], Junyue Cao[1], Zijian Tang[1,2], Siyuan Dai[1], Yishu He[1], Stephen Byers Sampson[1], Ivor J. Benjamin[3], Chengkai Dai[1]

[1]The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA; [2]Graduate programs, Department of Molecular & Biomedical Sciences, The University of Maine, 5735 Hitchner Hall, Orono, Maine 04469, USA; [3]Division of Cardiovascular Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA

To cope with proteotoxic stress, cells attenuate protein synthesis. However, the precise mechanisms underlying this fundamental adaptation remain poorly defined. Here we report that mechanistic target of rapamycin complex 1 (mTORC1) acts as an immediate cellular sensor of proteotoxic stress. Surprisingly, the multifaceted stress-responsive kinase c-JUN N-terminal kinase (JNK) constitutively associates with mTORC1 under normal growth conditions. On activation by proteotoxic stress, JNK phosphorylates both regulatory-associated protein of mTOR (RAPTOR) at Ser863 and mTOR at Ser567, causing partial disintegration of mTORC1 and subsequent translation inhibition. Importantly, heat shock factor 1 (HSF1), the central player in the proteotoxic stress response (PSR), preserves mTORC1 integrity and function by inactivating JNK, independently of its canonical transcriptional action. Thereby, HSF1 translationally augments the PSR. Beyond promoting stress resistance, this intricate HSF1-JNK-mTORC1 interplay, strikingly, regulates cell, organ and body sizes. Thus, these results illuminate a unifying mechanism that controls stress adaptation and growth.

Funding sources: The Jackson Laboratory Cancer Center Support Grant (3P30CA034196), and grants from the NIH (1DP2OD007070) and the Ellison Medical Foundation (AS-NS-0599-09).

Program Abstract #229

FOXF2 is a direct mesenchymal target of Hedgehog signaling during upper lip development and cleft lip pathogenesis

Josh Everson[1], Joon Won Yoon[2], Dustin Fink[1], Henry Kietzman[1], Robert Lipinski[1]

[1]University of Wisconsin - Madison, USA; [2]Northwestern University Feinberg School of Medicine and Children's Memorial Research Center, USA

The Hedgehog (Hh) signaling pathway is a conserved morphogenetic regulator of brain and face development. We have previously shown that *in utero* exposure to the Hh pathway inhibitor cyclopamine results in cleft lip with or without cleft palate (CL/P) in the mouse. Here, we aimed to identify Hh target genes involved in the initial pathogenesis of CL/P. DNA microarray networking and clustering analysis of significant differentially expressed genes revealed the Forkhead box (Fox) family of transcription factors as a broad, downstream target of Hh signaling during CL/P pathogenesis. Nine individual Fox gene family members (*Foxb2, Foxc1, Foxc2, Foxd1, Foxd2, Foxe1, Foxf1, Foxf2,* and *Foxl1*) were downregulated in cyclopamine-exposed embryos. *In situ* hybridization confirmed each of these Fox genes is co-expressed with the canonical Hh target gene *Gli1* in the neural crest-derived mesenchyme of the tissues that form the upper lip. *Foxf2* variants in humans were recently reported to be associated with CL/P, compelling us to further examine its regulation and role in lip development. *Foxf2* expression was directly induced by Hh ligand stimulation in a cranial neural crest cell line, while electrophoretic mobility shift assays confirmed a functional GLI1 binding site 834 bp downstream from *Foxf2*. The role of *Foxf2* in mediating proliferation and differentiation of neural crest-derived mesenchyme is currently being examined. Our study is the first to identify multiple Fox family members as targets of Hh signaling during the initial pathogenesis of CL/P. Functional assessment of these candidate human orofacial cleft genes in normal and abnormal development is a promising avenue to elucidate the complex etiology this common and morbid birth defect.

This work is supported by R00DE022010-02 and JE by T32ES007015-37

Program Abstract #230

Elucidating the Structure and Organization of the Transcription Factor p53 Tetramer Bound to Host Cellular Proteins with Cryo-EM.

Reginald McNulty, Alexander S. Krois, H. Jane Dyson, Peter E. Wright

TSRI, USA

Intrinsically disordered proteins (IDP's) are incompletely structured or contain disordered regions in solution yet remain perfectly functional. IDP's play a role in many biological processes, including regulation of senescence, the cell cycle, transcription activation, and metabolic signaling. The tumor suppressor p53, an IDP with functionally relevant disordered regions at its N- and C-termini, is a transcription factor that prevents cancer by promoting cell death. It interacts with a plethora of macromolecular complexes and is subject to substantial post-translational modifications which all influence its

structure and function. Although there are crystal structures for isolated domains of p53, it remains to be determined how the p53 tetramer forms complexes with cellular proteins. To deal with intrinsic disorder and heterogeneity, interactions of tetrameric p53 with portions of Creb-binding protein (CBP) have been examined with cryo-electron microscopy (Cryo-EM). The domain organization within the tetramer is visible in many orientations via 2D class averages. The structural information obtained via cryo-EM will enable us to probe the determinants of recognition and contribution of different residues in p53 binding. This work is funded by a Ruth L. Kirchstein NIH NRSA fellowship 3F32GM108310 (RM) and a NCI grant 5R01 CA096865 (PW).

Program Abstract #231

The availability of the embryonic TGF-ß protein Nodal is dynamically regulated during glioblastoma multiform tumorigenesis through an endocytic pathway

Maria Cecilia Oliveira-Nunes[1], Suzana Kahn[2], Ana Luiza Barbeitas[1], Willian Querido[1], Tania Spohr[1,3], Luiz Gustavo Dubois[1,3], Grasiella Ventura[1], Loraine Campanati[1], José Marques Brito[1], Flavia Regina Lima[1], Vivaldo Moura-Neto[3], Katia de Paula[1]

[1]Federal University of Rio de Janeiro, BR; [2]Stanford University, Institute for Stem Cell Biology & Regenerative Medicine, USA; [3]Instituto Estadual do Cérebo Paulo Niemeyer, Brazil

It is increasing the number of evidences showing that genes classically described performing critical roles during embryogenesis are also expressed during cancer development and progression, meant to be reactivated during tumorigenesis. Indeed, it has been shown that the embryonic microenvironment is able to reprogram multipotent cancer cells and in this scenario oncogenesis can be considered as a recapitulation of embryogenesis, except by the lack of its embryonic context including the microenvironment and its signaling molecules. In this sense, a lot of work has been investigating the similarities that might exist between embryonic development and cancer development. For instance, Glioblastoma (GBM) is the most common primary brain tumor presenting self-renewing cancer stem cells which are meant to recapitulate programs from embryogenesis. Among embryonic signaling molecules, the TGFβ-superfamily member Nodal has a preeminent role during both animal and cancer development. In the present work we investigated the mechanisms that dynamically control Nodal availability during GBM tumorigenesis in both stem (GBMsc) and more differentiated GBM cells (mdGBM) through morphological analysis and immunofluorescence of Nodal protein and of early and late endocytic markers. While Nodal-positive vesicle-like particles were symmetrically distributed in GBMsc they presented asymmetric perinuclear localization in mdGBM. Strikingly, when subjected to dedifferentiation, the distribution of Nodal in mdGBM shifted to a symmetric pattern. Moreover, the availability of both intracellular and secreted Nodal were downregulated upon GBMsc differentiation. Interestingly, the co-localization of Nodal with endosomal vesicles also depended on the differentiation status of the cells. These results shed light on a new approach to investigate tumorigenesis under the concepts first raised in developmental biology and that may emerge as suitable targets for GBM therapy.

Program Abstract #232

Investigation of Nicotine Exposure on Wound Response in *Drosophila*

Michelle Juarez, Chrissy Cherenfant

City College New York, USA

Our body has many cellular functions to maintain homeostasis. One of these cellular functions is the mechanism of cellular repair and wound response. When localization of a wound response occurs only in damaged tissue, the body is then able to recover from injury such as, surgery or disease. Wound response can be visualized in *Drosophila* embryos, which are 75% genetically similar to humans, by fluorescent wound reporter genes such as, *tyrosine hydroxylase (ple*) and *Dopa Decarboxylase (Ddc*). These genes each display their respective phenotypes when activated, being local or global, in epidermal cells. A localized wound response results from a "control" puncture injury. The local gene activation is limited to the cells surrounding the site of damage. A global response may result during "experimental" microinjection of chemicals. The global gene activation is unlimited and spreads beyond the cells surrounding the site of damage. While acknowledging wound response and its role in maintaining cellular balance, we must also be aware that external factors may affect wound response. The external factor that is specifically under investigation is nicotine and its affect on wound response in *Drosophila* embryos. Our hypothesis is that nicotine exposure to *Drosophila* embryos will activate a global response after puncture injury. Determining the affect of nicotine exposure on wound response in *Drosophila* will provide insight into nicotine's impact on localization and wound repair. We may gain improved understanding of how external factors and human actions, like smoking, may ultimately affect healing after injury. Future experiments will focus on

genetic components of nicotine signaling and wound response. Using studies in *Drosophila*, as a model organism, will provide new directions for clinical studies to improve recovery in humans following tissue damage.

Program Abstract #233
Exploring the mechanics of tissue separation behaviour at Brachet's cleft during gastrulation in *Xenopus laevis*
Debanjan Barua
University of Toronto, CA
Boundary formation between two distinct tissues is a crucial yet still poorly understood developmental process. In contrast to boundaries where cells are intimately attached, cleft-like boundaries allow for movement of tissues while preventing intermixing of the tissues. During gastrulation of the anuran *Xenopus laevis*, involuting mesoderm cells migrate across the ectodermal blastocoel roof through repeated cycles of attachment and detachment mediated by anti-parallel ephrin/Eph forward signaling between the two tissues. This leads to the formation of a cleft-like boundary called Brachet's cleft. Perturbation of molecular factors within the ectoderm and mesoderm tissues, such as: ephrins/Ephs, xC-cadherin and PAPC, can lead to the abolishment of the cleft. Although key molecular factors that play a role in the formation and maintenance of Brachet's cleft have been well-characterized, less is known about their effects on the mechanics underlying this design. Here I used transmission electron microscopy to explore intercellular contacts at the boundary as a way to quantitatively analyze the behavior of the ectoderm and mesoderm tissues at the cleft. I found that boundary cells exhibit close contacts at intercellular distances compatible with cadherin mediated adhesion, intermediate contacts at much greater distances which may be regulated by the ECM, and large gaps, all of which under normal conditions are maintained at a particular ratio. Disruption of the aforementioned molecular factors lead to drastic changes in this ratio between opposing tissues at the cleft. I propose that abolishment of the cleft leads to increased close contacts between opposing boundary cells which correlates with increased cadherin-mediated attachment between these cells. These findings emphasize the importance of establishing a link between the molecular and mechanical aspects of boundary formation during gastrulation.

Program Abstract #234
A Feature-Independent Method for the Analysis of Calcium Activity Time Series in Neural Development
John Marken, Andrew Halleran, Caroline Golino, Michael LeFew, Laura Odorizzi, Atiqur Rahman, Peter Kemper, Margaret Saha
College of William and Mary, USA
Calcium ions are essential messengers that perform diverse functional roles through distinct activity patterns across all phyla. Calcium activity is particularly important during the development of the nervous system where it has been shown to play a role in neural induction, phenotype specification, and synaptogenesis. Currently, the analysis of calcium activity time series is restricted to feature-dependent algorithms. These algorithms force the experimenter to make *a priori* assumptions about the characteristic shapes of features in their data, such as arbitrary amplitude thresholds for what constitutes a calcium spike. While such methods may be suitable for systems like the mature nervous system where features tend to closely follow a well-defined form, they are ill-suited for analyzing the developing nervous system as it displays a much wider range of features in its calcium dynamics. We present a simple, easy-to-use feature-independent calcium analysis method that bypasses the constraints of a feature-dependent method. We then apply our method to developing neuronal tissue from *Xenopus laevis* and find that the information entropy of a cell's calcium activity decreases as the cell matures. We pursue these results by investigating the relationship between the information entropy of a presumptive neuronal cell's calcium activity and its eventual phenotype.
Funding provided by NSF Grant 1257895 and NIH Grant 1 R15 NS067566 to MS.

Program Abstract #235
Neuromast Deposition and Patterning in the Zebrafish Lateral Line Primordium Described by Complementary Agent-based Models
Damian Dalle Nogare, Ajay Chitnis
NIH/NICHD, USA
Formation of the zebrafish Posterior Lateral Line (PLL) system is pioneered by the PLL primordium (PLLp), a group of cells that migrates along the horizontal myoseptum periodically depositing neuromasts. Establishment of polarized Wnt and FGF signaling systems in the PLLp coordinates morphogenesis of the PLLp. Wnt signaling, most active toward the leading end, promotes its own activity. At the same time it drives expression of both FGF ligands and FGF signaling inhibitors. This prevents leading cells from responding to the FGFs. Instead, they initiate FGF signaling at the trailing end,

where Wnt signaling is low. Activation of FGF receptors initiates expression of Wnt antagonists that help establishment of a stable FGF signaling center that coordinates formation of a protoneuromast by promoting morphogenesis of epithelial rosettes with a central hair cell progenitor. Over time the Wnt system shrinks and more protoneuromasts form in its wake. Proliferation adds to growth, nevertheless, as the PLLp migrates, cells incorporated into protoneuromasts are deposited from the trailing end as neuromasts, the rest are deposited in between as interneuromast cells, and the PLLp progressively shrinks. Throughout, however, the length of the migrating PLLp correlates with the size of the leading Wnt system. Using data related to initial size, proliferation rate, speed and the rate at which the Wnt system shrinks, we have built an agent-based model that effectively predicts how far the PLLp migrates, how may neuromasts it deposits and what their average spacing is. But what ties PLLp length to the size of the Wnt system? We provide an answer to this question with a different agent-based model that explores behavior of the Wnt-FGF signaling network. It shows how local activation of Wnt signaling, coupled with its long-range inhibition by FGF signaling, determines specification of a central hair cell progenitor and periodic formation of protoneuromasts in the migrating PLLp.

Program Abstract #236

Imaging the implanting embryo and the uterine environment in three-dimensions

Ripla Arora, Khalida Sabeur, Karina Oelerich, David H Rowitch, Linda C Giudice, Diana J Laird
University of California, San Francisco, USA

Although much is known about the embryo and changes that surround implantation, the structural uterine environment in which the early embryo develops is not well understood. By employing confocal imaging in combination with 3D image analysis we identify and quantify dynamic changes in murine uterine luminal structure in preparation for implantation. We extend this technique to image and develop 3D renderings of a full thickness segment of cycling human endometrium. When used to analyze mouse mutants in the non-canonical Wnt signaling pathway with known molecular implantation defects, we uncovered striking abnormalities in uterine structure at the time of implantation. Our imaging also identified stereotypical reorientation of mouse uterine glands towards the site of implantation. Analyzing the uterine and embryo structure in 3D for different genetic mutants and pathological conditions will help uncover novel molecular and structural pathways involved in successful implantation, uterine endometrial decidualization and placentation of the embryo.
Funding support: CIRM TG2-01153, UCSF PBBR and NIH T32HD007263 to RA, P50HD055764 to LCG and NIH 1DP2OD007420 to DJL

Program Abstract #237

Imaging how cells decide their fate, shape and position in live mouse embryos

Nicolas Plachta
*IMCB, A*STAR (Singapore), SG*

We first establish quantitative imaging techniques to show how transcription factors re-partition between specific and non-specific DNA binding sites, as cells decide their fate in live mouse embryos. We discover that differences in the binding of Sox2 to specific DNA sites 1) appear as early as the 4-cell stage, 2) are regulated by histone methylation and 3) predict cell fate. We then show that as cell choose their fates, they also extend long filopodia protrusions to pull their neighbor cells closer, revealing a mechanism for embryo compaction. Finally, we develop membrane segmentation and laser ablation methods to show how anisotropies in tensile forces generated by the acto-myosin cortex drive some cells inside the embryo to form the pluripotent inner mass.

References:

White et al (2016) *Cell*
Samarage et al (2015) *Dev Cell*
Fierro-Gonzalez et al (2013) *Nat Cell Biol*
Kaur et al (2013) *Nat Commns*

Program Abstract #238

The Rac/Cdc42 exchange factor beta-Pix is required for neural epithelial organization and collective mesoderm migration in early mouse embryo

Tatiana Omelchenko, Alan Hall, Kathryn Anderson
Memorial Sloan Kettering Cancer Center, USA

During embryonic development, cell movements and cell-cell adhesion mediated by spatially localized activation of Rho GTPase signaling is required for the formation of tissues and organs. However the cellular and biochemical basis of how cells self-organize and coordinate their behaviors in mammalian embryosis poorly understood. We previously showed that

beta-Pix, a small Rho GTPase Rac/Cdc42 exchange factor, is required for polarized protrusive activity and collective epithelial migration of the extraembryonic endoderm, which is required for establishment of the anterior-posterior body axis. Here, using live imaging of early mouse embryos expressing fluorescent reporters, we identify essential roles for beta-Pix in formation of the neural epithelium and in mesoderm migration. Deletion of beta-Pix in the epiblast (the embryo proper) blocks embryonic morphogenesis leading to early lethality at E8.5. The cranial neuroepithelium of epiblast-deleted *beta-Pix* embryos has abnormal folds with ectopic lumens associated with ectopic apical markers; live imaging shows that the formation of ectopic lumens is associated with inappropriate cell division. In the mesoderm, beta-Pix promotes directionality and persistence of migration. Intriguingly, beta-Pix null mesodermal cells show increased motility but not directional migration, leading to widening and thickening of the mutant embryo. Analyses using beta-Pix null MEFs and mesoderm explants define beta-Pix GEF-dependent and independent mechanisms of mesoderm migration. These studies provide cues to understanding mechanisms of collective cell behaviors that are disrupted in developmental diseases and in cancer.
The work was supported by National Institutes of Health (NIH) grants GM081435 and CA008748 (to AH) and NIH HD035455 to KVA.

Program Abstract #239
Single-cell analysis of progenitor and endocrine cells in the developing pancreas
Yung Hae Kim[1], Hjalte List Larsen[1], Pau Rué[2], Alfonso Martinez-Arias[2], Anne Grapin-Botton[1]
[1]University of Copenhagen, Denmark; [2]University of Cambridge, United Kingdom
Organogenesis relies on the spatiotemporal balancing of progenitor proliferation and differentiation of their progeny. We investigate how single cell decisions contribute to global organ growth and differentiation during pancreas development. In this organ, endocrine cells arise from NEUROG3^{+} endocrine progenitors, which emerge from pancreatic progenitors. Using 3-D live imaging and in vivo clonal analysis, we have previously shown that developing mouse pancreas balances progenitor expansion and endocrine differentiation by 3 modes of division: self-renewal of progenitors, asymmetric and symmetric endocrinogenic divisions. At E14.5 when beta-cell differentiation is initiated, endocrine differentiation occurs with a probability of 20%, and our data and mathematical model suggest that the timing of stochastic endocrine specification during the cell cycle of pancreatic progenitors determines asymmetric versus symmetric endocrine cell emergence. To further examine transcriptional progression of endocrine differentiation from pancreatic progenitors in the E14.5 pancreas, we sorted pancreatic progenitors and endocrine cells using lineage-specific reporter lines and performed single-cell PCR. Our analysis shows lineage trajectories within the E14.5 pancreas, as well as clustering of lineage-specific factors and cell cycle markers particularly in expanding progenitors. The data also indicate expression of specific Notch and Wnt pathway transcripts at specific stages. Single-cell RNA sequencing is ongoing to analyze pancreatic cells in an unbiased manner, and discover novel factors in different stages of endocrine differentiation. Our study brings our understanding of endocrine differentiation dynamics to a single cell level and provides insights to optimize the generation of β-cells *in vitro* as a therapeutic strategy to treat diabetes mellitus.

Program Abstract #240
Single Cell Muscle Sequencing of Schmidtea Mediterranea to Define Key Regulatory Players in the Regenerating Mediolateral and Dorsoventral Axes
Jennifer Cloutier[1,2,3], Isaac Oderberg[1,2], M. Lucila Scimone[1], Kwadwo Owusu-Boaitey[1,2,3], Peter Reddien[1,2]
[1]Whitehead Institute, USA; [2]Massachusetts Institute of Technology, USA; [3]Harvard Medical School, USA
Planarians (*Schmidtea mediterranea*) are an established, powerful model system to study regeneration. Planarians have the capacity to fully regenerate because of the presence of dividing cells called neoblasts. In order to properly regenerate missing tissues, neoblasts can not only specify into differentiated cell types but also require positional information to determine which tissues to form. Muscle cells express genes regionally along the different animal axes, termed position control genes (PCGs), which provide this positional information. Several of these genes are required for proper patterning of the different regions of the animal along the anteroposterior axis. However, which genes are required for establishing and maintaining the mediolateral and dorsoventral axes is less well understood. Here, we have devised a single-cell sequencing approach to interrogate this question and will discuss our progress on characterizing putative PCGs that regulate these two axes using RNA interference.

Program Abstract #241
Whole organism lineage tracing by multiplex in vivo genome editing
James Gagnon[1], Aaron McKenna[2], Greg Findlay[2], Marshall Horwitz[2], Jay Shendure[2,3], Alexander Schier[1]

[1]*Harvard University, USA;* [2]*University of Washington, USA;* [3]*Howard Hughes Medical Institute, USA*
Multicellular organisms develop from single cells by way of a lineage. The invariant cell lineage of the roundworm *C. elegans* was determined by visual observation, but tracing cell lineage in nearly all other multicellular organisms is vastly more challenging. Contemporary methods rely on genetic markers or somatic mutations, but these approaches have limitations that preclude their application at the level of a whole organism. Here we introduce genome editing of synthetic target arrays for lineage tracing (GESTALT) as a means of recording cell lineage relationships in complex multicellular systems. As a proof-of-concept, we use CRISPR/Cas9 to accumulate a combinatorial diversity of mutations in engineered target arrays that serve as densely informative barcodes. A key feature of this system is that lineage-informative barcodes can be read out by a single sequencing read per single cell. In a cell culture system, we show that patterns of target array editing are tunable, and that barcode relationships can be used to infer a synthetic lineage. In zebrafish, we induce and recover thousands of different barcode alleles from single animals that can be related to one another, with major clades making highly non-uniform contributions to organ systems. Many adult zebrafish organs exhibit clonal dominance, in that the majority of cells are derived from relatively few progenitors. We anticipate that whole organism lineage tracing by multiplex in vivo genome editing will help generate large-scale spatiotemporal maps of cell lineage in multicellular systems, in the context of both normal development as well as disease.
Funding provided by the Paul G. Allen Family Foundation, the American Cancer Society, the Howard Hughes Medical Institute, NIGMS, NICHD, NIMH, and NHLBI.

Program Abstract #242
An unexpected link between fusogen activity and intracellular lumen elongation
Fabien Soulavie[1], David H. Hall[2], Meera Sundaram[1]
[1]*Department of Genetics, University of Pennsylvania, Philadelphia, PA, USA;* [2]*Department of Neuroscience, Center for C. elegans Anatomy, Albert Einstein College of Medicine, USA*
Unicellular tubes with an intracellular lumen are found in the mammalian microvasculature and in some invertebrate organs, including the *C. elegans* excretory (renal-like) system. Many unicellular tubes are seamless –they lack autocellular junctions along their length – and they adopt elongated or branched shapes. How cells become seamless tubes with such complex shapes is poorly understood. One proposed mechanism for seamless tube formation involves uptake of basal membrane by macropinocytosis ("cell gulping") followed by cell hollowing. An alternative mechanism involves cell auto-fusion, a process related to cell-cell fusion. We've shown that the *C. elegans* excretory duct forms a seamless tube by cell wrapping to form an autocellular junction, followed by membrane auto-fusion to remove that junction and become a seamless toroid. The duct tube subsequently elongates more than five-fold and adopts an unusual asymmetric shape. Both auto-fusion and subsequent tube elongation depend on the EGF-Ras-ERK signaling cascade and its downstream target, the plasma membrane fusogen AFF-1. In the absence of *aff-1*, the duct retains an autocellular junction and has a dramatically shortened morphology, with the lumen only ~a third of its normal length. *aff-1* mutants also accumulate vesicular intermediates with apical cargoes adjacent to the main lumen, and inclusions with highly convoluted membranes adjacent to and continuous with the basal membrane. Based on the *aff-1* mutant phenotype, we propose that duct tube elongation involves macropinocytosis, and that the AFF-1 fusogen mediates scission of macropinocytic compartments.
This work was supported by NIH grant GM58540 to M.S.

Program Abstract #243
Capabilities and limitations of tissue size control through passive mechanical forces
Jochen Kursawe[1], Pavel Brodskiy[2], Ruth Baker[1], Jeremiah Zartman[2], Alex Fletcher[3]
[1]*University of Oxford, UK;* [2]*University of Notre Dame, USA;* [3]*University of Sheffield, UK*
Embryogenesis is an extraordinarily robust process, exhibiting the ability to control tissue size and repair patterning defects in the face of environmental and genetic perturbations. The size and shape of a developing tissue is a function of the number and size of its constituent cells, as well as their geometric packing. How these cellular properties are coordinated at the tissue level to ensure developmental robustness remains to a large extent a mystery. Understanding such control mechanisms requires studying multiple concurrent processes that make up morphogenesis, including the spatial patterning of cell fates and apoptosis, as well as cell intercalations. Here, we develop a computational model that aims to understand aspects of the robust pattern repair mechanisms of the *Drosophila* embryonic epidermal tissues. Size control in this system has previously been shown to rely on the regulation of apoptosis rather than proliferation; however, to date little work has been carried out to understand the role of cellular mechanics in this process. We employ a vertex model of an embryonic segment to test hypotheses about the emergence of this size control. Comparing the model to previously published data across wild type and genetic perturbations, we show that passive mechanical forces suffice to

explain the observed size control in the posterior (P) compartment of a segment. However, observed asymmetries in cell death frequencies across the segment are demonstrated to require patterning of cellular properties in the model. Finally, we show that distinct forms of mechanical regulation in the model may be distinguished by differences in cell shapes in the P compartment, as quantified through experimentally accessible summary statistics, as well as by tissue recoil after laser ablation. Funding from the Engineering and Physical Sciences Research Council, the Royal Society, the National Institutes of Health, and the National Science Foundation is gratefully acknowledged.

Program Abstract #244

Calcium dynamics reveal mechanisms of epithelial wound detection

Erica Shannon[1], Monica Lacy[2], M. Shane Hutson[2], Andrea Page-McCaw[1]

[1]Dept. of Cell and Developmental Biology, Vanderbilt University, USA; [2]Dept. of Physics, Vanderbilt University, USA

When an epithelial tissue is wounded, affected cells undergo a set of coordinated behaviors to close the wound and repair the tissue. Cells across the epithelium participate in this response, not just cells bordering the wound. We want to know how cells, including those away from the wound, get information that a wound has occurred. The earliest known wound response is a calcium wave, previously reported to originate in cells on the wound margin and expand outward several cells in diameter. This calcium wave is highly conserved and is important for wound healing. However, the mechanism of wave initiation remains unknown. Based on the kinetics we observe, we hypothesize that the calcium wave is a result of changes in tissue mechanics upon wounding. We analyze the kinetics of the calcium wave using live imaging of the *Drosophila* pupal notum, an epithelial monolayer of diploid cells that express the GFP-based GCaMP calcium reporter. Our data reveals three distinct stages of calcium dynamics upon wounding: initial release, radial spreading, and stochastic flares. During the initial release, calcium flows independently into multiple cells at varying distances from the wound margin, only milliseconds after wounding. The second stage of calcium release is consistent with diffusion of calcium to neighboring cells. The third stage occurs ~30-60 seconds after wounding and is characterized by random flashes of calcium that propagate around the wound for up to 30 minutes. These flashes are consistent with calcium induced calcium release. The initial independent release of calcium moves from proximal to distal cells in a wave traveling on the order of 1 μm/ms. Preliminary analysis suggests that diffusion cannot account for such a rate of spread. Thus we are testing the role of mechanotransduction in wound detection. We will use genetic tools to manipulate tissue mechanics and then assess how the calcium wave is affected by the mechanical perturbations.

Program Abstract #245

Positioning and morphogenesis of a neuron-glia attachment that shapes dendrite extension

Elizabeth Lamkin, Ian G McLachlan, Maxwell G Heiman

Harvard University/Boston Children's Hospital, USA

During organ assembly, specialized attachments among cells transmit mechanical forces that instruct changes in cell shape. Thus, it is critical that attachments form between appropriate partners, at the proper positions, and with the correct morphology. To study this process at the single-cell level, we have turned to the nematode *C. elegans*, in which cell shapes and attachments are nearly invariant. We focused on the attachment between the BAG sensory neuron and its neighboring glial cell because, in adult animals, this attachment has a unique and striking morphology in which the BAG dendrite ending precisely wraps a protrusion from the glial cell. This attachment had previously been visualized only by electron microscopy, and the molecular mechanisms that generate it were unknown. We used super-resolution optical microscopy to visualize the BAG-glia attachment in living animals. We also performed forward genetic screens to identify factors required for BAG dendrite development. We showed that the nascent dendrite ending attaches to the glial cell and is pulled to its final length by mechanical forces generated during embryo elongation. Our genetic screens revealed that this attachment requires the cytoskeletal adaptor protein GRDN-1/Girdin, which acts cell-autonomously in the glial cell, and the adhesion molecule SAX-7/L1CAM, which is localized via GRDN-1 to a specialized domain at the glial ending. Based on the partial penetrance of these mutants, we reasoned that additional factors may contribute to the specification and morphogenesis of the BAG-glia cell attachment. To identify such factors, we conducted a forward genetic screen for modifiers of the *sax-7* dendrite extension defect. The specialized attachment between BAG and its partner glial cell provides a model for how cell-cell attachments form at defined locations and how cells coordinate the morphologies of these contact sites.

This work was supported in part by an NSF GRFP to ERL.

Program Abstract #246

Development of organized stress fibers in a model contractile tube coincides with cell contraction following tissue stretch and is dependent on Ca2+ signaling and myosin II activity

Alison Wirshing, Erin J. Cram
Northeastern University, USA

Stress fibers are actomyosin structures found in contractile non-muscle cells such as myoepithelial cells in glandular tissue and the smooth muscle and endothelial cells of the vasculature. Stress fibers play an important role in cell contractility, motility, and perception and response to mechanical cues (mechanotransduction). Additionally, there is evidence that altered composition/organization of stress fibers contributes to the pathogenicity of diseases that impact these contractile tissues such as hypertension and asthma. Numerous studies have used *in vitro* or *ex vivo* models to investigate actin dynamics during changing mechanical stresses. However, 2D cell culture experiments are not able to recapitulate the complexity of *in vivo* 3D systems. At present, there are few *in vivo* systems that allow for real-time investigation of actin dynamics in intact contractile tissues. In this work, we use *C. elegans* spermatheca as a novel model for investigating stress fiber maturation. The spermatheca is a highly contractile tube of 24 myoepithelial cells that houses the sperm and is the site of fertilization during ovulation. In mature adults, spermathecal actin is organized into parallel, circumferential stress fibers. We use fluorescently labeled actin and confocal microscopy to capture 4D images of actin dynamics during cell stretch, contraction, and relaxation and show that parallel stress fibers develop from a loose meshwork of fibers during contraction. Furthermore, timely development and organization of stress fibers is influenced by Ca^{2+} signaling and myosin II activity. Both reduced and increased actomyosin tension cause changes in actin filament spacing, thickness, and tortuosity. We conclude that Ca^{2+} signaling activates tissue contraction and that myosin II activity is the primary force driving stress fiber development in the *C. elegans* spermatheca.

Program Abstract #247

The effect of simulated microgravity on neuronal function and development in *C. elegans*

Katherine Burgos, Amy C. Groth
Eastern Connecticut State University, USA

With commercial space flight and the prospect of colonizing Mars on the horizon, it is important to understand the effects of microgravity on both adult and developing tissues. Microgravity leads to a decrease in muscle strength in humans, as well as reduced neurocognitive performance. We have subjected *Caenorhabditis elegans* to simulated microgravity to study its effects on the nervous system and development of a living organism. In order to study changes in the function of olfactory neurons, adult worms were exposed to microgravity for three days by vertical rotation in a Rotatory Cell Culture System (RCCS) developed by NASA. Control worms were grown in a RCCS chamber with horizontal rotation for the same period of time. After exposure to simulated or normal microgravity, the worms were analyzed for their ability to properly chemotax to butanone. In order to determine if microgravity affects the ability of worms to fully develop from embryos to adults, embryos were grown in simulated and normal microgravity for three days. Preliminary data showed no significant changes in olfaction towards butanone, although chemotaxis tests have not been done to other chemicals. Interestingly, a strong difference in development between microgravity-exposed worms and control worms was observed. Microgravity exposed embryos developed much more slowly to an adult stage than control worms. Preliminary data also indicates that worms exposed to microgravity during development to L4 larvae had higher embryonic lethality than control worms. Information gathered in this study can be used to address the limitations of sending humans to space for extended periods of time. Funding was provided by the CT Space Grant Consortium, the ECSU Biology Department, a CSU-AAUP Faculty Research Grant and an ECSU-AAUP Jean H. Thoresen Scholarship.

Program Abstract #248

Notochord vacuoles play a key role in spine formation during vertebral bone growth

Jennifer Bagwell[1], Kathryn Ellis[3], Xiaoyan Ge[2], James Norman[1], Daniel Keeley[1], Didier Stainier[2], Michel Bagnat[1]
[1]Duke University, USA; [2]Max Planck Institute for Heart and Lung Research, Germany; [3]NIH, USA

The notochord plays critical structural roles during vertebrate development. At the center of the vertebrate notochord is a large fluid-filled organelle, the notochord vacuole. We have recently shown that zebrafish notochord vacuoles are specialized lysosome-related organelles required for AP axis elongation and spine morphogenesis. Disruption of notochord vacuoles results in larvae with a shortened anterior-posterior body axis and juveniles with spine kinks that are similar to those found in congenital scoliosis (CS) in humans. Using live imaging and genetic manipulations, we found that notochord vacuole function in spine morphogenesis is crucial during vertebral bone growth. To gain new insights into the processes that lead to CS, we identified mutants with severe scoliosis of the spine. One of these mutants, *spaetzle*

(*spzl*), shows disrupted notochord vacuoles in an otherwise straight notochord. Using live imaging we found that it is not until vertebrae maturation that the spine in *spzl* mutants becomes curved and kinked as bone grows into the notochord. Our data suggest that the lack of a hydrostatic scaffold for bone deposition in fish with disrupted notochord vacuoles leads to aberrant spine morphogenesis. Using exome sequencing we isolated the *spzl* mutation in a gene encoding a kinase of unknown tragets. Given the similarity of the phenotypes in *spzl* mutants and those of the vacuolar trafficking pathway, we performed genetic interaction studies and found that *spzl* functions in clathrin-dependent post-Golgi transport. Furthermore, phoso-proteomics revealed that a clathrin subunit is hypo-phosphorylated in *spzl* mutants. These studies offer mechanistic insights into spine morphogenesis and the etiology of CS.

Program Abstract #249
Cardiac function shapes the architecture of the cardiac outflow tract
Pragya Sidhwani, Deborah Yelon
University of California-San Diego, USA
"Form ever follows function, and this is the law," as stated by the architect Louis Sullivan, is highly relevant to the morphogenesis of developing organs. In the embryonic heart, for example, both cardiac contractility and blood flow have been shown to direct heart morphogenesis through modulation of cell shape, cell size, and proliferation. However, it is still unclear precisely how the biomechanical forces generated by cardiac function are sensed within the developing heart and translated into changes in cardiac cell behavior. In our studies, we aim to address this question in the context of the cardiac outflow tract (OFT), a small cylindrical structure positioned at the arterial pole, which acts as a primary portal for blood flow from the heart to the periphery. Importantly, the OFT is constructed after contractility and blood flow have already initiated, pointing to a potential influence of cardiac function not only on the final shape of the OFT, but also in its initial assembly. Through high-resolution morphometrics in the zebrafish embryo, we have characterized the stereotypical cellular architecture of the OFT and determined that its organization is severely perturbed when cardiac function is disrupted. Specifically, zebrafish mutants with defects in cardiac function exhibit an abnormally narrow cylinder of OFT myocardium, as well as a collapse of the OFT endocardium. Pharmacological inhibition of mechanosensitive channels leads to a similar narrowing of the OFT, implicating a role of biomechanical forces in OFT development. Together, our data suggest a model in which cardiac function leads to the activation of mechanosensitive channels within the endocardium, thereby inducing expansion of the OFT lumen and setting the dimensions for the OFT myocardium.

Program Abstract #250
Myocardial Afterload is a Key Epigenetic Regulator of Cardiac Valve Development
Neha Ahuja[1], David Bark[1], Prasad Dasi[2], Deborah Garrity[1]
[1]Colorado State University, United States; [2]The Ohio State University, United States
Congenital heart defects occur when the heart fails to undergo proper morphogenesis. It is estimated that a congenital heart defect occurs in 4 out of every 1000 live births (Pierpont et al., 2007). Currently, we cannot attribute a cause to over 80% of congenital heart defects. In order to understand how congenital heart defects arise, we must first understand how the heart develops. One emerging hypothesis is that biomechanical forces serve as epigenetic cues to facilitate proper cardiovascular development. One such biomechanical cue is myocardial afterload. Myocardial afterload can be thought of as the resistance that the ventricle must overcome in order to successfully pump blood throughout the body and has been shown to cause pathology in human adults. However, the effect of myocardial afterload on cardiac development has not yet been investigated. One reason for this knowledge gap is a lack of tools to manipulate afterload in a relevant model organism. Zebrafish have traditionally been used to study early cardiac development as their optical clarity and ease of genetic manipulation make them well-suited for the laboratory. We use a novel approach of applying vasopressin, a potent vasoconstrictor, to study the effects of increased myocardial afterload on development using zebrafish as a model organism. Our results suggest that increasing afterload causes valve elongation and increases the expression of several genes involved in valve development

Program Abstract #251
LINC-ing Lrmp structure and function to nuclear dynamics in zebrafish
Christina Hansen, Francisco Pelegri
University of Wisconsin - Madison, USA
LINC (LInker of Nucleoskeleton and Cytoskeleton) complexes are conserved structures consisting of inner nuclear membrane SUN-domain proteins and outer nuclear membrane KASH-domain proteins, which serve as a bridge between

the nuclear interior and structural elements in the cytoplasm. Despite being well characterized as critical players in the developmental processes of a number of model organisms, to date the function of the LINC complex in vertebrate embryonic development is poorly understood. We use the zebrafish as a model system to study the function of this complex. Our research aims to establish Lrmp, a maternally-inherited KASH-domain protein, as a member of the LINC complex necessary for pronuclear congression and fusion in zebrafish. The physical structure of Lrmp suggests that its C-terminus physically interacts with a SUN-domain protein, and that its N-terminus interacts with dynein in order to facilitate movement along microtubules. Additionally, *lrmp* RNA localization data suggests that Lrmp protein production is highly regulated spatially and temporally during the early embryonic cell cycles, likely to facilitate protein targeting to the outer nuclear envelope membrane. Through the use of *in situ* hybridization, immunofluorescence, and high-resolution microscopy, we will present progress towards addressing these hypotheses. This work is supported by NIH R01 GM065303.

Program Abstract #252

FGF-mediated tensional gradients drive collective cell movements to form the avian hindgut

Nandan Nerurkar[1], L Mahadevan[2], Cliff Tabin[1]

[1]Harvard Medical School, USA; [2]Harvard University, USA

The recent resurgent interest in physical aspects of morphogenesis has advanced our understanding of the types of forces involved, and the subcellular machinery that enact them. Ultimately, embryonic forces are under genetic control, but upstream signals that spatiotemporally regulate them remain largely unknown. Here we aim to address this in the context of gut tube formation in the chick embryo. The internalization of the endoderm to form the gut tube is a fundamental, yet poorly understood process in establishing the vertebrate body plan. We identified a collective polarized movement of endoderm cells that drives hindgut formation, and using a combination of biophysical and molecular approaches, determined that these movements arise through an FGF-mediated contractile gradient. FGF8 generates tension in the endoderm through RhoA-dependent contraction, and as a result, the posterior-to-anterior gradient in FGF8 generates a parallel gradient in tension. This force imbalance drives movement of cells from anterior (low tension/FGF8) to posterior (high tension/FGF8). A key feature of this mechanism, revealed by mathematical modeling, is the positive feedback that results, whereby passive anterior cells are pulled posteriorly by active contracting cells, and in doing so are exposed to increasing FGF8 concentrations, contract themselves, and pull more cells posteriorly. This is analogous to a tug of war game, where as one team begins to win, they recruit players from the opposing team. This is why posterior movement of the endoderm outpaces axis elongation despite the fact that both processes are coordinated by the same FGF8 gradient. This work represents some of the first mechanistic insight into how the regulation of tissue-scale physical forces in vertebrate development can be traced to signaling pathways. Specifically we show that modulation of tissue-level forces by diffusible signals represents a fundamental mechanism by which morphogenesis is carried out.

Program Abstract #253

Cell rearrangements shape the mandibular arch

Hirotaka Tao[1], Kimberly Lau[1], Owen Whitley[1], Min Zhu[1,2], Xiao Xiao[1], James Ferguson[3], Sidhartha Goyal[4], Radhika At it[3], Yu Sun[2], Sevan Hopyan[1,5,6]

[1]Program in Developmental and Stem Cell Biology, Research Institute, Hospital for Sick Children, Toronto, Canada; [2]Department of Mechanical and Industrial Engineering, University of Toronto, Canada; [3]Department of Biology, Case Western Reserve University, Cleveland, USA; [4]Department of Physics, University of Toronto, Canada; [5]Department of Molecular Genetics, University of Toronto, Canada; [6]Division of Orthopaedic Surgery, Hospital for Sick Children and University of Toronto, Canada

The nuanced shapes of organ primordia influence pattern formation and function. During outgrowth, the mandibular portion of the first branchial arch acquires a proximally narrow and distally bulbous shape. Although substantial progress has been made regarding the morphogenesis of epithelial sheets, mechanisms that shape a volume of tissue such as the arch are less understood. By combining time lapse light sheet microscopy of mouse embryos with custom 4D cell tracking, we observed that directional tissue growth is attributable to rearrangements among small groups of cells in 3D that are analogous to those observed in unstable foams. To measure cortical tension of individual cells associated with cell intercalations in vivo, we generated a transgenic FRET-based vinculin force sensor. Relatively high energy cell intercalations result in volumetric convergent extension that is characteristic of the narrow region of the arch. In contrast, lower energy intercellular movements are characteristic of the bulbous region. Based on these observations, we propose a basic thermodynamic model of branchial arch growth in which tissue entropy increases from proximal to distal. Analysis

of mutant embryos suggested that Wnt5a regulates orderly cell rearrangements that narrow and elongate the proximal arch. Therefore, distinct types of 3D cell rearrangements drive outgrowth and shape the madibular arch.

Program Abstract #254

Mechanical regulation of synovial joint development at molecular level

Pratik Singh[1], Claire Shea[2], Shashank Sonker[1], Rebecca Rolfe[2], Paula Murphy[2], Amitabha Bandyopadhyay[1]
[1]Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, India; [2]Department of Zoology, School of Natural Sciences, Trinity College Dublin, Ireland

Articular cartilage present at the ends of appendicular skeletal elements provides frictionless movement of synovial joints. Interestingly, sufficient movement of the foetus is also necessary for normal joint development as evidenced in certain clinical conditions, congenital disorders and animal models (immobilized chick and mutant muscle-less mouse embryos) where dynamic muscle contractions are reduced or absent. Although the importance of mechanical forces on joint development is unequivocal, little is known about the molecular mechanisms involved. Wnt signalling has been implicated in a response to mechanical stimulation in muscle-less mouse embryos although the mechanism involved is still elusive. Here, using DMB (decamethonium bromide) induced immobilized chick embryos as a model, we have identified genes that are abnormally expressed in knee joint tissues including a number of genes involved in Wnt and BMP pathways. We consequently examined Wnt and BMP pathway activity and found downregulation of β-catenin (with simultaneous upregulation of a gene encoding a pathway inhibitor (Sfrp2)) and ectopic pSMAD1/5/8 activity near the knee joint. Our results suggest that abnormal joint development in immobilised embryos might be due to inappropriate activities of Wnt and BMP signaling during definition of the emerging tissue territories i.e. reduced β-catenin activation and concomitant upregulation of pSMAD1/5/8 signaling. Moreover, our data suggests that the dynamic mechanical loading of the knee joints activates Smurf1 which keeps these joints insulated from the pSMAD1/5/8 signaling and is essential for joint cavitation and maintenance. The mechanosensitive genes identified in this study serve as a focus for further elucidation of the molecular basis of mechano-regulation of joint development.

Funded by India-Ireland Co-operative Science Programme 2011 (Grant reference: DST/INT/IRE/P-1/11)

Program Abstract #256

Dynamic changes to extracellular matrix composition direct airway epithelial branching through focal adhesion kinase

James Spurlin, Celeste Nelson
Princeton University, USA

Mechanical cues provided by the extracellular matrix (ECM) influence the development of several tissues, including the branching program in the lung. For instance, basement membrane (BM) thinning has been observed at the tips of embryonic lung branches, suggesting a role for BM turnover in propagating the airway branching program. Currently, it is unclear if BM degradation ensues prior to branch formation as a mechanism to specify branch sites. To address this, we characterized the distribution of BM proteins in embryonic chicken lungs prior to and during branch induction. Our data also shows BM thinning during branch outgrowth, however no discernable changes in BM protein distribution could predict incipient branch sites. Moreover, ex vivo lung culture experiments reveal that matrix metalloproteinase activity is not required for branch initiation, suggesting that BM thinning is a response to branch elongation. To further elucidate how dynamic changes in ECM distribution affects branch outgrowth, we investigated the spatiotemporal accumulation of mechanically induced ECM protein, tenascin-C (TNC), during airway branching. TNC is localized in the BM adjacent to the airway epithelium, which becomes enhanced in both epithelial and mesenchymal cells at the tips of growing branches. To test if local changes in mechanics could account for TNC accumulation at branch tips, we blocked the activity of the mechanotransduction protein, focal adhesion kinase (FAK), during lung morphogenesis. In FAK-inhibited lungs, TNC expression is significantly downregulated, branch initiation is significantly reduced, and elongating branches assume a wedge-shape morphology distinct from control lungs. Taken together, we propose a model by which the airway epithelium induces branch formation in a FAK-dependent manner. As branches elongate, the deformation of neighboring mesenchymal cells promotes a change in local ECM composition via FAK activation, ultimately shaping the growing branch.

Program Abstract #257

Optimization of culture condition for limb bud progenitors by exploiting the Hyaluronan-based 3D culture system

Charlotte Colle, Yuji Atsuta, Clifford Tabin
Harvard Medical School, USA

Defining optimum culture conditions for stem and progenitor cells is pivotal not only to the study of developmental biology, but also to promote potential medical applications such as cell therapy or regeneration. Limb progenitors (LPs) exist as a transient multipotent cell population competent to give rise to the majority of patterned limb structures including bones and cartilages. We previously showed that the administration of Wnt, FGF8 and retinoic acid (WFR) was sufficient to maintain the full differentiation and patterning potentials of in vitro cultured chicken LPs (cLPs), however, we were only able to maintain their potential within a short time frame (36h). Moreover, we observed a lot of cell detachment after 48h of culture. Here we attempt to establish long-term culture system of LPs by taking advantage of Hyaluronan (HA)-based hydrogel. As extracellular matrix (ECM) has been highlighted as a key player for stem/progenitor cells maintenance, we focused on HA, which mainly composes early limb ECM. Indeed we successfully cultured LPs for 14 days in the presence of WFR. The expression of proximodistal (PD) markers was nicely maintained until day4. Furthermore, when transplanted into chicken limb buds, retrieved cells from day2 hydrogels recapitulated differentiation and PD patterns of endogenous cLPs. We also tested mouse LPs (mLPs), and to our surprise, mLPs were more proliferative in HA-hydrogels than cLPs. As observed for cLPs, a fraction mLPs was also positive for a LP marker. Together, these results suggest that HA-hydrogel system holds a great promise to improve culture condition of LPs. We also aim to discuss the possible customization of the scaffolds to mimic native environment of the early limb bud, thereby enhancing our culture conditions.

Program Abstract #258

Induced in vivo cell reprogramming of muscle into endoderm by direct transdifferentiation

Clyde Campbell, Joseph Lancman, Michelle Mattson-Hoss, Jonatan Matalonga, Zach Achen, Duc Dong
Sanford Burnham Prebys Medical Discovery Institute, United States

The extent to which differentiated cells, while remaining in their native microenvironment, can be converted into unrelated cell types will reveal fundamental insight into cellular plasticity and impact regenerative medicine. We have identified two transcription factors that, when co-expressed in several non-endoderm lineages including skeletal muscle, are able to cell-autonomously induce the early endoderm program. Induced muscle cells can proceed to express key endoderm organogenesis genes including *hnf1b* and *pdx1*, and subsequently form organoids. Endoderm markers appearing prior to loss of muscle cell morphology, a lack of dependence on cell division, and a lack of pluripotency gene activation, together, suggests that lineage reprogramming occurred independent of a pluripotent intermediate. Importantly, lineage reprogramming can occur in *oct4* mutants, providing functional evidence that lineage conversion is via direct transdifferentiation. Our work demonstrates that within a vertebrate animal, differentiated cells originating from one germ layer can be induced to directly adopt lineages of a different germ layer – suggesting that differentiated cells *in vivo* are more plastic than previously assumed. This discovery may pave the way towards a vast new *in vivo* supply of replacement cells for degenerative diseases such as diabetes.

Program Abstract #259

Bioengineered Human Extracellular Matrix for Tissue Engineering and Regenerative Medicine

Michael Zimber[1], Mark Baumgartner[1], Rebecca Symons[1], Ryan Fernan[2], Gail Naughton[1,2]
[1]Histogen Inc., USA; [2]PUR Biologics, USA

The extracellular matrix (ECM) constitutes a critical element in providing structural support, relaying instructional cues, and guiding the growth and differentiation of cells to pattern and organize tissues and organs. ECM derived from animals is frequently used in cell biology research and as transplant material in human clinical settings, but there exists a need for non-xenogenic, human-derived ECM for tissue engineering and regenerative medicine purposes. We have generated a bioengineered human extracellular matrix (hECM) produced by human multipotent dermal fibroblasts cultured on microcarrier beads grown in suspension and under hypoxic (2-5% O_2) and serum-free culture conditions within a controlled bioreactor system. The insoluble fraction of hECM is harvested from the bioreactor and minimally processed to produce a liquid hECM-rich material which can be used in a variety of forms such as liquid injectables, device and substrate coatings, three-dimensional lyophilized scaffolds and reverse thermal hydrogels. In addition, the hECM can be combined with other molecules, polymers and inorganic materials to create hybrid, multiphasic scaffolds tailored for specific indications. The hECM supports proliferation and differentiation of several different cell types including skin cells, preosteoblasts, neural crest derivatives and mesenchymal stem cells. In animal models, the hECM has demonstrated a capacity to support in vivo regeneration of bone and cartilage tissue in critical-sized osteochondral defects as well as promoting the healing of full-thickness cutaneous wounds with minimal scarring and contraction. The hECM represents a human-source biomaterial with a range of potential uses that includes tissue engineering scaffolds for the study of cellular

dynamics and organogenesis, biointegrative coatings of medical implants, bioinks for three-dimensional printing, and as regenerative tissue fillers for human clinical indications.

Program Abstract #260
Paxillin in Myotome Morphogenesis
Andrew Jacob, Chris Turner, Jeff Amack
SUNY Upstate Medical University, USA
Paxillin (Pxn) is a key adapter protein and signaling regulator at sites of cell-extracellular matrix (ECM) adhesion that has been well studied using *in vitro* mammalian cell cultures. To investigate the role of Pxn during vertebrate development, we took a reverse genetic approach using the zebrafish embryo as a model system. We have characterized two paralogous Pxn genes, *pxna* and *pxnb*, in zebrafish that are maternally supplied and expressed in developing myotomes. CRISPR-Cas9 genome editing and antisense Morpholino gene knockdown approaches were used to identify Pxn functions during zebrafish development. While genome editing of either *pxna* or *pxnb* alone did not result in gross defects, double zygotic mutants (*pxna*$^{-/-}$;*pxnb*$^{-/-}$) that also lacked maternally supplied *pxna* or *pxnb* displayed embryonic phenotypes that included aberrant cardiovascular and myotome development. Morpholino knockdown of either *pxna* or *pxnb* alone resulted in similar, but more severe defects. Irregular myotome shape and decreased Laminin intensity were observed, suggesting an inside-out signaling role for *pxn* genes in ECM organization. Inhibition of non-muscle myosin II during somitogenesis altered the subcellular localization of Pxn protein and phenocopied *pxn* gene knockdowns. These data suggest that Paxillin genes are regulators of contractility-driven morphogenesis of the embryonic trunk musculature in zebrafish. Together, these results implicate Pxn in embryonic muscle development and provide useful genetic models for further analysis of Pxn function.

Program Abstract #261
FGFs direct muscle pathfinding during *Drosophila* development
Katrina Cable[1], Brenna Clay[2], Aaron Johnson[2]
[1]*University of Colorado Denver AMC, USA;* [2]*University of Colorado Denver, USA*
Skeletal muscle is derived from a pool of precursor cells known as myoblasts. Following specification, myoblasts fuse to form multinucleated myotubes, which then elongate and attach to tendons. Although, the molecular mechanisms involved in myoblast cell fate specification have been studied extensively, the processes by which the nascent myotubes elongate and find the proper tendon attachments are largely unknown. *Drosophila* embryonic body wall muscle is an excellent system to investigate myotube pathfinding because body wall muscle develops over just a few hours, which allows the entire process to be visualized in unperturbed embryos. In addition, the body wall muscles are functionally the same as vertebrate skeletal muscle in that they are multinucleate, attach to tendons, contain sarcomeres, and are voluntarily controlled. We hypothesize that tendon cells secrete chemotactic molecules that direct myotube pathfinding. To identify these molecules, we performed RNA-sequencing on FACS-sorted tendons and myotubes. We found a significant enrichment of FGF signaling components in sorted myotubes. FGF ligands play important roles in cell migration during *Drosophila* development, and we found that FGF ligands are expressed in tendon cells by *in situ* hybridization. We next analyzed myotube pathfinding defects in embryos with either hypomorphic or null mutations in two FGF8-like ligands. FGF8 null embryos showed a complete loss of myotube outgrowth and elongation. Embryos homozygous for a strong mutation in the FGF8-like receptor showed similar defects in myogenesis. Thus, FGF signaling is a key regulator of myotube pathfinding. This study is the first to identify a chemoattractive molecule that directs myotube pathfinding, and will be the basis for future studies investigating the mechanisms by which FGFs regulate cytoskeletal dynamics during myogenesis.

Program Abstract #262
The testis muscle sheath of *Drosophila melanogaster* arises by myoblast fusion and an independent migration process
Silke Rothenbusch-Fender, Jessica Kuckwa, Katharina Fritzen, Detlev Buttgereit, Renate Renkawitz-Pohl
Philipps Universität Marburg, DE
The inner male reproductive system of adult D. melanogaster consists of five organs: paired testes, seminal vesicles, paragonia, one ejaculatory duct and the sperm pump. The organs of somatic origin are surrounded by different muscle layers, the mono- and multinucleated striated muscles. In contrast the unique multinucleated testis musculature resembles vertebrate smooth muscles (Susic-Jung et al., 2012).
The reproductive organs develop from the gonads and a somatic genital imaginal disc, which forms during larval

development. During metamorphosis, the gonads and the genital disc grow towards each other and recognize. Finally the seminal vesicles fuse with the developing testes. The myoblasts which will form the muscles surrounding the reproductive organs proliferate during metamorphosis. A subgroup, that will form the testis muscle sheath migrates from the prospective seminal vesicle towards the gonad, contact the gonad at the distal end and migrate further to surround it (Kozopas et al., 1998, Kuckwa et al., 2016). The multinuclear state of the smooth-like testes muscles is achieved by fusion processes on the tip of the developing seminal vesicle. To characterize the fusion process we used myoblast-specific RNAi-mediated knockdown. We observed that reduced fusion of testes myoblasts does neither affect the filament arrangement nor the population of the testes with smooth-like muscles (Kuckwa et al., 2016). From these results we conclude that the fusion and migration processes proceed separately This enables us to analyze proteins like cytoskeleton regulators and components of signaling pathways regarding their role in migration of nascent myotubes disregarding their potential function during the fusion process. To get further insight into the migration of nascent myotubes from the genital disc onto the testes we established co-cultures of pupal genital discs and testes.
This work was supported by the Deutsche Forschungsgemeinschaft [Re 628/16-1 and GRK 1216].

Program Abstract #263
Diving into epithelial morphogenesis: How do centripetally migrating follicle cells cover the *Drosophila* oocyte?
Travis Parsons, Juan C. Duhart, Anna Kabanova, Laurel Raftery
University of Nevada, Las Vegas, USA
Collective cell migration occurs in normal morphogenetic processes as well as pathological processes such as tumor metastasis. During oogenesis in *Drosophila melanogaster*, a somatic epithelium composed of follicle cells (FCs) initially undergoes a collective migration by rotating circumferentially around a cluster of germ cells, depositing extracellular matrix that constricts the shape of the growing egg. Within hours after this process stops, two separate populations of somatic cells invade into the germ cell cluster. The border cells migrate as a cluster, whereas the ring of centripetal cells elongate apically between the oocyte and nurse cells to enclose the oocyte in an eggshell. Centripetally migrating FCs have been observed in developing follicles of more basal Dipterans as well as *D. melanogaster*, suggesting it is generally employed to form eggshell around a maturing egg in holometabolous insects with polytrophic ovarioles. The cell biological processes and the nature and identity of the cell communication signals that orchestrate the centripetal migration event are largely unknown. BMP signaling is active in these cells, both prior to and during centripetal migration. It remains unknown whether BMP signals are necessary for the events of centripetal migration, such as early patterning of the centripetal cell fate, or perhaps regulation of cellular movements. We are collecting tools to examine centripetal cell movements and changes in cell shape in order to distinguish between mechanisms such as infolding of a contiguous epithelium, versus elongation of a ring of cells that subsequently reorganize into an eggshell-secreting epithelium. To investigate these questions we are using time-lapse imaging of follicles in culture combined with mosaic analysis of mutations in various signaling pathways such as BMP. These studies will provide mechanistic insight into epithelial morphogenesis during formation of more complex organs. Funded by NSF grant IOS-1355091.

Program Abstract #264
The Migrations of *Drosophila* Muscle Founders and Primordial Germ Cells Are Interdependent
Angela Stathopoulos
Caltech, USA
Caudal visceral mesoderm (CVM) cells migrate from posterior to anterior of the *Drosophila* embryo as two bilateral streams of cells to support specification of longitudinal muscles along the gut. To accomplish this long-distance migration, CVM cells receive input from their environment, but little is known about how this collective cell migration process is regulated. In a screen, we found that *wunen* mutants exhibit CVM cell migration defects. Wunens are lipid phosphate phosphatases shown to regulate the directional migration of primordial germ cells (PGCs). PGC and CVM cell types interact during the period when PGCs are en route to the somatic gonadal mesoderm, and previous studies have shown that CVM cells impact PGC migration. In turn, we found that CVM cells exhibit an affinity for PGCs, localizing to the position of PGCs whether mislocalized in the ectoderm or trapped in the endoderm. Furthermore, CVM cell migration is delayed in mutants lacking PGCs. These data demonstrate PGC and CVM cell migrations are interdependent and suggest that distinct migrating cell types can coordinately influence each other to promote effective cell migration during development.

Program Abstract #265

Quantitative characterization of collective stem cell migration during olfactory neurogenesis

Vijay Warrier[1], Bi-Chang Chen[2], Eric Betzig[3], Marianne Bronner[4], Ankur Saxena[1]

[1]University of Illinois, Chicago, United States; [2]Academia Sinica, Taiwan; [3]HHMI Janelia Research Campus, United States; [4]California Institute of Technology, United States

The cranial ganglia and sense organs arise from two vertebrate-specific progenitor cell types: neural crest and ectodermal placodes. *In vivo* lattice light-sheet microscopy of subsets of these populations in zebrafish embryos has provided high-resolution spatiotemporal cell migration data. Algorithmic cell tracking based on nuclear-localized fluorescent markers enables simultaneous quantitation of hundreds of cell trajectories. Extracting biologically interpretible readouts from such high-resolution data requires innovative analytical approaches. To this end, we are applying statistical techniques from both machine learning (generalized Fisher discriminant analysis (FDA)) and econometrics (Wiener-Granger causality (WGC)) to our datasets. Specifically, we have used FDA to explore automation of cell type identification based on cell trajectory data and WGC to indirectly assay the mechanisms underpinning collective neural crest cell migration in cranial development. Preliminary results demonstrate successful quantitative discrimination of placode versus neural crest cell types and the ability to identify previously unobservable trends of collective neural crest migration. Future application of these techniques to non-wildtype datasets may provide further insights into stem cell migration and differentiation during vertebrate organogenesis.

Program Abstract #267

Morphological response of genetically identified, immature cortical neurons to cortical extracellular matrix and ethanol exposure

Eric Zluhan[1,2], Eric Olson[1,2]

[1]SUNY Upstate Medical University, USA; [2]Developmental Exposure to Ethanol Research Center, USA

In the developing cerebral cortex, nascent neurons are particularly sensitive to disruption of the extracellular environment and exposure to toxins. Here, we used a high-throughput *in vitro* approach to examine the effects of cortical extracellular matrix (cECM) and ethanol exposure on genetically identified, immature cortical neurons. The neurons express a plasmid encoding a red fluorescent protein (dsRed) driven by the doublecortin promoter/enhancer (Wang et. al., J. Nsci Res, 2007) and are thereby identified as early migrating and differentiating cortical neurons. To explicitly mimic the *in vivo* environment, the neurons are plated on cECM extracted from embryonic cortex that is then contained within a hydrogel. Time-lapse confocal imaging reveals that dsRed+ neurons on cECM are highly dynamic with active dendritic remodeling and axon extension. Consistent with hippocampal neuron studies, ethanol exposure alters the dynamic behaviors of axons during the period of active outgrowth. Addition of ethanol to dsRed+ neurons (400mg/dL) causes a dramatic (approximately two-fold) increase of collaterals and the occasional bifurcation of the developing axon. The rapidity (<20 min) of the axonal response implies that ethanol may directly disrupt signaling mechanisms involved in axonal pathfinding and could provide insight into some of the pathfinding defects described in FAS. The mechanisms by which ethanol disrupts neuronal development are varied and most of the known mechanisms disrupt specific cell classes in specific cellular environments. This novel primary cell culture platform should allow the dissection of cell-extrinsic and cell-intrinsic underpinnings of the ethanol response.

Program Abstract #268

Convergent effects of reelin-deletion and ethanol on Golgi apparatus positioning during dendritic initiation in the cerebral cortex

Eric Olson[1,2], Ryan O'Dell[1]

[1]SUNY Upstate Medical University, USA; [2]Developmental Exposure to Ethanol Research Center, USA

The mechanisms controlling cortical dendrite initiation and stabilization are not well described. Multiphoton imaging of developing mouse cortex revealed that dendrites emerge by direct transformation of the neuron's leading process during the terminal phase of radial migration. In the absence of the secreted protein reelin (*reeler* genotype), a subset of neurons complete migration but then retract and reorganize their neurites away from their normal target area. In both *reeler* cortices and ethanol-exposed cortices, neurons show polarization defects and aberrant Golgi positioning. To better understand the dynamics of Golgi positioning and its relation to dendrite initiation and stabilization, we labeled migrating neurons with a red fluorescent protein (tdTomato) to identify cellular morphology and a GalT-eGFP fusion protein to identify the trans-Golgi compartment. Whole hemisphere explants were prepared to examine deep-layer, excitatory neurons that are completing migration and initiating dendritic growth. In actively migrating neurons, the Golgi (GalT-eGFP signal) is localized in the leading process. Somal movements arrested at bifurcations in the leading process. Somal

movement resumed when one branch was retracted and the other branch showed exclusive Golgi-investment. During the final period of migration the Golgi extended into the leading process as it transformed into the nascent dendrite. Primary dendrites with associated Golgi were larger caliber and longer than neurites that lacked Golgi. For both ethanol-exposed and *reeler* neurons, Golgi were dysmorphic and postmigratory neurons had more primary process with shorter life-times. These findings suggest that Golgi-localization and function are critical for stabilizing the nascent cortical dendrite.
Support provided by the NIAAA (P50AA017823) to the Developmental Exposure to Ethanol Research Center (DEARC).

Program Abstract #269

ApoER2 and reelin are expressed in regenerating peripheral nerve and regulate Schwann cell migration by activating the Rac1 GEF Protein, Tiam1

Consuelo Pasten[1], Joaquín Cerda[2], Ignacio Jausoro[2], Felipe A. Court[4], Alfredo Cáceres[3], Maria-Paz Marzolo[2]
[1]University of the Andes, Facultad de Medicina, Centro de Investigación Biomédica (CIB), Chile; [2]Pontificia Universidad Católica, Facultad de Ciencias Biológicas; Laboratory of Intracellular Trafficking and Signaling, Chile; [3]Instituto Mercedes y Martin Ferreyra (INIMEC) CONICET, Laboratory of Neurobiology, Argentina; [4]Pontificia Universidad Católica, Facultad de Ciencias Biológicas, Departamento de Fisiología, Chile

ApoER2 and its ligand Reelin participate in neuronal migration during development. Upon receptor binding, Reelin induces the proteolytic processing of ApoER2 as well as the activation of signaling pathway, including small Rho GTPases. Besides its presence in the central nervous system (CNS), Reelin is also secreted by Schwann cells (SCs), the glial cells of the peripheral nervous system (PNS). Reelin deficient mice (reeler) show decreased axonal regeneration in the PNS; however neither the presence of ApoER2 nor the role of the reelin signaling pathway in the PNS have been evaluated. Interestingly SC migration occurs during PNS development and during injury-induced regeneration and involves activation of small Rho GTPases. Thus, Reelin-ApoER2 might regulate SC migration during axon regeneration in the PNS. Here we demonstrate the presence of ApoER2 in PNS. After sciatic nerve injury Reelin was induced and its receptor ApoER2 was proteolytically processed. In vitro, SCs express both Reelin and ApoER2 and Reelin induces SCs migration. To elucidate the molecular mechanism underlying Reelin-dependent SC migration, we examined the involvement of Rac1, a conspicuous small GTPase family member. FRET experiments revealed that Reelin activates Rac1 at the leading edge of SCs. In addition, Tiam1, a major Rac1-specific GEF was required for Reelin-induced SC migration. Moreover, Reelin-induced SC migration was decreased after suppression of the polarity protein PAR3, consistent with its association to Tiam1. Even more interesting, we demonstrated that PAR3 binds preferentially to the full-length cytoplasmic tail of ApoER2 corresponding to the splice-variant containing the exon 19 that encodes a proline-rich insert and that ApoER2 was required for SC migration. Our study reveals a novel function for Reelin/ApoER2 in PNS, inducing cell migration of SCs, a process relevant for PNS development and regeneration

Program Abstract #270

Vangl2 is required for intraretinal pathfinding of mouse retinal ganglion cell axons.

Vicki Leung[1], Alexandra Iliescu[1], Christine Jolicoeur[2], Michel Gravel[1], Sergio Apuzzo[1], Elena Torban[3], Michel Cayouette[2], Philippe Gros[1]
[1]McGill University, CA; [2]Institut de Recherches Cliniques de Montréal, CA; [3]McGill University Health Center, CA

Vangl2 plays a critical role in the establishment of planar cell polarity and is well characterized for its role in human and murine neural tube development. In mice, we have previously detected expression of Vangl2 in the developing embryonic retina, which led us to investigate the potential role of Vangl2 during retinal development. We generated a *Vangl2βGeo* knock-in mouse, and we used an isoform-specific antibody on retinal cryosections to evaluate Vangl2 mRNA and protein expression, respectively. To further investigate the role of Vangl2 in retinal development, we examined the retinas of embryos homozygous for mutant Vangl2 alleles. We found that Vangl2 mRNA and protein are dynamically expressed in the developing retina with expression becoming progressively restricted to the ganglion cell layer and optic nerve as the retina matures. The expression of Vangl2 is most prominent at the plasma membrane and the axons of retinal ganglion cells (RGCs). Additionally, we found that Vangl2 is essential for retinal and optic nerve development as $Vangl2^{Lp/Lp}$ mutant embryos display reduced eye size, thickening of the retina, and optic nerve hypoplasia. Notably, in $Vangl2^{Lp/Lp}$ mutants, we observed axon bundles that traverse throughout the entire retina without specific orientation. These ectopic axons ultimately become trapped within the sub-retinal space resulting in optic nerve hypoplasia. There was no observable increase in RGC generation, progenitor proliferation, or retinal axon outgrowth. Taken together, these results identify a severe intraretinal pathfinding defect of RGC axons in $Vangl2^{Lp/Lp}$ embryos, highlighting a novel and essential role for Vangl2 in retinal axon guidance.
This study was supported by The Canadian Institutes of Health Research.

Program Abstract #271

Using Cytokeratins to probe mechanisms that coordinate collective cell migration in the zebrafish posterior lateral line primordium

Harsha Mahabaleshwar, Ajay Chitnis
NICHD - NIH, United States of America

The zebrafish posterior lateral line primordium (PLLp) is a multicellular cohort of ~120 cells that migrates along the horizontal myoseptum whilst being deposited as a series of epithelial rosettes called the neuromasts. While the migratory cues and the signals controlling the epithelial rosette formation – provided by the chemokine Cxcl12a and the ligands FGF3/FGF10 respectively - have been well studied, very little is known about mechanisms that coordinate the collective migration via mechanical coupling. Cytokeratins (CKs), the members of the intermediate filament family have been shown to be involved in the mechanical coupling of gastrulating mesendodermal cells, thus influencing their collective movement. In this system, the CK8, localizes to regions of cadherin based adhesion via plakoglobins while the resultant adhesion based intercellular tension, promotes cellular protrusions and migration in the direction opposite to the exerted pull. As keratins, plakoglobins, and cadherins are expressed in the primordium, we asked if CKs have a similar role in coordinating collective migration in the PLLp. In this ongoing study we compare the cellular localization dynamics of the CK8 and endogenously expressed zebrifish keratins (Ker15/Ker18) along the length of the actively migrating PLLp, to identify regions of keratin enrichment. Our analysis reveals the association of CKs with the apical constrictions of maturing neuromasts in the migrating PLLp and, in some contexts, with the "feet" of cells at the baso-lateral edges of the migrating PLLp. We further characterize how the localization of keratins dynamically changes with manipulations expected to perturb tension or adhesive interaction between the cells of the migrating cluster. Finally we describe changes in neuromast stablity in the absence of Ker15/Ker18 and the resultant reduction in neuromast deposition frequency.

Program Abstract #272

Characterizing the roles of Prickle and Dishevelled in regulating cell adhesion processes during *Xenopus* gastrulation

Yunyun Huang
University of Toronto, Canada

In *Xenopus laevis* embryos, mesodermal cells undergo radial intercalation and migrate along the inner surface of the blastocoel roof while remaining separate from the ectoderm. These gastrulation movements require continuous cell adhesion as well as repeated attachment and detachment, the regulation of which has yet to be fully elucidated. Previous work has shown participation of planar cell polarity (PCP) signalling proteins in regulating cell adhesion and separation. Using Prickle1 (Pk1) and Dishevelled2 (Dvl2) as two examples, I study the role of PCP components in local polarized regulation of cell attachment and detachment during mesodermal cell directional migration. I found a correlation of Pk1 and Dvl2 with cell detachment behaviour at the rear and lateral sides, respectively, consistent with their localization. I also found that Pk1 and Dvl2 knockdown both reduced cell motility on fibronectin. One possibility is that Pk1 and Dvl2 regulate cell cortex rearrangements for proper attachment and detachment. Quantifications of cell cortical F-actin and cortical tension showed that Pk1 and Dvl2 modulate cell adhesion strength and cortical contractility through up- and down-regulation of cortical F-actin density. I propose that Pk1 accumulates at the cell trailing edge, and locally up-regulates cortical F-actin to increase contractility of the retraction fibre. I also propose that Dvl2 promotes lateral cell-cell adhesion by down-regulating cell cortex contractility and stabilizing lateral adhesion sites between cells. My results revealed the role of PCP signalling proteins in local polarized regulation of cell attachment and detachment, and the contribution of cell contractility to overall cell adhesion and directional migration.

Program Abstract #273

Directed migration in the *Xenopus* endoderm is correlated with the polarized distribution of alpha-Catenin

Jason Wen
University of Toronto, CA

Vegetal rotation is a morphogenetic process that drives internalization of the vegetal cell mass along with the adjacent mesoderm during amphibian gastrulation. While a yolk-rich multilayered vegetal cell mass is a common feature of amphibian embryos, vegetal rotation was first characterized in *Xenopus laevis*. Despite knowledge of where endoderm cells move, the mechanism by which these cells move is not yet understood. We show here that endoderm cells translocate using a mode of intercellular migration called differential migration. In this process cell migration occurs asynchronously at spatially graded velocities with respect to neighbouring cells. At the cellular level, differential migration proceeds in a cyclic fashion whereby endoderm cells first elongate their cell bodies, followed by the

simultaneous expansion of the leading edge domain in tandem with shrinkage of the trailing domain. Finally, this process is completed by the retraction of the trailing edge, leading to cell rearrangement. Molecularly, we show that a biased enrichment of alpha-Catenin near the cell cortex coincides with expansion of the leading edge domain. We propose that the asymmetric distribution of alpha-Catenin mediates differential migration of endoderm cells. Together, these observations provide a basic schematic for endoderm cell movement within the endoderm during vegetal rotation.

Program Abstract #274

A Left-Right differential cell migration drives heart bending in vertebrates

Oscar Ocaña[1], Hakan Cockun[1], Carolina Minguillón[2], Prayag Murawala[3], Elly Tanaka[3], Juan Galceran[1], Ramón Muñoz-Chápuli[4], Angela Nieto[1]

[1]Instituto de Neurociencias de Alicante CSIC-UMH, San Juan de Alicante, Spain; [2]Institut de Biologia Molecular de Barcelona, Parc Científic de Barcelona, Spain; [3]DFG Center for Regenerative Therapies, Dresden (CRTD), Technische Universität Dresden, Germany; [4]University of Málaga, Faculty of Science, Department of Animal Biology, Málaga, Spain

The establishment of a left-right asymmetric pathway is a central event during embryo development for proper positioning, morphogenesis and function of internal organs. Activation of Nodal-Pitx2 axis specifically within the left lateral plate mesoderm confers left identity during organ positioning and differentiation. The epithelial-mesenchymal transition (EMT) inducer Snail represses Pitx2 on the right. Whether in addition to the repression of the left cascade an informative right-derived information operates in the embryo has remained elusive. Here we show that in vertebrates, BMP signaling activates EMT inducers preferentially on the right that promote differential L/R cell movements and heart bending through an actomyosin-dependent mechanism. Downregulation of EMT prevents heart looping leading to mesocardia, one of the most severe congenital heart defects. This indicates that a right-handed informative cascade also exists in vertebrates and therefore, that two parallel left and right pathways, respectively driven by Nodal and BMP integrate left and right information to govern the morphogenesis and positioning of the heart.

Program Abstract #275

Analysis of Migration, Proliferation, and Apoptosis in Phenylalanine Treated Cells

Jordan Guffey, Kody Harvey, Mary Thetford, Nikki J Seagraves

University of Central Oklahoma, USA

Maternal phenylketonuria [MPKU] is a syndrome of multiple congenital anomalies including cardiovascular malformations [CVMs], 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. Previous studies have shown that cardiac neural crest cells are important in formation of the outflow tract (OFT) and aortic arch arteries (AAA). Cell migration of the neural crest cells is a central process in the development of the heart. Study Objective: Since congenital CVM of the OFT and AAA are often observed in maternal PKU, in this study we aimed to determine if exposure to high Phe levels perturbs cell migration, proliferation, and apoptosis. Methods: We conducted in vitro silicon elastomeric masks migration assays on several cell types to determine if migration, was affected by Phe exposure and analyzed apoptosis, and proliferation by IHC. We also conducted neural tube explant migration assays in a collagen matrix in the absence or presence of Phe 1500 μM followed by analysis of apoptosis, and proliferation by IHC Results: Phe exposure causes a significant increase in migration of cells. IHC results are currently undergoing analysis to determine the effect of Phe on proliferation and apoptosis.

Program Abstract #276

The Role of Decorin or Biglycan in First Contact During Palate Fusion

Kathy Svoboda, Isra Ibrahim, Maria Serrano, L-Bruno Ruest

Texas A&M Univ Baylor College of Dentistry, USA

The secondary palate in humans and mice forms from maxillary process shelves that are mesenchyme covered with epithelium. Chondroitin sulphate proteoglycans (CSPG) on the apical surface of the medial edge epithelia (MEE) were necessary for palatal shelf adhesion (Gato et al, 2002 Dev Biol). However, the identity of the CSPGs involved in palate fusion was unknown. The objective of this study was to investigate the expression of two specific proteoglycans with chondroitin (CS) or dermatan sulfate (DS) side chains, decorin and biglycan, in palatal shelf adhesion. We also asked if TGFβ signaling was necessary for decorin and biglycan expression. Mouse palatal shelves from three stages of palatal shelf development (E13.5-14.5) were used for immunohistochemistry (IHC) and laser capture microdissection to collect MEE cells for RT-PCR. Cultured mouse palates treated with TGFβ RI kinase inhibitor (SB431542) for 48 hours were used to investigate the effects on blocking TGFβ signaling. The expression of biglycan was detected on the lateral surface

MEE cells at E13.5, when the palatal shelves were elevated. As palatal shelves approached, a thin layer of decorin and biglycan were distributed on the apical and lateral surfaces of MEE cells. Palatal shelves in close contact had abundant expression of both proteins on the MEE apical and lateral surfaces. The expression continued on the lateral surfaces of the midline epithelial seam (MES) cells after fusion. The staining for biglycan was more intense than decorin at all stages. Both proteoglycans' mRNA levels coincided with the protein expression. Palatal shelves treated with TGFβRI Kinase Inhibitor failed to fuse and had a persistent MES. Decorin and biglycan proteins were not expressed in the MEE/MES cells. In conclusion, the expression pattern of decorin and biglycan during palatal adhesion indicates they may have a role in normal palatal fusion and they are dependent on TGFβ signaling. Support: Baylor Oral Heath Foundation

Program Abstract #277

Proteolytic control of neural crest cell migration.

Dominique Alfandari, Ketan Mathavan, Vikram Khedgikar
Univ of Massachusetts, USA

Cranial Neural Crest (CNC) cells are a transient population of pluripotent stem cells that are induced at the border of the neural and non-neural ectoderm during early embryogenesis. They must migrate through precise route to invade the ventral tissues and contribute to the formation of the face. We have previously shown that CNC migrate first collectively prior to segregating into single cells. To achieve this process a fine control of cell-cell and cell matrix adhesion must be achieved. ADAM cell surface metalloproteases are enzymes that are involved in this process. We have shown previously that ADAM9 and 13 cleave the extracellular domain of Cadherin-11 to produce a fragment that can inhibit contact inhibition of locomotion and promote cell migration. In addition we have shown that the ADAM13 cytoplasmic domain is cleaved by gamma-secretase translocates into the nucleus where it regulates multiple gene expression. Using Proteomics, we have identified new substrates that are cleaved from the neural crest cell surface during migration. In particular the protocadherin PCNS (pcdh8l) expression is controlled at both transcriptional and post transcriptional levels by the ADAM13 metalloprotease. Previous work has shown that PCNS is absolutely critical for CNC migration. Here we show that the proteolytic activity and the cytoplasmic domain of ADAM13 regulate PCNS gene expression and protein level to support CNC migration *in vivo*. To better understand ADAM13's ability to regulate gene expression we used Mass Spectrometry to identify proteins that interact with the cytoplasmic domain and regulate nuclear translocation and nuclear function. We also investigated the role of the extracellular fragment of the cleaved cell adhesion molecules in regulating signaling pathways that mediate cellular migration.

Program Abstract #278

Cadherin-6B proteolytic fragments promote cranial neural crest cell EMT and delamination

Andrew Schiffmacher, Vivien Xie, Lisa Taneyhill
University of Maryland, College Park, USA

During epithelial-to-mesenchymal transitions (EMTs), cells disassemble cadherin-based intercellular junctions to permit their segregation from the epithelia. Chick premigratory cranial neural crest cells reduce existing Cadherin-6B (Cad6B) levels through several mechanisms, including proteolysis, to permit their EMT and migration. Proteolysis of Cad6B by ADAMs produces shed N-terminal fragments (NTFs), while further processing by γ-secretase generates intracellular C-terminal fragments (CTF2s). We hypothesize that Cad6B NTFs and CTF2s provide positive regulatory input into the premigratory cranial neural crest cell transition into a motile state. Here we report that Cad6B NTFs and CTF2s both possess novel, adhesion-independent roles in the cranial neural crest *in vivo*. Following proteolysis, CTF2 remains associated with β-catenin, co-imports into the nucleus, and upregulates *β-catenin*, *CyclinD1*, *Snail2*, and Snail2 promoter-based *GFP* reporter expression *in vivo*. A CTF2 β-catenin-binding mutant, however, fails to alter gene expression, indicating that CTF2s play a pro-EMT role by modulating β-catenin-responsive EMT effector genes. ChIP assays will further reveal how CTF2 controls gene expression *in vivo*. Cad6B NTFs impact the delamination of premigratory neural crest cells by prematurely compromising basement membrane integrity, as demonstrated by a specific loss of laminin. NTF-overexpressing cells also tend to delaminate from neighboring untreated neural crest cells. Zymography assays will determine if NTFs regulate protease activity to promote basement membrane degradation. Taken together, these findings reveal how Cad6B proteolysis orchestrates multiple pro-EMT regulatory inputs via the generation of distinct fragments, and provide insight into how cadherins regulate normal developmental and aberrant EMTs that underlie human disease. This work is supported by grants to AS (F32DE022990) and LAT (R01DE024217; ACS Research Scholar Grant, RSG-15-023-01-CSM).

Program Abstract #279

Twist1 and Snail1b Cooperate to Promote Epithelial-to-Mesenchymal Transition in the Neural Crest Cells of Zebrafish (*Danio rerio*).

Cristian Marchant[1], Jaime Espina[1], Elias Barriga[2], Ariel Reyes[1]

[1]Laboratorio Biología del desarrollo, Facultad de Ciencias Biológicas, Universidad Andres Bello., Chile; [2]University College London, England

Neural crest cells (NCCs) are a highly migratory, multipotent, embryonic cell population unique to vertebrates that undergo epithelial-to-mesenchymal transition (EMT) during development. The EMT process has been best studied in the context of cancer and involves several important transcription factors known as *e-cadherin* transcriptional repressors, including *Twist, Snail,* and *Sip1/Zeb2*. Experimental loss-of-function assays in different model organisms have established a pan-vertebrate neural crest gene regulatory network of NCCs during development. However, the molecular mechanisms that control EMT processes in model organisms evidence species-specific differences. In chicken, for example, Sip1 and Snail2 control the EMT of NCCs (Taneyhill *et al*., 2007; Rogers *et al*., 2103). Our group previously found that the transcriptional repression of *e-cadherin* in the NCCs of *Xenopus leavis* depends on Twist1 and not Snail1/2 (Barriga *et al*., 2013). In zebrafish (*Danio rerio*), while the repressors of transcriptional expression in NCCs are known (Twist1a/b and Snail1b), the promoter of EMT is unknown. Using immunofluorescence, we found that efficient *e-cadherin* repression in *D. rerio* is necessary for the EMT of NCCs, similar to findings in *X. leavis*. Dominant-negative Twist1a/b and Snail1b inducible by dexamethasone, used to prevent mesoderm defects, were injected separately and together. Partial defects occurred when separately injected, but a more robust response in NCC migration occurred when injected together. This suggests that both transcription factors work together to promote EMT in zebrafish NCCs. These data contribute to understanding part of the complex transcriptional mechanisms that orchestrate the EMT of NCCs.

Funding: FONDECYT 1150816.

Program Abstract #280

Neural crest cells modify their extracellular environment during enteric nervous system development by producing collagen XVIII

Nandor Nagy[1,2], Csilla Barad[2], David Dora[2], Hannah K Graham[1], Ryo Hotta[1], Jaime Belkind-Gerson[1], Allan M Goldstein[1]

[1]1Department of Pediatric Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, USA; [2]2Department of Anatomy, Histology and Embryology, Faculty of Medicine, Semmelweis University, Budapest, HU

The enteric nervous system (ENS) is derived from neural crest cells that migrate, proliferate, and differentiate into enteric neurons and glia within the intestinal wall. Many extracellular matrix (ECM) components are present in the embryonic gut, but how they promote or inhibit enteric neural crest cells (ENCC) migration is largely unknown. Chick embryos provide an attractive model system to study ECM components and their roles for ENS formation. Here, we identified heparan sulfate proteoglycan (HSPG) proteins, including collagen XVIII and agrin as important regulators of ENS development. Collagen XVIII is dynamically expressed during ENS development in the chick gut. This member of the HSPG family is expressed in preganglionic hindgut mesenchyme and its expression becomes limited to the region surrounding enteric ganglia in addition to the basement membranes of the blood vessels and gut epithelium. The source of collagen XVIII around the developing ENS is unknown. Aganglionic hindgut leads to the loss of collagen XVIII expression in the submucosal and myenteric area, but remains strongly expressed in the epithelial basement membrane. Neurospheres were prepared from embryonic and adult mouse ENCC and demonstrate collagen XVIII expression within the neurospheres. Chick-mouse intestinal chimeras were generated by implanting preganglionic mouse gut into the chick coelom. The results show that chick ENCCs colonize the mouse graft and produce collagen XVIII. We conclude that ENCCs modify their microenvironment by producing collagen XVIII which may regulate the ENCC migration by modulating the adhesiveness of ENCCs to their substratum.

Program Abstract #281

Planar Cell Polarity factor Prickle1b Regulates the Polarity and Directed Migration of Cranial Neural Crest Cells

Kamil Ahsan[1], Christina Huang[1], Ankur Saxena[2], Victoria Prince[1]

[1]University of Chicago, US; [2]University of Illinois at Chicago, US

The neural crest is an embryonic multipotent stem cell population unique to vertebrates. The migratory behavior of neural crest cells (NCCs), which has been likened to metastasis, has been studied extensively. During vertebrate neurulation, cranial neural crest cells undergo an epithelial-to-mesenchymal transition (EMT), delaminate from the dorsal aspect of the neural tube and migrate ventrolaterally into different cranial regions where they contribute to a variety of cell types including melanocytes, cartilage and neuronal cell types in the head periphery. Non-canonical Wnt/Planar Cell Polarity

(PCP) signaling has previously been described to play a role in NCC migration by regulating the dynamic and long-range migratory behavior of NCCs. Here we report a role for core PCP factor Prickle1b in regulating the morphology, polarity, and migratory behavior of NCCs in zebrafish embryos. We use live imaging of NCC behavior *in vivo* to show that Prickle1b is required for directed ventrolateral migration of cranial NCCs. Furthermore, we demonstrate that Prickle1b affects the spatiotemporal dynamics of F-actin, which is known to be regulated during EMT, during delamination as well as migration of NCCs, suggesting that Prickle1b promotes NCC migration in an EMT-dependent fashion. Together, our data indicate that Prickle1b may provide a link between EMT behavior and migration of NCCs.
This work was funded in part by the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust and by the Elias D. Joury endowment.

Program Abstract #282
Requirement for Bmp signaling in endoderm and jaw development identified from a gene-ethanol screen in zebrafish
Charles Lovely, Mary Swartz, Neil McCarthy, Jacqueline Norrie, Taylor Henegar, Johann Eberhart
University of Texas At Austin, USA
Most craniofacial birth defects are likely due to complex interactions between genes and the environment. In a series of genetic screens for gene-environment interactions, we found that *bmp4* and a forward genetic mutant, *au15*, interacted synergistically with ethanol to cause lower jaw defects. Neither of these mutants has facial defects in control conditions, but their ethanol-induced phenotypes mirror one another and phenocopy defects observed in endoderm mutants. Because the role of Bmp signaling in endoderm development is poorly understood, we first analyzed Bmp activity using a Bmp response transgenic line. We found that the endoderm receives Bmp signaling from 10-18 hours post fertilization (hpf), the same time period when *bmp4* mutants are sensitive to ethanol. Using genetic and chemical inhibitor approaches, we show that morphogenesis of the endodermal pouches and craniofacial skeleton requires Bmp signaling during this time window. Genetic chimeras show that Bmp signals directly to the endoderm for proper morphogenesis and cells that lack Bmp signaling are excluded from the pouches. Instead, these cells are retained in the medial endoderm. Time-lapse imaging demonstrates that loss of Bmp signaling results in failure of pouch out-pocketing, similar to what is seen in Fibroblast Growth Factor (Fgf) signaling mutants. Using an Fgf response transgenic line, we show that Bmp signaling regulates Fgf responses within the pharyngeal pouches. Double loss-of-function analyses demonstrate that Bmp and Fgf signaling interact synergistically in craniofacial development. We are currently determining how ethanol interacts with both *bmp4* and *au15* to disrupt lower jaw development. Unlike *bmp4*, *au15* mutants are sensitive to ethanol from 24 to 48 hpf, suggesting that it functions downstream of Bmp signaling. Overall, these data suggest that the Bmp pathway and *au15* are part of an ethanol-sensitive genetic pathway regulating jaw development.

Program Abstract #283
Investigating the role of Pcdh10a in melanocyte migration in zebrafish
Jason Williams
University of Colorado Denver, USA
Neural crest derived melanocyte precursors migrate along discrete pathways to reach their final destination in the skin. A mechanism by which neural crest cells undergo directed migration is via contact inhibition of locomotion (CIL), where weaker adhesion between cells is required for cells to move collectively forward. How neural crest cells maintain a weaker adhesion is not well understood. Cell adhesion proteins such as Protocadherins, similar to classic cadherins in that they function in cell adhesion and cell guidance, are good candidates to mediate a weaker adhesion required for contact inhibition. Here we tested the hypothesis that *pcdh10a* functionsin zebrafish neural crest derived melanocyte precursors migration by regulating actin distribution thereby promoting CIL. Through expression and loss of function analysis, we have determined that *protocadherin10a* (*pcdh10a)* is expressed in dct+ melanoctyes during neural crest migration. Loss of *pcdh10a* function results in the development of fully melanized melanocytes within the ventral pathway adjacent to the notochord and fail to reach their final position in the skin. Live cell imaging analysis suggests two phenotypes in melanocyte precursor migration: 1) dorsally located cells aggregate and cluster together; and 2) cells that are able to migrate ventrally detach from the migrating stream. In addition, actin localization in *pcdh10a*-/- neural crest cells migrating in the ventral pathway is disrupted in that actin localization along the medial cell membrane closest to the neural tube is increased. These data in combination suggest that *pcdh10a* controls migration via CIL, and in the absence of pcdh10a, a stronger adhesion between neural crest cells is observed, resulting in clumping of cells during migration and differentiation of melanocytes in ectopic locations.
This work is funded by a pre-doctoral NRSA fellowship from NIDCR (1F31DE024953) to J.S. Williams

Program Abstract #284

Defining the essential role of NSD3-mediated methylation during neural crest specification and migration

Bridget Jacques-Fricke[1,2], Jeff Barcus[2], Laura S. Gammill[2]

[1]Carleton College, USA; [2]University of Minnesota, USA

Neural crest cells are a vertebrate stem cell population that arise from the dorsal neural tube, migrate extensively to reach their final targets, and form a variety of structures. Methylation is crucial to regulate gene expression and protein activity during neural crest development; however, the full spectrum of methyltransferases involved and their impact on the neural crest developmental program remains unresolved. We recently showed that the lysine methyltransferase NSD3 is necessary for neural crest specification and independently required for neural crest migration. While NSD3 is dispensable for gene expression generally, NSD3 is essential for the expression of several key neural crest transcription factors, including *Sox10*, *Snail2*, *Sox9* and *FoxD3*, and the neural plate border gene *Msx1*. Although NSD3 is a histone H3 lysine 36 (H3K36) dimethylase (me2), surprisingly, only *Sox10* H3K36me2 occupancy requires NSD3 when select sites in these genes are analyzed. We are currently profiling H3K36me2 genome-wide from control and NSD3-deficient neural folds to identify H3K36me2 that is NSD3-dependent (ChIPseq), and how this histone methylation corresponds to NSD3-dependent gene expression (RNAseq). Interestingly, temporally restricting NSD3 loss of function to migratory stages reveals that NSD3 directly regulates neural crest migration subsequent to its essential role in neural crest gene expression. We have confirmed this crucial role for NSD3 in cell migration using C8161 human metastatic melanoma cells, a neural crest-derived cancer that serves as a cell culture proxy for neural crest cells. Upon NSD3 knockdown, C8161 cells fail to migrate, unlike wildtype cells. Together, our work reveals an essential, complex role for NSD3-mediated methylation in neural crest gene expression and cell migration.

Funding provided by MOD 5-FY-0-39, NSF IOS-1052102 and 1354809, NIH F32 DE021651, U of MN DBC Training Grant, and Carleton College Biology Dept.

Program Abstract #285

TACC3, a microtubule plus-end tracking protein, affects neural crest migration in embryonic development.

Elizabeth Bearce, Erin Rutherford, Andrew Francl, Leslie Carandang, Claire Stauffer, Matt Evans, Laura Anne Lowery

Boston College, USA

Cell migration is a pivotal aspect of embryogenesis, immune response, and wound healing, and relies upon intricate and dynamic coordination of the cytoskeleton. Microtubules (MTs) perform a myriad of critical functions during migration, due to their commanding involvement in trafficking, cell polarization, and focal adhesion (FA) turnover. MT plus-end dynamics are regulated by a conserved family of proteins called 'plus-end-tracking proteins' (+TIPs). In addition to regulating MT dynamic instability, a number of +TIPs have been demonstrated to serve distinct roles in cell motility. Our lab has recently shown that the centrosome-associated protein TACC3, a member of the transforming acidic coiled coil (TACC) domain family, can behave as a +TIP in multiple embryonic cell types, and facilitate axon elongation during neural development. Additionally, previous research indicates that TACC3 dysregulation in cancer cells can induce morphological changes consistent with EMT and an increase in invasive capacity in vitro; suggesting a role for TACC3 in cancer metastasis. However, no mechanistic role has been established for TACC3 in cytoskeletal dynamics associated with cell migration. We demonstrate that TACC3 knockdown and overexpression impacts velocity and directionality of cranial neural crest cells migrating in vitro. To explore whether these motility phenotypes can be attributed to a TACC3's function as a +TIP, we use high-resolution microscopy to monitor changes in MT stability. Finally, we demonstrate that TACC3 manipulation is sufficient to induce abnormal neural crest migration into the pharyngeal arches in vivo, supporting a role for TACC3 during developmental cell migration.

Program Abstract #286

Control of Cytoskeletal Dynamics via Light-Mediated Microtubule-Actin Crosslinking

Rebecca Adikes, Ryan Hallett, Brian Kuhlman, Kevin Slep

University of North Carolina at Chapel Hill, USA

The cytoskeleton is a dynamic network composed of microtubules (MTs), actin and intermediate filaments (IFs), which are integral in a multitude of cellular processes. Spectraplakins are the primary class of proteins that crosslink and integrate the different cytoskeletal filaments, aiding in the dynamic remodeling of the cytoskeleton. Studies in multiple model systems have reveled key roles for members of the spectraplakin family in fundamental cellular processes including polarity, morphogenesis, migration and intracellular trafficking. How the physical coupling of the cytoskeletal filaments dictates down stream cell morphological changes remains poorly understood. To begin to address this question, we developed an optogenetic tool, SXIP-iLID, to spatially and temporally crosslink MT plus ends to actin within a

subcellular region of interest. The optogenetic tool, SXIP-iLID, is based on iLID light-induced dimerization (Guntas et al., PNAS 2015) and facilitates the spatial and temporal recruitment of factors to MT plus ends. SXIP-iLID constitutively tracks MT plus ends via an interaction with end binding protein 1 (EB1). SXIP-iLID can be used to spatially and temporally recruit an SspB-tagged protein to MT plus ends when activated with 405-500 nm light. Post activation, SXIP-iLID returns to the dark-state and releases SspB-tagged proteins from MT plus ends. Here, we validate this tool in *Drosophila* S2 cells and show that it can be used to crosslinking the MT and actin networks in space and time. Light-mediated MT-actin crosslinking decreases MT growth velocities and creates a MT exclusion zone in the lamella. We are now positioned to test photo-induced crosslinking *in vivo*. Moving forward, we aim to introduce this modular system into *Drosophila* to probe how actin/MT crosslinking regulates key developmental processes, including dorsal closure.
Funding Sources: NIH F31 GM116476 to R.C.A, NIH R03 HD084980 to K.C.S

Program Abstract #287
Mical2 mutation alters actin polymerization and is associated with human ectodermal dysplasia.
Georgia Buscaglia, Jayne Aiken, Adam Almeida, Steven Rose, John Keoni Kauwe, Emily Bates
University of Colorado Anschutz Medical Campus, USA
Ectodermal dysplasia is a group of genetic syndromes characterized by abnormal development of two or more structures derived from ectoderm: skin, hair, teeth, sweat glands, tear ducts, or finger/ toe nails. Here, we characterize a case of dominantly inherited ectodermal dysplasia that is co-morbid with autism and other morphological abnormalities in some family members. We used whole exome sequencing to identify a mutation in the LIM domain of Mical2 (R1014Q) that was shared between nine affected family members and one unaffected carrier. Mical2 has a flavin adenine dinucleotide (FAD) domain for redox activity, a Calponin homology (CH) actin-binding domain, and a LIM domain with unknown function. Mical2 has a known role in regulation of the actin cytoskeleton dynamics, however, the LIM domain has not been implicated in this role. We found that expression of the mutant form of Mical2 reduced the number of neuronal precursor cells with actin stress fibers and increased the number of cells with actin bundles around the cell periphery. Immunohistochemistry suggests that the R1014Q mutation alters subcellular localization of the protein. Preliminary data suggests that ectopic expression of mutant human Mical2^{R1014Q} impedes migration of neurons in the mouse cortex, while ectopic expression of wild type human Mical2 does not significantly alter the migration of neurons.

Program Abstract #288
Wnt5a is required for proper interkinetic nuclear migration in the early fetal intestinal epithelium
Sha Wang, Deborah Gumucio
Department of Cell and Developmental Biology, University of Michigan, USA
The remarkable length of the small intestine contributes greatly to its large absorptive surface area that is required for efficient nutrient absorption. The intestinal elongation prior to birth is critical to establish proper intestinal length. Human infants born with abnormally short intestines, known as congenital short bowel syndrome (CSBS), have very high mortality rates. However, little is known about how proper intestinal length is achieved during embryogenesis at both cellular and molecular levels. Wnt5a is a representative non-canonical Wnt ligand expressed in the intestinal mesenchyme. Previous investigators found that Wnt5a is required for gut elongation (Cervantes et al., 2009). Failure of radial intercalation of stratified epithelial cells was postulated to contribute to the short Wnt5a$^{-/-}$ gut. However, it has been recently demonstrated that intestinal epithelium is pseudostratified, not stratified (Grosse et al., 2011). Cells, in the early pseudostratified epithelium, are actively cycling and exhibit interkinetic nuclear migration (IKNM, nuclei move up and down in the epithelium, in accord with the cell cycle), which was misinterpreted as radial intercalation. After mitosis happening at apical zone, daughter cells must connect *de novo* with the basement membrane. Absence of Wnt5a impairs this basal attachment and results in anoikis. We found that loss of Wnt5a does not perturb proliferation but instead increases apoptosis and randomizes the distribution of two daughter cells along the anterior-posterior axis, which cause the severely shortened intestines.

Program Abstract #289
Collective Cell Migration of the Nephric Duct
Lital Attia, Ronit Yelin, Tom Schultheiss
Technion-Israel Institute of Technology, IL
During the course of development, the vertebrate nephric duct (ND) extends and migrates from the place of its initial formation, adjacent to the anterior somites, until it inserts into the bladder or cloaca in the posterior region of the embryo. The molecular mechanisms that guide ND migration are still poorly understood. First, a system was developed for live

imaging of ND migration in the chick embryo at the individual cell level through use of GFP driven by a ND-specific enhancer element of the Gata3 gene. Use of this system on normal embryos revealed that cells in different regions of the duct migrate at different rates, with cells at the leading posterior duct tip migrating 4-fold faster than more anterior duct cells. This system and other approaches were then used to examine the role of FGF signaling during ND migration. It was found that FGF receptor inhibition blocks nephric duct migration. In combination with data showing expression of FGF Receptors and FGF response genes in the ND, and FGF ligands in surrounding tissues, these results indicate that FGF signaling is required for nephric duct migration. Placement of a localized source of FGF signal adjacent to the nephric duct did not affect the duct migration path, indicating that FGF signaling is not sufficient to determine the migration pathway of the nephric duct. Taken together, these studies indicate that FGF signaling acts as a "motor" that is required for duct migration, but that other signals are likely needed to determine the directionality of the duct migration pathway. We have also used this experimental system to investigate the role of canonical Wnt signaling in ND migration. We find that Wnt signaling is required for cohesiveness of migrating ND cells, and that when Wnt signaling is inhibited, individual cells wander away from the ND structure. We are currently investigating the cellular and molecular basis of the loss of ND cell cohesiveness upon the lowering of Wnt signaling.

Program Abstract #290

Zebrafish lines with lymphocyte cytosolic protein (lcp1) loss-of-function.

Elizabeth LeClair[1], Rachel Riccio[1], Emily Baumgartner[1], Jacek Topczewski[2]
[1]DePaul University, USA; [2]Department of Pediatrics, Northwestern University Feinberg School of Medicine, USA

Cell movement is essential for the normal development and function of the immune system, but can be destructive in diseases such as cancer. How cells regulate motility is therefore an important issue. In this study, we investigate the actin-bundling peptide lymphocyte cytosolic protein 1 (L-plastin, Lcp1), a highly conserved component of the eukaryotic cytoskeleton. This gene was originally discovered in neoplastic human fibroblasts, and is significantly upregulated in many cancer cell lines and solid tumors. Interestingly, L-plastin is also abundantly expressed by normal leukocytes, including macrophages, monocytes, and neutrophils. Zebrafish have only one ortholog of *lcp1*, facilitating gene targeting of this locus. Using CRISPR/Cas9 mutagenesis, we modified Exon2 of *lcp1*, generating four novel alleles. Three alleles are predicted to cause severe, premature truncations of the protein; the fourth modifies one of the N-terminal 'EF-hand' calcium-binding domains, which may affect the tertiary structure of the peptide. A comparison of two of the truncation alleles shows that 1) all homozygote *lcp1* mutant embryos (-/-) completely lack Lcp1 immunostaining, but 2) that these mutants can survive in normal Mendelian ratios. These novel, viable mutants should allow further analyses of cell-motility phenotypes relating to development, immunity, infection, and cancer.

Program Abstract #291

VegF signaling is required for PMC migration from the ventrolateral clusters in the sea urchin *Lytechinus variegatus*

James Huth, Daniel Zuch, Cynthia Bradham
Boston University, USA

Vascular endothelial growth factor (VegF) signaling has been implicated in regulating both biomineralization and skeletal patterning in sea urchin embryos. Inhibition of VegF signaling disrupts the arrangement of primary mesenchyme cells (PMCs), which are responsible for skeleton formation, although this has not been studied in detail. To rigorously assess the effect of VegF signaling on PMC positioning within the blastocoel, we assessed PMC positioning and marker gene expression at different stages of development in *Lytechinus variegatus* embryos treated with axitinib, a VegF Receptor antagonist. We found that PMCs in treated embryos remained primarily in the ventrolateral clusters during gastrulation, failing to migrate into the typical ring and cords arrangement. PMCs that did exit the clusters tended to remain clumped in small groups. Axitinib-treated embryos continued to show clustered PMCs into the pluteus stage, suggesting that VegF signaling is required for PMCs to shift from cluster- to linear-type adhesion.

This work was supported by the National Science Foundation (NSF) [IOS 950030254 to C.B.].

Program Abstract #292

Dynamics of Pigment Cell EMT and MET in the Sea Urchin *Lytechinus variegatus*

Andrew George
Duke University, USA

Epithelial-to-mesenchymal transition (EMT) is a dynamic cellular process that occurs normally during early development and is similarly re-deployed during cancer metastasis. The sea urchin embryo provides a tractable system to observe this

process in vivo. Previous work has identified gene regulatory networks that govern different aspects of this cellular process in the primary mesenchymal cells. Much like EMT, mesenchymal-to-epithelial transition (MET), occurs normally throughout development during somitogenesis, cardiogenesis, hepatogenesis and in the neural crest. There is however, little mechanistic understanding of the dynamics of MET. Pigment cells of the sea urchin embryo undergo both an EMT and MET allowing us to study the in vivo cellular dynamics and generate gene regulatory networks that govern these processes. At the onset of gastrulation, pigment cell precursors undergo an EMT at the tip of the archenteron and begin to migrate down the side of the gut where they undergo a MET and incorporate back into the ectoderm. Using a combination of live imaging, gene knockdowns, and in situ hybridization of previously identified markers of pigment cells, as well as novel markers; we can begin to dissect the regulation of pigment cell MET. By understanding the molecular mechanisms that govern MET in the sea urchin, we will gain valuable insights into the genetic control of an essential developmental process, allowing for a better understanding of many diseases.

Program Abstract #293

Lipoxygenase activity regulates PMC positioning during secondary skeletogenesis in the sea urchin *Lytechinus variegatus*

Daniel Zuch, Michael Piacentino, Kanwal Aziz, Sviatlana Rose, Cynthia Bradham
Boston University, USA

Patterning of the sea urchin larval endoskeleton offers a unique platform to gauge developmental plasticity *in vivo*. The 3D configuration of skeletal elements in a mature larva is delimited by the migration of primary mesenchyme cells (PMCs), which integrate local positional cues within the blastocoel and arrange into a stereotypic pattern, along which they secrete calcium carbonate biomineral. From an RNA-Seq screen for transcripts involved in PMC positioning, we have identified LvLipoxygenase (LOX) as a potent regulator of PMC migration toward and with respect to the bilateral midline. LOX is expressed in the apical and ventrolateral ectoderm, where it converts arachidonic acid to hydroxyeicosatetraenoic acids (HETEs). HETEs and their hormone-like metabolites exhibit a range of autocrine and paracrine signaling properties in several species. In *Lytechinus variegatus*, knockdown of LOX expression typically produces embryos with missing bilateral midline skeletal elements or elements rotated with respect to the midline. Pharmacological inhibition of LOX with MK886 mimics these effects. In contrast, ectopic introduction of HETEs typically results in embryos with right-side skeletal defects. The spectrum of observed skeletal defects suggests that HETEs signal to PMCs both locally and at a distance. While early PMC positioning is generally predictive of downstream skeletal pattern formation, LOX perturbation impacts PMC migration only after the primary skeleton has been initiated. These results suggest that tight spatiotemporal regulation of migratory cues throughout skeletogenesis is crucial for appropriate positioning of PMCs and patterning of the resulting larval skeleton. Funding provided by the National Science Foundation.

Program Abstract #295

Mitotic safeguards of postmitotic cortical neurons

Itsuki Ajioka, Mio Oshikawa
Tokyo Medical and Dental University, JP

Although neurons are able to re-enter the cell cycle in pathological situations such as neurodegeneration and stroke, they are tightly protected from cell division and undergo cell death after S phase progression. Neurons become permanently post-mitotic immediately after cell cycle exit during development, however, the mechanism underlying non-dividing feature is mostly unknown.

The Retinoblastoma protein family (Rb, p107, p130) plays a central role in preventing cells from entering the S phase. When Rb family expression is lost in neuronal progenitor cells, the subsequent coordination of cell-cycle exit and neuronal differentiation is lost, and neurons can divide in some cases such as retinoblastoma (Ajioka et al., ***Cell*** 2007). We recently developed a technique for conditionally inactivating all of the Rb family members in mouse cortical progenitors, either before or immediately after cell-cycle exit, by electroporation with Cre-expressing plasmids containing a ubiquitous pCAG promoter or a neuron-specific pMAP2 promoter (Oshikawa et al., ***Development*** 2013). When the Rb family is inactivated using pCAG-Cre, immature neurons generated from the pCAG-induced Rb-TKO ($Rb^{-/-}; p107^{-/-}; p130^{-/-}$) progenitors divide. In contrast, the pMAP2-induced Rb-TKO immature neurons enter the S phase, but undergo cell death. Thus, once progenitor daughter cells exit the cell-cycle and initiate neuronal differentiation, they are prevented from undergoing cell division, and maintain mitotic resistance even after acute Rb family inactivation. These findings led us to hypothesize that pathological neurons in the S phase undergo cell death by activating mitotic safeguards.

Here, we identified such mitotic safeguards. We will also demonstrate the cell division of dying hypoxic cortical neurons

in the S phase by inactivating the mitotic safeguards. Our results may represent a novel strategy for treating neurological disorders.

Program Abstract #296
Control of Pancreatic Endocrine Differentiation by the Planar Cell Polarity Pathway
Lydie Flasse[1], Cédric Cortijo[2], Manuel Figueiredo-Larsen[1], Anne Grapin-Botton[1]
[1]Danish Stem Cell center, Denmark; [2]Swiss Institute for Experimental Cancer Research, Switzerland
Processes that coordinate cellular behaviors or cell polarity within the plane of the epithelium are essential for tissue morphogenesis. Recent work in the laboratory has demonstrated that the planar cell polarity pathway (PCP) is required in the developing pancreas for the differentiation of endocrine cells from epithelial progenitors lining a network of ducts (*Cortijo et al., 2012*). In this project, we are investigating how the PCP components organize pancreatic progenitors in space and how this spatial organization can control the fate of endocrine progenitors during embryogenesis. To address these questions, using *in vivo* and *ex vivo* approaches, we are performing a comparative analysis of mutant mice harboring inactivation of different members of the core PCP pathway (Vangl mutant, Fz3/6 DKO). We show that Looptail mice, in which the function of Vangl1 and 2 is inactivated, exhibit morphological defects including an abnormal ductal tree and an enlargement of the ducts; as well as a decrease by 60% of the number of insulin expressing cells. This analysis is complemented by the characterization of a transgenic line where a Vangl2-cherry fusion protein is expressed in pancreatic progenitors. These mice exhibit mislocalisation of the Vangl2-Cherry protein in the progenitors which leads to hypoplasia. Taken together, our experiments show an important role of the PCP pathway in pancreatic growth and start to decipher the molecular components mediating this pathway in the control of cell differentiation. This work highlights the importance of the 3D organization and collective communication of cells within the pancreatic epithelium to generate appropriate numbers of endocrine cells.

Program Abstract #297
Investigate the Function of Wnt/ Planar Cell Polarity Signaling in Skeletal Development
Kun Yang
Harvard School of Dental Medicine, United States
Planar Cell Polarity (PCP) is emerging as an essential mechanism whereby embryonic morphogenesis is controlled. Regulation of PCP is conserved from *Drosophila* to human, and is regulated by Wnt ligands. Wnt signaling gradients act through PCP to provide directional information in many fundamentally critical processes of embryonic morphogenesis including cartilage elongation in the limb. Our lab has previously established that Vangl2, a core PCP component, is asymmetrically localized on cell membrane in developing mouse embryo digit. Wnt5a signaling is required to regulate Vangl2 asymmetrical localization by inducing a receptor complex that contains Vangl2; and Vangl2 is phosphorylated in a Wnt5a dose-dependent manner. To further understand how Wnt gradient and Vangl2 asymmetrical localization guide cartilage morphogenesis, here we show that Cadherins as the cell-cell adhesion molecules are internalized by Vangl2. We further identified that N-cadherin asymmetrically localized in mouse embryonic limb digits on the sides of the cell without Vangl2 localization. These findings suggest that Wnt5a, as a global tissue morphogenesis cue, recruit cells into tissues by regulating cadherin-based cell adhesion.
Funding source: Harvard School of Dental Medicine Start-up Funding

Program Abstract #298
Astrotactin2 regulates planar cell polarity in mammalian skin
Hao Chang, Jeremy Nathans
Johns Hopkins University, United States
Planar cell polarity (PCP) signaling controls the global orientation of surface structures, such as hairs and bristles, in both vertebrates and invertebrates. In *Frizzled6*$^{-/-}$ (*Fz6*$^{-/-}$) mice, hair follicle orientations on the head and back are nearly random at birth, but reorient during early postnatal development to eventually generate a wild-type-like anterior-to-posterior array. We recently identified that deletion of *Astrotactin2* (*Astn2*) exon5 modifies the hair follicle orientation phenotype in *Fz6*$^{-/-}$ mice. In *Fz6*$^{-/-}$*;Astn2*$^{ex5del/del}$ mice, hair orientation on the lower back is subtly biased from posterior-to-anterior at birth, leading to a 180-degree orientation reversal in mature mice. How the exon5 deletion of *Astn2* alters skin development and thus modifies the hair follicle orientations in *Fz6*$^{-/-}$ mice is not clear. Astn2 has a signal peptide, two transmembrane domains, and an unusual transmembrane topography in which both N- and C-termini reside on the extracellular face of the membrane. Deletion of *Astn2* exon5 leads to an in-frame deletion of 36 amino acids in the predicted cytosolic domain. In vitro over-expression experiments with an Astn2 cDNA that carries the exon5 deletion in

HEK293 cells indicate that the mutant protein is stable. These data suggest that deletion of exon5 may alter but not abolish Astn2 function. To fully abolish Astn2 function and elucidate its role in PCP, we have made an *Astn2* conditional knockout mouse line in which the ATG-containing exon1 is flanked by two *lox*P sites. Together, these studies will lead a better understanding on the role of Astn2 in the regulation of mammalian PCP signaling.

Program Abstract #299

The adaptor protein Cindr fine-tunes JNK activity to maintain tissue integrity and regulate cell movement during morphogenesis.

Hannah Yasin, Sam van Rensburg, Christina Feiler, Ruth Johnson
Wesleyan University, USA

The conserved adaptor protein Cindr (Cd2ap/Cin85) regulates adhesion and the actin cytoskeleton. Reducing Cindr during development disrupts *Drosophila* eye patterning because cells become ectopically motile and fail to acquire correct stereotypical positions. In the fly wing, loss of Cindr similarly disrupts cells but in this tissue the predominant phenotype is dramatic loss of epithelial integrity: cells delaminate from the epithelium and migrate short distances before dying. These observations lead us to explore the role that Cindr plays in maintaining appropriate adhesion and cytoskeletal architecture in the dynamic context of epithelial morphogenesis. We find that Cindr interacts with Basket, the *Drosophila* Jun N-terminal Kinase (JNK). This interaction represses JNK activity that would otherwise induce cell delamination, migration and death. In genetic interactions we observed that the effects of reducing Cindr in epithelia are rescued when JNK signaling is genetically compromised. Together, our genetic and biochemical data suggest that Cindr is required to limit JNK signaling and we are testing models to determine the mechanism Cindr uses to do this.

Program Abstract #300

Semaphorin-1b is required for oocyte polarization in *Drosophila melanogaster*

Julia Wittes
Princeton University, United States

During *Drosophila* oogenesis, signaling between the germline and somatic follicle cells organizes the future embryonic axes. At mid-oogenesis, Gurken protein in the oocyte signals through EGFR/Torpedo to specify the posterior follicle cells (PFCs). Shortly thereafter, the PFCs signal back to the oocyte, triggering the repolarization of the oocyte microtubule cytoskeleton and the proper localization of the posterior determinant *oskar* and anterior determinant *bicoid*. This signal, known as the posterior or polarizing signal, has yet to be identified. We performed microarray and RNAi screens to discover novel posterior signaling genes and identified *Semaphorin-1b* (*Sema-1b*). Sema-1b is a member of the Semaphorin family of signaling proteins, which have been implicated in several developmental processes, but which have not yet been shown to function during oogenesis. We report that *Sema-1b* RNAi in the follicle cells causes a failure of oocyte repolarization but does not compromise overall epithelial integrity or interfere with specification of the PFCs. We will also present evidence suggesting that Sema-1b protein is expressed in the PFCs and that it may be activated in response to EGFR signaling. Our findings suggest that the *Drosophila* egg chamber may provide a new system in which to study the activation, mechanism, and downstream effectors of Semaphorin signaling. This work is supported by National Institutes of Health Grant R01 GM077620.

Program Abstract #301

Single-particle dynamics underlying the segregation of GFP::PIE-1 during asymmetric division of the *C. elegans* zygote

Youjun Wu, Erik Griffin
Dartmouth College, Hanover NH 03755, USA

During the asymmetric division of the *C. elegans* zygote, the cytoplasmic RNA-binding proteins MEX-5/6 and PIE-1, which are initially uniformly distributed, redistribute to form anterior-rich and posterior-rich concentration gradients, respectively (Mello 1996; Schubert 2000; Tenenhaus 1998). The posterior kinase PAR-1 controls the segregation of MEX-5 by increasing MEX-5 mobility in the posterior cytoplasm, causing the preferential retention of MEX-5 in the anterior cytoplasm (Tenlen, 2008; Daniels, 2010; Griffin, 2011). In turn, MEX-5/6 act to increase the mobility of PIE-1 in the anterior cytoplasm, resulting in the preferential retention of PIE-1 in the posterior cytoplasm (Wu, 2015). In order to determine the mechanisms by which MEX-5/6 control the mobility of PIE-1, we have used Near-TIRF imaging to characterize the dynamics of individual GFP::PIE-1 particles in the polarized zygote. We find that GFP::PIE-1 is present in two classes of particles: a rapidly diffusing population that is uniformly distributed and a slow-diffusing population that is enriched in the posterior cytoplasm. Slow-diffusing GFP::PIE-1 particles appear and subsequently disappear from the

same position in the embryo, suggesting fast and slow-diffusing particles frequently interconvert. Slow-diffusing GFP::PIE-1 particles appear four times more frequently and persist significantly longer in the posterior cytoplasm, leading to their enrichment in the posterior. Interestingly, we find that MEX-5/6 controls both the appearance rate and persistence of slow-diffusing GFP::PIE-1 particles. Furthermore, GFP::MEX-5 is present in anteriorly-enriched slow-diffusing particles whose dynamics are similar to slow-diffusing GFP::PIE-1 particles. Based on these results, we propose that MEX-5/6 compete with PIE-1 for interaction with a relatively static cytoplasmic structure such that PIE-1 binding is biased to the posterior where MEX-5 concentration is low.

Program Abstract #302

Flexibility of a wave-inducing genetic cascade explains evolutionary diversity in insect early embryogenesis

Ezzat El-Sherif[1], Xin Zhu[2], Heike Rudolf[1], Jutta Distler[1], Paul Francois[3], Susan Brown[2], Martin Klingler[1]
[1]Friedrich-Alexander Universität Erlangen-Nürnberg, DE; [2]Kansas State University, US; [3]McGill University, Canada

While the basic body plan of animals is highly conserved, earlier and later embryonic events are quite diverse, a phenomenon termed 'the hourglass model'. All insects have a segmented body plan, where anterior segments arise in a non-growing 'blastoderm', and posterior segments arise in a growing 'germband'. However, insects differ widely in the number of blastodermal versus germband segments. Here we show, using *in vivo* and *in silico* evidence, that patterning in both blastderm and germband of the beetle *Tribolium* is based on the same flexible mechanism: a genetic cascade of gap genes that induces waves of gene expression. The mechanism is capable of converting blastodermal to germband fates and vice versa. Using RNAi and heat shock perturbations, the mechanism was dissected and balstodermal fates were induced in the germband, and germband fates were indeuced in a blastoderm-like morphology. Computational modeling suggests two genetic programs are involved, in line with the recent finding of non-redundant shadow enhancers in *Drosophila*. Our work suggests a simple mechanism for evolutionary flexibility in early insect development.

Program Abstract #303

TGF beta signaling and axis formation in the annelid *Capitella teleta*

Alexis Lanza, Elaine Seaver
University of Florida, USA

Embryonic organizers are signaling centers that coordinate developmental events within an embryo. Localized to either an individual cell or group of cells, embryonic organizing activity induces the specification of other cells in the embryo and can influence formation of the body axes. In the spiralian *Capitella teleta*, previous cell deletion studies have shown that organizing activity is localized to a single cell, 2d, and this cell induces the formation of the dorsal-ventral and left-right axes. In this study, we attempt to identify the signaling pathway responsible for the organizing activity of 2d. Embryos at stages when organizing activity is occurring were exposed to various small molecule inhibitors, raised to larval stages, and scored for axial anomalies analogous to previously described phenotypes. Our results suggest that the MAPK, Notch, and Wnt/β-catenin signaling pathways do not play a role in 2d's specification of the dorsal-ventral axis. However, interference with the TGF beta signaling pathway through a short 3 hr. exposure to the inhibitor SB431542 results in larvae that lack bilateral symmetry and a detectable dorsal-ventral axis. These and further investigations will shed light on the identity of the 2d signaling pathway involved with *Capitella* axes formation, and contribute to our understanding of the evolution of body plan diversity.

Funding source: NSF

Program Abstract #304

Quantitative aspects of NFκB activity in *Drosophila melanogaster* DV patterning.

Maira Cardoso[1], Desiree Nunes[1], Marcio Fontenele[1], Francisco Lopes[1], Stanislav Shvartsman[2], Paulo Bisch[1], Helena Araujo[1]
[1]Federal University of Rio de Janeiro, BR; [2]Princeton University, USA

The Dorsal (Dl) transcription factor, member of the NFkappaB family, regulates dorsal-ventral patterning of the *Drosophila* embryo, as well as antimicrobial and anti-fungal peptide production during the innate immune response. In both contexts, Cactus (Cact), the sole IkappaB homologue in *Drosophila*, inhibits Dl activity by preventing Dl nuclear translocation. Two pathways regulate Cact levels and thus regulate this ability of Cact to inhibit Dl: Toll pathway activation generates a signaling cascade that promotes phosphorylation and ubiquitination of Cact molecules complexed to Dl (Cact/Dl) and consequent Cact degradation by the proteasome. The second mechanism that regulates Cact levels is mediated by Calpain A (CalpA), a Calcium-dependent cysteine protease that cleaves free Cact molecules present as dimers in the cytosol. We hypothesize that CalpA cleaves the Cact N-terminal region releasing a fragment that is

unresponsive to the Toll Pathway (CactE10) and a Cact monomer ready to complex with new Dl molecules. Both pathways may be required to promote the correct balance of Cact molecules in the cytoplasm in order to refine the Dl Gradient during embryogenesis. To test this hypothesis, we have quantitatively analyzed the Dl gradient during embryogenesis, as well dorsal-ventral expression domains regulated by Dl, in embryos expressing different doses of *cact* hypomorphic alleles or overexpressing *cactE10* and full-length *cact*. These genetic contexts allow us to disturb the balance between endogenous Cact and Dl levels, and analyze the ability of CactE10 to rescue this equilibrium as well as its impact on the Dl gradient. Interestingly, *cactE10* overexpression alters the Dl gradient only when Dl levels are reduced. Moreover, decreased Dl and Cact levels lead to stochastic expression of Dl target genes in ventral regions of the embryo. These unexpected results suggest that the balance between free and Dl-bound Cact levels has greater importance than previously described.

Program Abstract #305
A New Role for Raf in Dorsal-Ventral Patterning of the *Drosophila* Embryo
Jay B. Lusk[1], Nicholas S. Tolwinski[1,2]
[1]Yale-NUS College, Singapore; [2]National University of Singapore, Singapore
The Ras-Raf-MEK-ERK pathway was one of the first major oncogenic pathways to be discovered, and in recent years a great deal of research on drug development has been done in the hope of developing more effective cancer treatments. However, there remain significant gaps in our knowledge about the precise mechanism of action used in the pathway. Study of its effects in model organisms would inform human therapy; therefore, understanding the contribution the pathway has in early *Drosophila* embryogenesis is of significant clinical value. Here we show that a new allele of the *Drosophila* homolog of Raf, D-Raf, results in very early dorsalization of the developing embryo. The allele causes a change in protein folding in a conserved region of Raf which has been shown in humans to be the site of Ras binding. This finding implicates Raf in a new role in determining Dorsal-Ventral polarity in the early stages of embryogenesis, and provides new clues to the mechanism of the function of the Ras-Raf binding site, a finding which will help inform future therapies targeting this pathway. This work was supported by the Yale-NUS College Student External Presentation Award, the Yale-NUS College Student Associate Program, and an Academic Research Fund (AcRF) grant (MOE2014-T2-2-039) of the Ministry of Education, Singapore to N. Tolwinski.

Program Abstract #306
V-type H+ ATPase activity is required for dorsal-ventral specification in sea urchin embryos
Daphne Schatzberg, Sarah Hadyniak, Luz Dojer, Patrick Reidy, Cynthia Bradham
Boston University, USA
Specification of the dorsal-ventral (DV) body axis during embryogenesis is a fundamental event that is required for body patterning and embryonic viability. Specification of the ventral domain in the ectoderm of sea urchin embryos is a symmetry-breaking event in which a subset of the pre-DV ectoderm begins to express Nodal, a TGF-beta ligand. Asymmetric Nodal expression is proposed to be initiated downstream of asymmetric p38 activity and/or asymmetric distribution of mitochondria; however, this event is not well understood. We have identified an endogenous voltage gradient that is present prior to the initiation of Nodal expression. The voltage gradient is present across the embryonic axis which becomes specified as the DV axis. V-type H^+ ATPase (VHA) activity is required for the presence of the voltage gradient. Treatment of sea urchin embryos with VHA inhibitors radializes *nodal* expression from the earliest time points at which it can be detected by in situ hybridization. In fact, all ventrally expressed genes we have assayed are radialized, including the Nodal inhibitor *lefty* and *bmp2/4*, which is required for dorsal specification. Embryos treated with VHA inhibitors develop a ventralized phenotype at pluteus stage that phenocopies Nodal overexpression. Experimental restriction of Nodal expression by zygotic injection of Nodal morpholino followed by injection of the constitutively active Nodal receptor Alk4/5/7 Q271D in one blastomere at the four cell stage is sufficient to rescue the effects of VHA inhibition and allow the development of morphologically normal larvae. These results suggest that an endogenous voltage gradient is required for the asymmetric expression of Nodal, without which DV axis specification does not occur. This work was supported by the National Science Foundation (CAB IOS 950030254) and start-up funds from Boston University (CAB).

Program Abstract #307
Proteinase K treatment radializes the dorsal-ventral axis in sea urchin embryos
Lina Soto, Daphne Schatzberg, Cynthia Bradham
Boston University, USA

Mechanisms of dorsal-ventral (DV) specification are conserved throughout the animal kingdom. In sea urchins, the DV axis is specified early in development with the expression of Nodal, a TGF-beta signaling ligand, in the ventral region of the embryo. As Nodal signals locally, it activates the expression of BMP2/4 and Lefty. BMP2/4, another TGF-beta signaling ligand, is expressed ventrally, but travels to the dorsal region of the embryo to signal and specify dorsal tissues. As Lefty diffuses away from the ventral territory where it is expressed, it inhibits the expansion of Nodal dorsally by restricting it to the ventral region of the embryo. The antagonistic relationship between Nodal and Lefty allows the embryo to establish the dorsal-ventral axis. When we bathe sea urchin embryos with proteinase K at fertilization they develop a radialized phenotype at pluteus stage, which is characteristic of perturbations to the DV axis. We show that Lefty is sensitive to proteinase K degradation by proteolytic digest of the protein on a western blot. Ciliary band and neural stains of treated embryos suggests that the embryos are ventralized and resemble a Nodal gain-of-function phenotype as opposed to a BMP2/4 loss-of-function phenotype. In situ hybridization of proteinase K treated embryos at mesenchyme blastula stage reveals that ventral genes such as *chordin, nodal, bmp2/4,* and *lefty* are radialized, while dorsal markers such as *tbx2/3* and *irxA* are not present. Quantitative analysis of mRNA levels by qPCR agree with the in situ hybridization results. This data suggests that proteinase K treatmentperturbs the dorsal-ventral axis by degrading lefty, thereby allowing Nodal to expand into the unspecified dorsal region of the embryo where it preempts BMP2/4 signaling. This project was supported by NSF (CAB IOS 950030254), start up funds from Boston University (CAB), and Boston University programs SURF and UROP.

Program Abstract #308

Collinear expression of the HoxD cluster is controlled by short and long-range regulation in the embryonic reproductive system.

Christopher Bolt

École Polytechnique Fédérale de Lausanne, Switzerland

The vertebrate *Hox* gene clusters exhibit a remarkable property where the position of each gene in the cluster corresponds to its position of expression along the primary body axis. This property, called collinearity, is essential to the proper organization of the body plan. Mutations that disrupt collinearity produce homeotic transformations, that is, where body portions incorrectly expressing *Hox* genes improperly adopt a fate reminiscent of adjacent body sections. Enhancer-reporter studies demonstrate that genetic elements located directly adjacent to the *Hoxd* gene promoters are sufficient for expression along the primary body axis while elements for more recent evolutionary productions such as limbs and external genitalia reside in the surrounding gene deserts. The intermediate mesoderm, which extends along the posterior portion of the primary body axis, gives rise to the internal reproductive organs. Interestingly, the expression domains of many *Hoxd* genes along the intermediate mesoderm correspond to the ontogenic domains of the reproductive organs. However, promoter-proximal elements are not sufficient to fully recapitulate expression of native *Hoxd* genes in these tissues and *Hox* gene loss-of-function mutations produce homeotic transformations in the reproductive system suggesting the operation of a distinct collinearity program. Using 4C-Seq and ATAC-Seq we are evaluating the *HoxD* gene deserts for regulatory elements to determine the influence of these enhancer-rich environments on *HoxD* expression during the formation of the mouse reproductive systems. Our data indicate a complex mechanism of regulation where positive and negative influences extend from the 5' gene desert and from within the cluster itself, while the 3' desert has no apparent activity. This mechanism appears to have a similar influence on the male and female reproductive organs even though the majority of these tissues have separate positions and times of formation. Funding provided by ÉPFL.

Program Abstract #309

Regulation of microtubule reorganization required for primary embryonic axis induction in zebrafish

Elaine Welch, Francisco Pelegri

University of Wisconsin-Madison, USA

In vertebrates such as amphibians and fish, axis induction involves the transport of maternally-derived factors from the vegetal pole of the egg to the prospective organizer in the animal region of the embryo. We previously reported that the maternal-effect gene *hecate*, which when mutated causes axis induction defects, encodes Glutamate receptor interacting protein 2a (Grip2a), and that *grip2a* mRNA is localized to the vegetal pole of the oocyte. Upon egg activation, mRNAs for *grip2a* and the proposed dorsal determinant *wnt8a*, as well as the kinesin adaptor protein Syntabulin, undergo an off-center shift consistent with a proposed cortical rotation-like movement. *hecate* functions in microtubule reorganization and bundling essential for this off-center shift. We also identified and characterized the maternal gene *too much information (tmi)* and found that it encodes Protein regulator of cytokinesis 1-like (Prc1L), known to have a role in spindle microtubule overlap during cell division. Embryos from females homozygous for *tmi/pcr1L* mutation are defective in

cytokinesis and exhibit a shortened spindle. Unexpectedly, we find that *tmi/prc1L* mutants also exhibit a disruption of the parallel microtubule network at the vegetal pole, thereby leading to defects in axis induction. Further studies using maternal-effect mutants and small molecule inhibitors identified other midbody regulators, in addition to Prc1, important for vegetal microtubule reorganization at the vegetal pole, such as Aurora Kinase B and Polo-like Kinase 1. Our studies identify factors that mediate microtubule reorganization at the vegetal pole involved in axis induction, revealing a role for midbody-associated factors in this process.
This work is supported by NIH RO1 GM065303

Program Abstract #310
A Wnt signaling network that directs anterior-posterior patterning in the sea urchin embryo
Ryan Range
Mississippi State University, USA
Studies in several deuterostome developmental models, including the sea urchin, suggest that an early, broad regulatory state initiates specification of the anterior neuroectoderm (ANE). During early development, a posterior-to-anterior wave of inductive signaling progressively positions this broad ANE potential along the anterior-posterior (AP) axis to a territory around the anterior pole. However, the molecular mechanisms used during ANE positioning are incompletely understood in any deuterostome embryo. Our recent results indicate that the ANE positioning mechanism in the sea urchin embryo involves integration of information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways. We have also found that secreted Wnt signaling modulators at the anterior pole act as a signaling center that is integrated into this Wnt network and establishes the ANE boundary and subsequently patterns the ANE territory. These studies provide the framework for our current focus on functional characterization of several transcription factors identified in whole-transcriptome differential screens whose expression is driven by the Fzl5/8 (Wnt/JNK) and Fzl1/2/7 (Wnt/PKC) signaling pathways, looking for functional interactions at the transcriptional level during ANE positioning. In addition, we are performing functional analyses on several potential extracellular and intracellular modulators of Wnt signaling in an effort to determine potential roles for these factors in the ANE positioning mechanism. These are the first steps in an our strategy to use a combination of high throughput genome-wide assays, molecular manipulations, and gene regulatory network analysis to produce a systems-level model of how a Wnt network governs a fundamental deuterostome developmental process.

Program Abstract #311
Collinear Hox-Hox Interactions Are Involved in Patterning the Vertebrate Anteroposterior (A-P) Axis
Kongju Zhu, Herman Spaink, Antony Durston
Institute of Biology, Leiden University, NL
Understanding the regulation and function of the Hox genes, key regulators of positional identity in the embryo, is of crucial importance for emergent medical technologies: stem cell therapy, in vitro organoid culture and targeted cancer therapies. One striking feature of Hox genes is collinearity: the temporal and spatial orders of expression of these clustered genes match their 3' to 5' order on the chromosome. Despite recent progress, the mechanism underlying collinearity is still not well understood. Here we use ectopic expression in wild-type and *noggin*-injected (Hox-free) *Xenopus* embryos, to show that two Hox-Hox interactions are crucial. Posterior induction (induction of posterior genes by anterior ones), drives Hox temporal collinearity (Hox timer), which itself drives anteroposterior (A-P) patterning. Posterior prevalence (repression of anterior Hox genes by posterior ones) is important in translating temporal to spatial collinearity. We show for the first time that collinear Hox interactions are the key to vertebrate axial patterning.

Program Abstract #312
A pre-gastrulation damage response uncovered by single-cell RNAseq
Jeffrey Farrell[1], Andrea Pauli[2], Alexander F. Schier[1]
[1]Harvard University, USA; [2]Institute of Molecular Pathology, Austria
Developing embryos nearly always manage to produce viable and phenotypically wild-type embryos, despite facing challenges from their environment (such as DNA damage), which suggests a remarkable capacity to recover from errors and damage. In most situations, cells with unrepaired DNA damage would be eliminated by apoptosis to prevent deleterious genomic changes that can result in uncontrolled growth and potentially tumorigenesis. However, embryos across many phyla are incapable of committing apoptosis until during gastrulation. This raises the question: what happens to damaged cells from pre-gastrulation embryos? Through single-cell RNAseq of zebrafish embryos just prior to gastrulation, we uncovered a previously undescribed cellular expression program that may hold the answer, which we call

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

Program Abstract #313
Vg1/Dvr1 is an essential regulator of vertebrate germ layer formation
Tessa Montague, Alexander Schier
Harvard University, USA
Vg1, a member of the TGF beta family, was discovered almost 30 years ago as a maternally localized mRNA in *Xenopus*. It has been implicated in mesoderm induction, yet overexpression studies have not yielded phenotypes, and knockdowns in zebrafish have suggested only a later role during left-right patterning. We created a null mutation in zebrafish *vg1*. The resulting embryos have a severe, lethal phenotype: they lack endoderm as well as mesoderm in the head and trunk, strongly resembling embryos with defects in the Nodal signaling pathway. Nodal target genes are not induced in *vg1* mutants, confirming that Vg1 is required for the Nodal signaling pathway. Nodal is still expressed in *vg1* mutants, and it can be cleaved and secreted in the absence of Vg1, indicating that Vg1 is not required for Nodal processing. We find that Vg1 is only required in the cells that express Nodal, and in the presence of Nodal Vg1 is cleaved and localizes to the extracellular space. These results suggest that Nodal and Vg1 might form heterodimers in a codependent relationship: Nodal is required for Vg1 to be cleaved, and Vg1 is required for Nodal to activate target receptors.

Program Abstract #314
Regulation of Wnt/ß-catenin target genes during *Xenopus* gastrulation
Rachel Kjolby, Richard Harland
UC Berkeley, USA
During *Xenopus* gastrulation, canonical Wnt/β-catenin signaling posteriorizes the neural plate, patterns the mesoderm, and induces the neural crest. While the mechanisms of Wnt signal transduction are well characterized, it remains poorly understood how Wnt instructs so many processes at this stage. I have identified novel transcriptional targets of the Wnt signaling pathway using a genome-wide approach using ChIPseq of a tagged β-catenin and RNAseq on Wnt knockdown embryos. Most of these novel Wnt targets are expressed in a horseshoe pattern around the blastopore at gastrula stages, similar to the expression of *wnt8*. However, the size of the gap in the horseshoe expression pattern differs amongst the novel targets. In addition, putative regulatory sites identified by ChIPseq not only have TCF motifs, but also motifs for other transcription factors. These differences in binding landscapes and expression patterns of the novel Wnt targets suggest that different co-regulators contribute to forming their distinct patterns. Here we assess the influence of other transcription factors on the regulation of Wnt target genes. We first quantitatively characterize the differences in patterns of target genes by measuring the gap in the horseshoe pattern. We then correlate the expression pattern with motifs found in the regulatory landscapes to predict how changing the binding of different transcription factors might affect the resulting expression pattern. To directly test the influence of other transcription factors identified in my motif analysis on the regulation of Wnt target genes, we have designed a small CRISPR screen to knock out each putative coregulator and assay the effect on expression of each Wnt target gene. Together, these findings will provide key insights to how Wnt/β-catenin signaling, with inputs from other transcription factors, can confer the unique expression patterns of target genes, thus enabling the many roles of Wnt signaling in the early embryo.

Program Abstract #315
Foxa2 is recruited to Foxh1-primed cis-regulatory sequences to refine endoderm specification during *Xenopus* gastrulation
Jin Cho, Rebekah Charney, Jessica Cheung, Elmira Forouzmand, Margaret Fish, Ira Blitz, Ken Cho
University of California, Irvine, USA

Embryonic development depends on precise programs of gene expression orchestrated by key transcription factors such as pioneer transcription factors. Foxa1 and 2 are well-known pioneer factors for specifying the liver, which is derived from the endoderm. To investigate whether Foxa2 also has a pioneering role during germ layer specification, we performed Foxa2 ChIP-seq and DNase-seq to understand chromatin architecture at the gastrula stage in *Xenopus tropicalis* embryos. More than half of Foxa2 ChIP-seq peaks show DNase hypersensitivity and enhancer activity (H3K4me1 enrichment). Surprisingly Foxh1, a maternal transcription factor, is already bound to a subset of Foxa2 binding sites before gastrulation. Foxh1 interactions were also detected earlier, during cleavage stages. We also found that Foxh1 binding occurs before RNAPII recruitment at the promoters of target genes. Our results suggest that Foxh1 has a pioneering function in blastula embryos to affect the epigenetic status of early vertebrate embryos. Following Foxh1 priming, the zygotically expressed transcription factor Foxa2 is recruited to maintain proper temporal and spatial regulation of endodermal gene expression during germ layer specification.

Program Abstract #316

The epigenetic modifier Fam208a is essential for mouse gastrulation

Shohag Bhattacharyya[1,2,3], Christiana Polydorou[1,2], Veronika Gresakova[1,2], Radislav Sedlacek[1,2], Trevor Allan Epp[1,2], Kallayanee Chawengsaksophak[1,2]

[1]Institute of Molecular Genetics of the AS CR, Prague, Czech Republic; [2]Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec, Czech Republic; [3]Faculty of Science, Charles University in Prague, Czech Republic

Gastrulation initiates with the formation of the primitive streak and, during which, cells of the epiblast delaminate and ingress through the primitive streak to form the mesoderm and definitive endoderm. At this stage, the pluripotent cell population of the epiblast undergoes very rapid cellular proliferation and extensive epigenetic programming. Previously, we identified a novel gene, *Fam208a*, as a new epigenetic modifier essential for early post-implantation mouse development. Causative mutations in this gene were identified in two mouse strains obtained by an N-ethyl-N-nitrosourea (ENU) screen for modifiers of transgene variegation. These mutant strains, both suppressors of transgene variegation, were termed *MommeD6* & *MommeD20* and are both homozygous lethal. Recently, the human orthologue *FAM208A* was highlighted as an essential member of a new epigenetic silencing complex (termed the HUSH complex), which includes *PPHLN1* and the chromodomain protein *MPHOSPH8* that together recruits the H3K9 methyltransferase *SETDB1*. In our ongoing study, we conducted a detailed investigation of the signaling pathways that are disrupted by *Fam208a* mutation during early post-implantation stages, and which may further explain the failure of these mouse mutants to gastrulate. We observed that homozygote mutants first appear growth retarded around E6.75, remain retarded leading to their eventual resorption around E9.5. At E6.5, mutant embryos exhibited significantly delayed epithelial-mesenchymal transition (EMT) as evidenced by diminished Snail expression. They also had delayed formation of the node, as shown by whole mount in-situ hybridization with Noto, Brachyury & Shh. We further observed several genes, being mis-expressed and delayed suggestive of incorrect anterior-posterior (A-P) patterning. Funding: GACR 15-23165S, OP RDI CZ.1.05/1.1.00/02.0109, GaUK 1000216.

Program Abstract #317

Integration of orthogonal signaling by the Notch and BMP pathways in *Drosophila*

Albert Erives, Elizabeth Stroebele

University of Iowa, USA

How transcriptional enhancers read out and integrate nearly ubiquitous signaling activities in a tissue- and stage- specific manner is a key question. To address this, we desired a novel enhancer that integrates orthogonal developmental signals in a tissue-specific compartment. The transcription factor Su(H) and its co-activator, the Notch intracellular domain, are polyglutamine (polyQ)-rich factors that target enhancers, where they interact with other locally-bound polyQ-rich factors. We identified conserved regulatory belts with binding sites for the polyQ-rich effectors of both Notch and BMP/Dpp signaling, and the polyQ-deficient dorsal wing compartment selector Apterous (Ap) and other selectors. We find that the densest binding site cluster is located in the BMP-inducible *nab* locus, a homolog of the vertebrate transcriptional co-factors *NAB1*/*NAB2*. This *nab* regulatory belt is a novel enhancer driving dorsal wing margin expression in regions of peak phosphorylated-Mad in wing imaginal discs. By virtue of its obligate Notch signaling input, the DWME's transcriptional readout of the orthogonal BMP morphogen gradient is an idealized two-dimensional graph of pMad levels (y-axis) at different A–P positions (x-axis). We then show that Ap is developmentally required to license the *nab* dorsal wing margin enhancer (DWME) to read-out Notch and Dpp signaling in the dorsal compartment. Last, we find that the *nab* DWME is embedded in a complex of intronic enhancers, including a wing quadrant enhancer, a proximal wing disc

enhancer, and a larval brain enhancer. This enhancer complex coordinates global *nab* expression via both tissue-specific activation and inter-enhancer silencing mediated in part by Su(H) binding sites. We suggest that DWME integration of BMP signaling maintains *nab* expression in proliferating margin descendants that have divided away from Notch-Delta boundary signaling.

Program Abstract #318

Hedgehog-dependent patterning and intraflagellar transport require IFT56

Daixi Xin, Kasey J. Christopher, Lewie Zeng, Yong Kong, Scott D. Weatherbee
Yale University, USA

Almost all aspects of vertebrate development are influenced by cilia including left-right specification, organogenesis, skeletal patterning, and neural development. Intraflagellar transport (IFT) is required to build cilia, maintain their unique protein composition, and ensure their proper function in development. IFT56 is part of the IFTB complex but is one of the least understood IFT proteins with an unclear role in primary cilia. We took advantage of the classic *hop-sterile* (*hop*) mouse, which harbors a nonsense mutation in *Ift56*, to resolve the function of this gene. *Ift56*^{hop} mice display preaxial polydactyly, and our analyses revealed expansion of Gli3 repressor targets in the limb. Additionally, we identified neural patterning defects in *Ift56*^{hop} mice indicative of reduced Shh signaling. At the cellular level, we found that IFT56 is required for the accumulation of Gli proteins at ciliary tips, a process that is essential for normal signaling transduction. Unlike other IFTB mutants, we found that *Ift56*^{hop} mice retain normal cilia but display ciliary microtubule defects including abnormal number and positioning of the axonemal doublets. Strikingly, the loss of ciliary IFT56 leads to abnormal localization of specific IFTB components including IFT88, IFT81, and IFT27 in *Ift56*^{hop} cilia. In particular, IFT27, a critical ciliary transporter of Shh pathway components, is almost completely absent from *Ift56*^{hop} cilia, which likely explains the Shh-related patterning defects in *Ift56*^{hop} mice. Here, we demonstrate a critical role for IFT56 in the stability and localization of the IFTB complex in mammalian primary cilia. This process is essential for the proper function of ciliary IFT in ensuring normal developmental patterning of tissues including the limb and neural tube.
This research was supported by NIGMS/NIH T32GM007499 and T32HD007149.

Program Abstract #319

Cilia are involved in multiple steps of establishing left-right asymmetry.

Natalia Shylo, Scott Weatherbee
Yale University, USA

Vertebrate primary cilia are near-ubiquitous and act in numerous developmental processes including sonic hedgehog (Shh) signaling and left-right (L-R) asymmetry specification. L-R establishment is critical for organogenesis, including heart looping, lung formation and stomach placement. In mice, motile cilia at the embryonic node create a leftward flow that triggers asymmetric expression of *Cerl2* and *Nodal*, and subsequent activation of the Nodal cascade in the lateral plate mesoderm. Absence or immotility of node cilia results in a randomized L-R axis. However, most mutations in cilia genes do not completely ablate cilia, but rather disrupt their function, and how this affects L-R specification is unclear. Ciliary transition zone protein Tmem107 is essential for the normal complement of proteins within cilia. *Tmem107* null mice have random heart and stomach positioning, and severely hypoplastic lungs with apparent left isomerism, indicating a key role for Tmem107 in L-R patterning. Few cilia are formed in *Tmem107* null embryos, but a subset of node cilia remain, and they appear to have normal protein composition. The Nodal cascade pattern in *Tmem107* mutants is unlike other described cilia mutants. We found bilateral *Cerl2* expression in mutants, which sharply contrasts with the left isomerism lung phenotype. *Nodal* expression at the node is randomized, but, surprisingly, is largely bilateral in the lateral plate mesoderm. *Lefty1* is absent from the mutant midline, suggesting a midline barrier defect. *Shh* expression in the midline appears normal, pointing to a failure by *Tmem107* null cilia to relay the Shh signal and induce *Lefty1* expression. Together, our data suggest that Tmem107 and more broadly, cilia, are required early to establish asymmetric *Cerl2* and *Nodal* around the node, but also later to "enforce" the laterality via maintenance of the midline barrier. Supported by NIGMS/NIH T32GM007499 and NSF DGE-1122492.

Program Abstract #320

Intraciliary calcium oscillations initiate cardiac left-right asymmetry

Shiaulou Yuan, Lu Zhao, Martina Brueckner, Zhaoxia Sun
Yale University School of Medicine, USA

Background: Bilateral symmetry during vertebrate development is broken at the left-right organizer (LRO) by ciliary motility and the resultant directional flow of extracellular fluid. However, how ciliary motility and flow is perceived and

transduced into asymmetrical intracellular signaling at the LRO remains controversial. As we have previously implicated calcium signaling with cilia in the LRO, we hypothesized that the cilium functions as a unique calcium-signaling compartment that directly responds to extracellular fluid flow during LR axis development. **Methods:** In order to visualize and measure intraciliary calcium during LR development, we targeted genetically encoded calcium indicators into cilia of intact zebrafish embryos. We then utilized these tools to spatiotemporally map intraciliary calcium dynamics in wildtype embryos and embryos that lack fluid flow or polycystin-2, a cation channel that localizes to cilia. To assess the requirement of intraciliary calcium signaling during cardiac LR patterning, we suppressed intraciliary calcium by targeting a genetically encoded calcium sink into cilia of zebrafish embryos. **Results:** We have identified the existence of high levels of calcium within the cilium that display a surprising oscillatory-like behavior in vivo during early left-right axis development. These intraciliary calcium oscillations are: (1) preferentially localized on the left-side of the embryo, (2) dependent on fluid flow and polycystin-2, and (3) the earliest known molecular LR asymmetry. Further, we have demonstrated that intraciliary calcium oscillations are essential for proper cardiac LR development. **Conclusions:** These findings reveal that the cilium is a novel and indispensable calcium-signaling compartment, and suggests that intraciliary calcium signaling is critical for the pathophysiology underlying heterotaxy and other cilia-related disorders, collectively coined as 'ciliopathies.'

Program Abstract #321

Imaging Cardiovascular Asymmetry Defects in Live Mouse Embryos

Andrew Lopez[1], Shang Wang[1], Kirill Larin[2], Paul Overbeek[1], Irina Larina[1]

[1]Baylor College of Medicine, USA; [2]University of Houston, USA

The loss of left-right (L-R) asymmetry results in heterotaxia syndromes. In humans, the most severe form of heterotaxia is congenital heart disease (CHD) where the combination of several heart malformations makes it difficult to diagnose and treat in patients. Our ability to develop effective diagnostic tools and provide successful treatment options for CHD rely heavily upon our understanding of early embryonic heart function and its regulatory mechanisms. Recently, we have developed a set of unique tools using state-of-the-art optical coherence tomography (OCT), static live mouse embryo culture protocols, and computational methods directed at the visualization of the beating heart in live mouse embryos. This allows us to visualize beating hearts volumetrically with cellular resolution and analyze blood flow and heart wall dynamics. Here, for the first time, we have implemented 4D (3D+time) OCT imaging and analysis of the embryonic heart in a novel mouse mutated for Wdr19 and revealed a cardiac L-R asymmetry defect. Our results indicate that live 4-D OCT imaging provides a powerful phenotyping approach to characterize embryonic cardiac function and L-R asymmetry in mouse models.

This work is supported by the National Institutes of Health (R01HL120140, U54HG006348, and R25GM056929) and the Optical Imaging and Vital Microscopy Core at the Baylor College of Medicine.

Program Abstract #322

Investigating the role of HCN4 during cardiogenesis

Hannah Harris, Justine Epiney, Emily Pitcairn, Michael Levin, Kelly A. McLaughlin

Tufts University, United States

Cardiogenesis occurs via a series of carefully controlled steps and the coordination of multiple progenitor cell populations to create a functional organ. In vertebrates, this process begins as two dorsal bilateral patches of cardiac field progenitor cells migrate to the ventral midline and fuse, forming a single heart field. After the migration and differentiation of the myocardium, the heart undergoes intense morphological changes as the linear heart tube is formed, rightward looping occurs, and the chambers septate. The roles of transcription factors in these processes have been extensively studied; however, recent studies suggest ion channels, such as the family of hyperpolarization-activated cyclic nucleotide-gated (HCN) proteins, may also act as regulators of heart development. In particular, the role of one isoform, HCN4, in modulating the pacemaker function in adults has been studied for decades, but HCN4 expression has been observed well before cardiac tissue begins to beat, suggesting additional roles for this channel. Our lab has demonstrated that in *Xenopus laevis* the HCN4 channel, which has not been previously characterized in *Xenopus*, is expressed during early stages of heart development. Using both chemical and genetic manipulations, we characterized the effects of this ion channel on the differentiation and development of the individual tissue layers of the heart and observed that the mature hearts of tadpoles exhibit severely mispatterned phenotypes when HCN4 function is altered during embryogenesis.

Program Abstract #323
A novel role for HCN4 during cardiogenesis in *Xenopus laevis*
Emily Pitcairn, Hannah Harris, Michael Levin, Kelly McLaughlin
Tufts University, USA
Cardiogenesis requires strict spatial and temporal control of genetic cascades, biochemical signaling, and morphogenetic movements to generate a functional, mature heart. While the transcriptional factors that coordinate cardiac differentiation and development have been extensively investigated, recent studies suggest other cellular components, such as ion channels, may also help regulate organogenesis. In this study we demonstrate that one such ion channel, hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4), is present in *Xenopus laevis* well before the completion of heart development and prior to the onset of heart contractions. In addition, we characterize HCN4's role during cardiogenesis both phenotypically and genetically, and have shown that altering channel function (through both overexpression and knockdown of the channel) induces severely malformed heart phenotypes. This study adds evidence to the growing body of literature implicating ion channels as regulators of pattern formation and organogenesis.

Program Abstract #324
Split Hand/-Foot Malformation Genetics Supports the Chromosome 7 Copy Segregation Mechanism for Human Limb Development
Amar Klar
National Cancer Institute at Frederick, USA
Genetic aberrations of several unlinked loci cause human congenital split-hand/-foot malformation (SHFM) development. Mutations of the DLX5 (distal-less) transcription factor–encoding gene of chromosome 7 cause SHFM through haploinsufficiency. Inexplicably, vast majority of cases result from heterozygous chromosomal aberrations of the region without mutating DLX5 gene. To resolve these genetics and developmental biology paradoxes, we invoke chromosomal epigenetic mechanism for limb development. It is composed of monochromatid gene expression phenomenon, first discovered in two fission yeasts, with the selective chromosome copy segregation phenomenon, first discovered in mouse cell. Accordingly, one daughter cell inherits both expressed DLX5 copies while the other daughter inherits both epigenetically silenced copies from a single deterministic cell dividing in each developing limb. Thus differentiated daughter cells will respectively produce proximal- or distal-limb tissues after further proliferation. Published results of a translocation with a centromere-proximal breakpoint situated over 41 million bases away from the DLX locus and of two centromeric inversions that satisfied key genetic and developmental biology predictions of the mechanism will be presented. Such a mechanism has explained the congenital developmental disorders phenotype due to mutations or chromosomal rearrangements in diverse organisms. We propose that the double-helix structure of DNA forms the physical basis of generating sister cells gene regulation asymmetry required for eukaryotic development. Note that the conventional morphogen paradigm of developmental research was withdrawn in 2009. (Supported by NCI.)

Program Abstract #325
Pitx1 Promotes Chondrocyte and Myoblast Differentiation in Mouse Hindlimb through Conserved Regulatory Targets
Jialiang Wang[1], Sungdae Park[1], Carlos Infante[2], Douglas Menke[1]
[1]The University of Georgia, USA; [2]The University of Arizona, USA
The Pitx1 transcription factor is expressed during hindlimb development, where it plays a critical role in directing hindlimb growth and specification of hindlimb morphology. While it is known that Pitx1 regulates hindlimb formation, in part, through activation of the *Tbx4* gene, other transcriptional targets remain to be elucidated. We have used a combination of ChIP-Seq and RNA-Seq to investigate enhancer regions and target genes that are directly regulated by Pitx1 in embryonic mouse hindlimbs. In addition, we have analyzed Pitx1 binding in hindlimbs of *Anolis* lizards to identify ancient Pitx1 regulatory targets. Active enhancers and Pitx1 binding regions were mapped in the mouse and *Anolis* lizard genomes by performing H3K27ac and Pitx1 ChIP-Seq on embryonic hindlimbs of both species. We found that Pitx1 peaks called from both mouse and *Anolis* hindlimbs are strongly associated with limb and skeletal system development. RNA-Seq performed on wild-type and Pitx1 mutant mouse hindlimbs revealed a large number of misexpressed genes in *Pitx1*-/-. Top enriched terms of misexpressed genes are related to limb patterning, cartilage and skeletal muscle development. Expression of mesenchymal condensation-related genes, such as *Sox9* and *Pax9*, is strongly reduced in the anterior hindlimb. Expression of myogenic determination factors, such as *Myod1* and *Myog*, is absent from the proximal hindlimb. By intersecting misexpressed genes with genes that have neighboring mouse Pitx1 ChIP-Seq peaks, we identified 353 candidate targets of Pitx1. Of these candidates, 54 exhibit ultra-conserved Pitx1 binding events

that are shared between mouse and *Anolis* hindlimbs. Among these ancient targets of Pitx1 are master regulators of cartilage and skeletal muscle development, including Sox9, Pax9 and Six1. Our data suggest that Pitx1 promotes chondrocyte and myoblast differentiation in the hindlimb by direct regulation of several key members of the chondrocyte and muscle transcriptional networks.

Program Abstract #326
Using mathematics to distinguish between the many models for periodic patterning
Tom Hiscock
Harvard Medical School, USA
Many organisms develop patterns that are periodic in space – most commonly, stripes or spots. Some well-known examples include: regularly spaced hair follicles on the skin; striped or spotted animal coat patterns; periodic undulations (villi) in the gut; and the digit/non-digit pattern in the limb. The formation of these patterns is commonly identified as a reaction-diffusion ("Turing") mechanism, where periodicity is achieved by a system of diffusing molecules (e.g. Wnts and BMPs in the limb). Despite their prominence, Turing-like reaction diffusion models are not the only mechanisms that can make periodic patterns. Models based on cell movement, cell growth or signaling via long cellular protrusions can also explain periodicity, as can simple mechanical mechanisms, such as tissue buckling. This raises the important question: for a given tissue, how can we design experiments to distinguish between the many models for periodic patterning? Here, we develop a mathematical framework to guide the design of such experiments. First, we formulate a general model that encompasses Turing-like reaction-diffusion as well as cell-based and mechanical mechanisms allowing them to be directly compared. We find that many different mechanisms generate qualitatively similar final patterns, and thus the similarity of *in vivo* and *in silico* patterns (a common comparison in the literature) is not good evidence for a given mechanism. Therefore we sought quantitative features that would be different between the mechanisms. Through modeling, we suggest experiments to capture these quantitative differences: (1) measurement of pattern spacing following carefully chosen perturbations; (2) analysis of pattern dynamics; and (3) measurement of key system parameters. In each case, we describe how a mathematical model predicts the utility of the experiment and illustrate our approach using the limb as a biological case study.
Funded by NIH grant DC010791.

Program Abstract #327
Bmp signaling, governed by 5'Hoxd-Gli3 antagonism in interdigits, regulates digit joint formation
Bau-Lin Huang
National Cancer Institute-Frederick, USA
Joint and phalange number are key features of digit 'identity' that are central to limb functionality and adaptation, varying from extreme hyperphalangy in whale fins to a reduced phalangeal formula in certain bat wing digits. How joint-phalanx patterning is regulated remains unclear. We are using mouse genetic models to investigate the role of 5'Hoxd (Hoxd11-d13) and Gli3 genes in controlling joint-phalanx formation. Digits are bi-phalangeal and lose joint formation in 5'Hoxd mutant, while Gli3 deletion produces a joint-like transformation in proximal phalanges. We find that 5'Hoxd and Gli3 interact genetically in a dosage-dependent manner, and the interaction in interdigit acts non-autonomously to regulate normal joint formation, suggesting that interdigit mesenchyme functions as a signaling center to regulate digit patterning. Further Manipulating Bmp activity in opposite directions corrects digit phenotypes in the Gli3 and 5'Hoxd mutants, suggesting that 5'Hoxd-Gli3 interaction controls interzone formation by modulating BMP signaling. BMP-responsive progenitors at the digit distal tip are proposed to form phalanges proximal-distal fashion. We observe that newly formed Gdf5+ interzones arise in the digit distal tip, and are accompanied by alternating BMP activity levels in the sub-AER region. In addition, reduction of BMP activity in 5'Hoxd mutant corrects bi-phalangeal phenotype to tri-phalanges with restored sub-AER Fgf10 expression, suggesting Bmp-Fgf interplay controls differentiation-survival states of digit distal-tip progenitors. We find that the stoichiometry of 5'Hoxd and Gli3 expression in interdigits correlate to digit identity in chick and mouse. Thus, we propose that the outcome of 5'Hoxd-Gli3 interaction determines digit identity through modulating the level of inerdigital BMP activity and it regulates the periodicity of joint-phalanx cell fate determination together with the BMP modulator from proximal emerging cartilage condensates.

Program Abstract #328
Over-expression of HES1 in skeletogenic mesenchyme results in preaxial polydactyly
Deepika Sharma[1,2], Timothy Rutkowski[2], Anthony Mirando[1], Matthew Hilton[1]
[1]Duke University, USA; [2]University of Rochester, USA

Preaxial polydactyly (PPD) is a common congenital birth defect characterized by extra digits in the anterior autopod (hand/foot). Sonic Hedgehog (SHH) signaling is critical in establishing proper digit identity and number. Mouse models of increased SHH signaling ($Gli3^{xt}$) produce a PPD phenotype, although the mechanism(s) are unclear. Interestingly, $Gli3^{xt}$ mice exhibit expanded *Hes1* expression within the anterior autopod. To determine whether altered *Hes1* expression is relevant to PPD, we generated *Hes1* gain-of-function (GOF) mice that over-express *Hes1* within the limb bud mesenchyme (*Prx1cre;Rosa-Hes1*$^{f/f}$)($Hes1^{GOF}$). Skeletal staining and radiographs demonstrate that $Hes1^{GOF}$ mice develop PPD. To dissect the role of HES1 in PPD and its interaction with SHH signaling, we performed RNAseq, qPCR, whole mount *in situ* hybridization, and Western analyses on E10.5 and E11.5 limb buds. These data indicate that HES1 regulates digit number by enhancing mesenchymal cell proliferation via cell cycle protein regulation and delays chondrogenesis via transcriptional regulation. While HES1 activationpromotes *Shh* expression and regulates known SHH effectors such as *Grem1, Pax9,* and *Alx4*, SHH signaling appears to be unaffected in $HES1^{GOF}$ mice since GLI3 processing and canonical target gene expression (*Ptch1* and *Gli1*) is not altered. To determine whether HES1 may act downstream of SHH, we generated $HES1^{GOF}$ mutant mice in the absence of SHH (*Prx1Cre;Rosa-Hes1*$^{f/f}$*;Shh*$^{f/f}$). Interestingly, *Hes1* over-expression partially overcame the constrained digit number phenotype of SHH mutant mice, suggesting HES1 likely functions downstream of SHH in regulating digit number. Consistent with this data, $Hes1^{GOF}$ mice in a $Gli3^{xt}$ heterozygous background (*Prx1Cre;Rosa-Hes1*$^{f/f}$*;Gli3*$^{xt/+}$) demonstrate an additive effect on digit number. Collectively, our data suggest that HES1 regulates digit number by acting downstream of SHH signaling to promote mesenchymal cell proliferation and delay chondrogenesis.

Program Abstract #329
Investigating mechanisms of ribosome protein specificity in mammalian development
Gerald Tiu
Stanford University, USA
Although great strides have been made to understand how gene expression is regulated at the level of transcription, less is known about post-transcriptional control. Until recently, the ribosome has been viewed as constitutive and passive machinery in control of gene regulation. However, ribosome protein (RP) mutations in human and mice manifest in specific phenotypes, such as selective anemias, isolated asplenia, and homeotic transformations. These observations suggest that the ribosome plays an active and specific role in regulating gene expression. However, the mechanisms for this specificity are not well understood. To explore these mechanisms, we have conditionally knocked out with T^{Cre} one allele of a small subunit RP in the mesoderm lineage, including within the lateral plate mesoderm (LPM) that gives rise to developing limb buds. This led to pronounced forelimb versus hindlimb specific patterning defects in which forelimbs exhibit polydactyly (increased digit numbers) whereas hindlimbs display dramatic proximal hypoplasia as well as oligodactyly (loss of digits). In addition, we have also found that the timing of RP loss-of-function also plays a crucial role in the manifestation of this limb patterning phenotype. Surprisingly, employing $Prx1^{Cre}$ to conditionally knock out the RP once limb buds have initiated produces little to no phenotype. By employing other Cre lines, active at different time points during development, we have found that the limb phenotypes are only manifest when the RP is lost at very early stages of limb development within the LPM. We are now carrying out experiments to determine the translational landscape of gene regulation at these early time points to assess what genes are perturbed and what features of these genes make them susceptible to RP loss-of-function.
We acknowledge NIH Director's New Innovator Award (DP2OD008509-2), NIH R01 (1R01HD086634-01), Stanford MSTP, and PD Soros Fellowship for support.

Program Abstract #330
Using *Xenopus laevis* as a model for pharyngeal development
Breanna Bond
Northern Kentucky University, USA
During embryonic development, pharyngeal arches form various parts of the face, neck, and skull. Proteins made by cells in the embryo help these areas develop properly, and their absence or mutation can cause complications such as craniofacial malformations. *Xenopus laevis* is a vertebrate frog species whose embryonic development can be used as a model for human development. We hypothesize that *Iroquois* transcription factors play an important role in pharyngeal development. We analyzed several sets of *Xenopus laevis* embryos injected with *Iroquois* morpholinos using immunohistochemistry, which uses fluorescent antibodies to visualize the location of different proteins in the embryos. We found that the pharyngeal endoderm may be disrupted by a loss of *Iroquois* transcription factors. Future work will

help identify the role *Iroquois* factors play and what interactions they have with various genetic signaling pathways during pharyngeal development. This will possibly help aid in the understanding of human craniofacial malformations.

Program Abstract #331

Multiple signaling pathways involved in patterning the *Xenopus* pharynx

Sarah Kunkler, Emily Shifley

Northern Kentucky University, USA

The pharynx is a region in vertebrate embryos that gives rise to multiple organs including the cartilage of the head and neck, thymus and parathyroid glands. If the pharynx does not develop properly, it can cause birth defects such as DiGeorge Syndrome. We hypothesized that the FGF signaling pathway plays an important role in the development of the *Xenopus* pharynx as in other vertebrates. We inhibited FGF signaling in developing *Xenopus* embryos at various stages of development. *In situ* hybridization of control and FGF inhibited embryos showed a down-regulation of RA pathway gene expression in the FGF inhibited embryos. These results suggest that FGF signaling plays a key role in *Xenopus* pharyngeal development at least partially by regulating RA signaling. Future work will determine the exact temporal roles of FGF and RA signaling pathways during pharyngeal development. These results will aid in our understanding of the genetic cues that guide vertebrate pharyngeal development and how they might be disregulated causing birth defects.

Program Abstract #332

Exclusion of Dlx5/6 expression from the distal-most mandibular arches enables Bmp-mediated specification of the distal cap.

Joshua Vincentz[1], Jose J. Casasnovas[1], Ralston M. Barnes[2], Jianwen Que[3], David E. Clouthier[4], Jun Wang[5], Anthony B. Firulli[1]

[1]Riley Heart Research Center, Herman B Wells Center for Pediatric Research Division of Pediatric Cardiology, Departments of Anatomy, Biochemistry, and Medical and Molecular Genetics, Indiana University Medical School, 1044 W. Walnut St., Indianapolis, IN 46202-5225, USA; [2]Bristol-Myers-Squibb, Biologics Discovery California, 700 Bay Rd, Redwood City, CA 94063, USA; [3]Department of Medicine, Columbia University Medical Center, 650 West 168th Street BB 8-801A, New York, NY 10032, USA; [4]Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, Aurora, CO 80108, USA; [5]Department of Molecular Physiology and Biophysics, Baylor College of Medicine and the Texas Heart Institute, Houston, TX 77030, USA

Cranial Neural Crest Cells (crNCCs) migrate from the neural tube to the pharyngeal arches (PAs) of the developing embryo, where they ultimately compose the bone and connective tissue of the mandible. Proper differentiation and positioning of bone, tongue, and teeth within the developing mandible is controlled by secreted morphogens. Among these, Endothelin (EDN1) and Bone Morphogenetic Proteins (BMPs) divide the nascent mandible into subdomains along a proximo-distal axis; however, the transcriptional mechanisms by which mandibular progenitor cells interpret these signals to establish these subdomains are poorly understood. Here, we show that the distal-most of these subdomains, the distal cap, is marked by expression of the transcription factor *Hand1*, and gives rise to the ectomesenchymal derivatives of the lower incisors. We characterize a *Hand1* enhancer that drives gene expression within the crNCCs populating the distal cap. We show, both *in vitro* and *in vivo*, that BMP signaling and the transcription factor Hand2 synergistically regulate distal cap expression of this enhancer. Conversely, the EDN1-dependent homeodomain transcription factors DLX5 and DLX6 reciprocally inhibit BMP/HAND2-mediated *Hand1* enhancer regulation. Further findings indicate that direct positive transcriptional inputs from GATA transcription factors inputs utilize a site shared with DLX5/6 to drive *Hand1* enhancer activity. Our findings reveal that, within the distal cap, BMP-dependent factors provide positive transcriptional inputs that counteract the repressive activity of EDN1-dependent transcriptional effectors. This integrated communication between BMP and EDN1 signaling then patterns the mandible.

This work is supported by the Riley Children's Foundation, Carrolton Buehl McCulloch Chair of Pediatrics, R01 HL122123-02, R01 HL120920-03, 1R0AR061392-05 (ABF), R01DK100342 (JQ), R01DE018899 (DEC) 16SDG27260072, IU Health – IU School of Medicine Strategic Research Initiative (JWV).

Program Abstract #333

Orphan nuclear receptor, GCNF, is required for early neural crest cell induction and survival

William Munoz[1], Shachi Bhatt[1], Jennifer Crane-Dennis[2], Annita Achilleos[1], Daisuke Sakai[3], Austin Cooney[4], Paul Trainor[1]

[1]Stowers Institute for Medical Research, USA; [2]Missouri Southern State University, USA; [3]Graduate School of Brain Science, Japan; [4]Baylor College of Medicine, USA

Neural crest cells (NCC) are considered to be a vertebrate innovation that significantly contributed to the evolution, predation, radiation and adaptation of vertebrates to most niches of the planet. NCC comprise a unique vertebrate cell population that is frequently termed the "fourth germ layer" because they form in conjunction with the other germ layers and give rise to a diverse array of cell types and tissues including most of the craniofacial skeleton, peripheral nervous system, and pigment cells amongst many others. NCC development is dependent on gene regulatory network (GRN) control of several cellular mechanisms including induction, migration and differentiation with defects resulting in clinical manifestations termed neurocristopathies. In contrast to aquatic and avian species, we currently have a very poor understanding of the factors that regulate mammalian neural crest cell induction and specification. Here we describe molecular analyses of a mouse neurocristopathy model that revealed a critical role for the orphan nuclear receptor, *Germ cell nuclear factor* (*Gcnf/Nr6a1*) in mammalian neural crest cell formation and survival. Comparison of null and conditional *Gcnf* mutant embryos indicates that *Gcnf* is required prior to E8.0 for proper neural crest cell specification and induction. Furthermore, *Gcnf* functions as a bimodal switch to repress neural stem cell fate and promote the acquisition of neural crest cell identity. Thus our findings have identified a novel regulator of mammalian neural crest cell development and defined a temporal window for mammalian neural crest cell formation which is earlier than previously thought and raises important questions regarding the appropriateness of *Wnt1Cre* in studies of mammalian neural crest cell specification and induction.

Program Abstract #334

Neural crest cell emigration in turtle embryos

Matthew Smith[1], Jennifer Spengler[1,2], Ritva Rice[3], Rebecca McLennan[2], Paul Kulesa[2], Scott Gilbert[3,4], Judith Cebra-Thomas[1]

[1]Millersville University, USA; [2]Stowers Institute, USA; [3]University of Helsinki, Finland; [4]Swarthmore College, USA

Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like the facial bones, from neural crest cells. Using cell-labeling and neural tube explant cultures, we have shown that cells expressing neural crest markers emerge from the trunk neural tube in the turtle *Trachemys scripta* in two migratory phases over a greatly extended period. The early phase of migration (comparable to the period of neural crest cell (NCC) migration in other amniotes) extends until stage G10-11. These cells give rise to typical NCC derivatives. The NCCs that emerge late (beginning in stage G15-16 turtle embryos, well beyond the stage of neural crest migration in chick or mouse embryos), appear to migrate ventrally to form an ectomesenchymal dermis that gives rise to the bones of the plastron. Thus, there appears to be two distinct migratory phases *in vivo.* The specification of premigratory NCCs, and the epithelial-mesenchymal transition that produces migratory NCCs, is controlled by a gene regulatory network. We are currently comparing the expression of markers of premigratory and early migratory NCCs in G10 and later turtle embryos to examine whether the premigratory NCC domain persists during the period between the early and late migratory phases. We are using antibody staining and *in situ* hybridization to examine the expression of genes expressed in premigratory and migratory NCCs, including the transcription factors *FoxD3*, *Snail, Snail2, Sox9,* and *Sox10*. If the expression of premigratory NCC markers persists after the first wave of NCC migration, it will suggest that the premigratory NCC region is maintained longer than in other model amniotes, and that the lack of NCC migration in stage G12-14 turtle embryos may be due to the absence of a supportive environment.

Funding sources: National Science Foundation, Pennsylvania State System of Higher Education, Millersville University, Stowers Institute and Academy of Finland.

Program Abstract #335

Scaling Shh Morphogen Gradients in the Developing Neural Tube

Zach Collins, Tony Tsai, Kana Ishimatsu, Ian Swinburne, Tom Hiscock, Sean Megason

Harvard Medical School, USA

Both within species and between closely related taxa, body size can vary drastically while patterning occurs with consistent proportionality. This form of patterning robustness, termed scaling, has been a central puzzle of modern developmental biology and remains unaccounted-for in many patterning systems. To study scaling of vertebrate organogenesis, we have developed a novel surgical method for reducing the size of zebrafish embryos. Following size reduction, patterning of the ventral neural tube by the Shh morphogen scales to match tissue size. In addition, our work indicates that the recently-characterized protein Scube2 is an excellent candidate regulator of scaling. Scube2 expands the signaling range of Shh ligands in a dose-dependent manner and is known to be down-regulated by Shh signaling. This feedback regulation may enable sensing of tissue size during patterning of the vertebrate neural tube. Our

work demonstrates the remarkable robustness of embryonic patterning, and will yield further insight into the regulation of Shh signaling gradients.

Program Abstract #336

RA is a conserved signaling pathway for photoreceptor patterning in retinal areas for high acuity vision

Susana da Silva[1,2],Connie Cepko[1,2]

[1]*Department of Genetics and Department of Ophthalmology, Harvard Medical School, USA;* [2]*Howard Hughes Medical Institute, USA*

Our high acuity vision relies on a small retinal specialization, the fovea. The photoreceptors of the fovea comprise only cones, which operate during the high light intensities of daylight, and which are responsible for color vision. The fovea has no rod photoreceptors, which endow vision in dim light conditions. In addition, the fovea has very few of the other retinal cell types, as they are spaced out laterally to the fovea in order to allow for more light to reach the fovea. The fovea is particularly sensitive to mechanical lesions and neurodegenerative diseases, such as age-macular degeneration (AMD), which affects 15% of people older than 80 years old. Despite this high prevalence in the elderly population, the etiology of AMD remains unclear, particularly regarding the selective vulnerability of the fovea. The design of successful therapeutic strategies has been hampered by our lack of understanding of the fovea, including the basic molecular mechanisms underlying photoreceptor patterning and differentiation. We have previously described that chickens have a retinal region that functionally resembles the primate/human fovea, comprising only cone photoreceptors, i.e. devoid of rods, and hence the name, the rod-free zone (RFZ). Here we show that the Retinoic Acid (RA) signaling pathway is involved in the development and patterning of the RFZ. RA-degrading and synthetic enzymes exhibit specific expression patterns during photoreceptor neurogenesis that suggest a role in the development of the RFZ. Manipulation of RA signaling *in vivo* was carried out, where an induction of rods in the RFZ was observed. RA-mediated photoreceptor patterning was found to act through Fgf8, by repressing FGF8 expression. The patterned expression of RA signaling components is preserved in fetal human retinal tissue. These findings provide the first mechanistic insight regarding the development of a high acuity vision area.

Program Abstract #337

Regulation of signaling pathways by microRNAs impacts early development

Jia Song

University of Delaware, USA

Development of animal embryos from fertilization through gastrulation requires multiple cell fate decisions under the control of Wnt and growth factor signaling pathways. We and others have found that components of these signaling pathways are regulated by microRNAs (miRNAs) which are small non-coding RNAs that fine tune gene expression by repressing translation and/or induce target mRNA degradation. The sea urchin skeletogenic primary mesenchyme cells (PMCs) give rise to the larval skeleton important for swimming and feeding. PMCs serve as excellent model for examining cross regulation of signaling pathways and miRNAs, because they undergo cellular specification and patterning in response to Wnt, VEGF, FGF signaling and miRNAs. Our results indicate that *Disheveled* (*Dvl)* and *β-catenin* are regulated directly by at least one shared miRNA. Dvl is upstream of β-catenin of the canonical Wnt signaling pathway (cWnt) and transduces Wnt ligand signals of both cWnt and non-canonical Wnt pathways. We observed that while blocking miRNA regulation of *β-catenin* of the cWnt pathway did not affect skeletogenesis, blockage of miRNA regulation of *Dvl* led to defects in PMC development. In addition, we found a highly conserved miRNA, miR-31, to target various components of the PMC gene regulatory network (GRN) and growth factor signaling. Blockage of miR-31 regulation of genes in the PMC GRN and VEGF receptor results in defective PMC morphogenesis. Thus, our results indicate that miRNAs regulate Wnt and growth factor signaling pathways that are critical for PMC patterning. Since the PMCs undergo epithelial-to-mesenchymal transition, directional migration, cell-to-cell fusion, and morphogenetic behaviors, our study contributes to the fundamental understanding of how an embryo uses miRNAs to regulate signaling pathways in directing cellular specification and morphogenesis.

Funding sources: NSF CAREER (IOS 1553338) to JLS

Program Abstract #338

A fate map of Hoxa5 expressing cells in somites shows tissue-type restriction

Maya Feldman[1], Dima Chaar[1], Danah Screen[1], Thibaut Leclercq[1], Madeline Pantalena[1], Lucie Jeannotte[2,3], Jennifer Mansfield[1]

[1]*Department of Biology, Barnard College, Columbia University, USA;* [2]*Centre de recherche sur le cancer de l'Université*

Laval, Centre Hospitalier Universitaire de Québec, L'Hôtel-Dieu de Québec, Canada; [3]Department of Molecular Biology, Medical Biochemistry and Pathology, Université Laval, Canada
Hox transcription factors pattern the body axis of most animal embryos. They confer anterior-posterior identity on embryonic segments and play direct roles in tissue morphogenesis later in development. In most cases, it is unknown what cellular processes they regulate to produce morphological differences among vertebrate musculoskeletal elements. *Hoxa5* non-redundantly patterns musculoskeletal elements spanning the mouse cervical-thoracic transition, including vertebrae, ribs, sternum, and pectoral girdle. To characterize the cell types and time-points at which *Hoxa5* acts, we used Cre/lox and inducible Cre/lox lineage labeling to fate map the descendants of *Hoxa5* expressing cells in the somites. We found that *Hoxa5* descendants contribute to a restricted subset of tissue types derived from somites. *Hoxa5* descendant cells are abundant in cartilage and perichondrium, but are absent from some other tissue types such as muscle. This restriction of *Hoxa5* descendant fate differs from that described for other Hox genes. Characterizing the fate and cell behaviors of *Hoxa5* descendants can shed light on its patterning role.

Program Abstract #339
Ephrin-B2 functions at the interface between dorsoventral patterning and morphogenesis in tracheoesophageal separation.
Ace Lewis, Jeffrey Bush
UCSF, USA
Tracheoesophageal (TE) fistula is a human birth defect defined by a connection between the esophagus and trachea, compromising normal feeding and respiration in neonates. The formation of a discrete trachea and esophagus, which must divide from a single, common foregut tube, is known to be dependent on the correct dorsoventral (DV) patterning of the foregut as induced by a milieu of extrinsic signaling cues. The ventral and dorsal domains of the foregut tube have distinct molecular identities, where the ventral, presumptive trachea, is defined by Nkx2.1 expression and the dorsal, presumptive esophagus, is marked by Sox2, and the separation into two tubes is thought to proceed along the boundary between these opposing domains. Although the importance of DV patterning has been established, little is understood regarding the molecular players directing this morphogenesis or the cell and tissue behaviors involved. Ephrin-B2, a member of the Eph/ephrin family of membrane-bound cell signaling molecules, has previously been implicated in TE separation. Eph/ephrin signaling is well-known for its roles in boundary formation between tissue compartments and providing cellular guidance cues, suggesting it as a candidate effector of TE separation. Using mouse genetic models, we show that dorsal, esophageal foregut cells express ephrin-B2 while ventral, tracheal foregut cells do not, and that this patterned endodermal expression of ephrin-B2 is necessary for TE separation but not for DV patterning of the foregut. Instead, we show that ephrin-B2 expression is regulated by upstream dorsoventral patterning of the foregut, to generate a dorsoventral ephrin-B2 differential. We hypothesize that ephrin-B2 functions at the interface of dorsoventral patterning and morphogenesis in TE separation.
Supported by R03 HD084895 from NIH/NICHD to J.O.B.

Program Abstract #340
Embryonic origins of adult stem cells in the acoel *Hofstenia miamia*
Silvia Golumbeanu
Harvard University, USA
Planarians (e.g., *Schmidtea mediterranea*) and acoels (e.g., *Hofstenia miamia*) are distantly related animals that are both capable of whole-body regeneration and possess populations of pluripotent adult stem cells called “neoblasts”. The developmental origins of neoblasts are unknown. The acoel *Hofstenia miamia* is particularly useful in addressing this question as the animals produce abundant embryos that are amenable to experimental investigations. We seek to understand the origins of the stem cell population by studying the embryonic development of *Hofstenia*, using *piwi*-1, a known adult neoblast marker. In the adult, *piwi*-1 is expressed throughout the parenchyma of the animal, notably missing from the anterior. We are generating a staged series as a reference for embryonic development in *Hofstenia*. We are also developing methods to use *in situ* hybridization to determine the expression pattern of *piwi*-1 mRNA in embryos at different stages. Our data will allow us to distinguish between two alternative hypotheses for the developmental origin of pluripotent stem cells: either *piwi-1* is expressed from very early on in the embryo’s life and is subsequently restricted, or it begins expression later on specifically in the cell lineage that gives rise to neoblasts.

Program Abstract #341

Putative role for progesterone in *Monodelphis domestica* embryogenesis and pregnancy

Kobi Griffith, Karin Yoshida, Joanna Johnson, Yolanda Cruz

Oberlin Col Sci Ctr, USA

Progesterone (P) has an unusual role in didelphid marsupials in that pheromonally induced levels of pro-estrous P trigger ovulation. Work in our lab reveals that serum P levels are significantly higher on pregnancy day 3 in the didelphid, *Monodelphis domestica,* plummet to extremely low levels during pregnancy day 5, and return to day-3 levels on day 9, before gradually declining as pregnancy advances. Because this precipitous decline coincides with the highest levels of embryonic mortality during the 14-day gestation period in *M. domestica*, we investigated the possibility that these unusual events may be related. We analyzed, at the histological level, uterine samples from days 1, 3, 5, 7, 9, 11 and 13 of pregnancy and compared them to samples from non-pregnant females. To understand the pattern of progesterone receptor (PR) expression in the uterus during pregnancy, we used HRP-mediated immunoassay of histological samples from these pregnancy stages. Our results indicate that P levels fluctuated in a manner coincident with that of thickness of the endometrial epithelium, both parameters being highest on days 5 and 9. Additionally, on day 9, the uterine stroma as well as the uterine glands in them were at their thickest. PR was clearly detectable in the cytoplasm and stroma of uterine gland cells at all pregnancy stages, declining gradually as pregnancy progressed, with one exception: day 5. PR expression was extremely faint, if at all detectable, at this pregnancy stage. Low PR expression would thus seem to exacerbate the effects of low P on day 5 of pregnancy, suggesting that low or absent progesterone signaling may have adverse effects on embryonic survival. Funding: Oberlin College.

Program Abstract #342

hmmr facilitates planar cell polarity-mediated radial intercalation to drive anterior neurulation and forebrain morphogenesis

Angela Prager[1], Cathrin Hagenlocher[1], Tim Ott[1], Thomas Thumberger[2], Kerstin Feistel[1]

[1]University of Hohenheim, DE; [2]Heidelberg University, DE

During neurulation, neural fold (NF) convergence and fusion depends on the interplay of cell polarization, adhesion, migration and intercalation. We have analyzed the function of Hmmr (hyaluronan-mediated motility receptor), a microtubule (MT)-associated protein implicated in cell division, cell migration and metastasis, for neurulation movements in *Xenopus laevis*. Loss of *hmmr* function (*hmmr* LOF) induced by morpholino oligomer injection severely affected the forebrain roof plate. This prevented forebrain hemisphere separation, producing a holoprosencephaly-like phenotype. The defective forebrain roof plate was the result of morphogenetic problems during neural tube closure (NTC). The timing of NTC in the spinal cord region was similar to wild type embryos, suggesting that *hmmr* did not affect planar cell polarity (PCP)-mediated mediolateral cell intercalation which governs posterior NTC. Instead, NTC was severely delayed in the future brain region. NF elevation, driven by MT-mediated cell elongation, was impaired along the entire anterior-posterior axis. In NF cells, Hmmr co-localized with MTs and was required for adhesion, elongation, apico-basal polarization of the MT network and radial cell intercalation. This suggested an interaction of *hmmr* with the PCP pathway, which in addition to mediolateral intercalation also governs the radial intercalation of cells. Indeed, *hmmr* LOF altered the subcellular localization of Rac1, a well-known PCP effector. The core PCP protein Vangl2 rescued MT arrangement and cell shape as well as NF morphology in *hmmr* morphants. This study thus reveals a novel role for Hmmr as a PCP-interacting protein essential for anterior NTC and forebrain development. Our data suggest that *hmmr* facilitates PCP-controlled radial cell intercalation. *hmmr* may thus act as a mediator between two PCP-governed cell intercalation behaviors, both of which are essential for morphogenetic cell rearrangements during neurulation.

Program Abstract #343

Anterior-Posterior Neural Axis Plasticity in the Developing Central Nervous System of *Xenopus laevis*

Lyuba Bolkhovitinov, Eri Anastas, Janhavi Giribhattanavar, Charith Ratnayake, Gladys Shaw, Kendrick Wiggins, Margaret Saha

College of William and Mary, USA

The regionalization of the anterior-posterior (AP) axis is an essential step in the development of the central nervous system. The molecular mechanisms of neural axis patterning have been extensively studied, but less is known about how this system responds to perturbations. The present study explores how gastrula-stage *Xenopus laevis* embryos respond to a physical perturbation of their developing AP neural axis. During either the mid- or late gastrula stage, a square of presumptive neural ectoderm was transplanted from a fluorescently-labeled donor embryo to the corresponding region on an unlabeled host embryo. The orientation of the transplanted ectoderm was either maintained, or rotated 180 degrees

along the AP axis. Embryos were assayed for the expression of AP axis regional marker genes (*XCG-1*, *Otx2*, *En-2*, and *Krox20*) at either the late neurula or hatching stage. Histological analysis of marker genes shows that neural ectoderm rotated at the mid-gastrula stage is able to adopt the positional identity of the host environment, while ectoderm rotated at the late-gastrula stage tends to maintain its previous AP identity. After mid-gastrula rotations, transplanted tissue correctly brings up regional marker gene expression and embryos develop with normal morphology. In contrast, late-gastrula rotated embryos have morphological abnormalities and show higher levels of ectopic gene expression along the AP axis, i.e. anterior genes in posterior regions and vice versa. Although all four regional marker genes generally follow this trend, they show slightly different patterns of regulation. Control embryos from both mid- and late-gastrula stages develop normally, showing that the abnormal development of late-gastrula rotated embryos is not caused by a simple loss in healing ability as embryos proceed through gastrulation. Instead, these results indicate that there is a loss in plasticity of the AP axis during a defined window of development.

Program Abstract #344

Endogenous Bioelectric Gradients Control Brain Development via Notch signaling and Regulation of Proliferation: impairment and rescue of neural defects

Vaibhav Pai[1], Joan Lemire[1], Jean-Francois Pare[1], Gufa Lin[2], Ying Chen[2], Michael Levin[1]

[1]Tufts University, Center for Regenerative and Developmental Biology, USA; [2]University of Minnesota, Stem Cell Institute, USA

Biophysical forces play important roles in pattern formation during embryogenesis and regeneration. Previously we showed that endogenous bioelectric signals are crucial eye patterning signals, being necessary and sufficient for eye induction. Here, we identify a new role for cell resting potentials (V_{mem}) as an instructive factor during brain patterning in *Xenopus laevis*. Early frog embryos exhibit a characteristic polarization of a key group of cells in the neural tube; disruption of this stereotypic bioelectric gradient induces changes in the expression of early brain markers and thus anatomical mispatterning of the brain. This effect is mediated by voltage-gated calcium signaling and gap-junctional communication. Neural cell proliferation within the developing brain is regulated by both the local V_{mem} states within the neural tube, and, strikingly, the V_{mem} states of distant ventral regions. Misexpression of the constitutively-active form of Notch, a suppressor of neural induction, alters the normal voltage pattern and drastically impairs neural patterning. However, reinforcing proper polarization by overexpression of specific ion channels rescues brain defects despite the presence of mutated Notch. Interestingly, hyperpolarization of regions outside the head induces ectopic neural tissue. Moreover, polarization states synergize with the actions of canonical reprogramming factors that promote an undifferentiated cell state to direct ectopic tissues toward neural fate *in vivo*. These data identify a new functional role for bioelectric signaling in brain patterning, reveal long-range coordination of cell behavior during brain development, characterize the interaction between V_{mem} and key biochemical pathways (Notch and Ca^{2+} signaling) during organogenesis of the vertebrate brain, and suggest voltage modulation as a tractable strategy for intervention in certain classes of neural birth defects.

Program Abstract #345

Serotonergic signaling and membrane potential promote innervation of eye grafts in *Xenopus* tadpoles

Douglas Blackiston, Michael Levin

Tufts University, USA

Serotonin is a well-known monoamine neurotransmitter found throughout the central nervous system. However, long before functional synapses are present in the developing embryo, serotonin and its related signaling molecules are present in a variety of cells and tissues, with maternal stores of serotonin present even at the one cell stage. What is the purpose of embryonic serotonin if not for neurotransmission? Our lab has uncovered a number of novel serotonin-related signaling events during development, including the establishment of the left-right axis and regulation of melanocyte proliferation, shape, and migration. Here we report the ability of serotonin to act as a pathfinding molecule for the growth cones of retinal ganglion cells. Using a transplant method, developing eye tissue can be grafted into blinded recipients, creating a morphologically complete eye at the site of the graft. Leveraging a variety of serotonergic and bioelectrical pathways, these grafts can be induced to innervate the host, even when the eyes are transplanted far from the head of the animal. Further, using a custom built behavior platform, we have for the first time trained *Xenopus* tadpoles in an associative assay. Utilizing this assay, we have demonstrated that animals receiving eye grafts can distinguish wavelengths, and that learning rates increase when innervation is promoted through serotonergic and bioelectric activation. Taken together, these results identify a novel serotonin signaling modality and reveal the remarkable plasticity of form and function which has implications for the regenerative medicine of repaired or implanted tissues, as well as for the evolution of pattern in

sensory-motor systems. This work was supported by the NIH (EY018168, MH081842, and 5T32DE007327-09) and the Leila Y. Mathers Charitable Foundation.

Program Abstract #346

Phosphorylation of hnRNP K at a site targeted by ERK promotes neurofilament protein expression and axon outgrowth

Ben Szaro, Erica Hutchins, Jamie Belrose
State University of New York at Albany, USA

The establishment and maintenance of brain circuitry depends on precise connections being made between neurons by their axons contacting the appropriate synaptic sites. During development, the synthesis of axonal cytoskeletal proteins must be precisely coordinated with the dynamics of axon elongation through cell signaling pathways, to match protein supply with demand and prevent the formation of the cytoskeletal protein aggregates that cause neurodegeneration. This coordination is accomplished in part through kinases acting on RNA-binding proteins like hnRNP K, which regulates the mRNAs of neurofilaments and other cytoskeletal-associated proteins that organize axonal cytoskeletal polymers. In *Xenopus* embryos, knocking down hnRNP K inhibits axonogenesis by interfering with the nuclear export and translation of these mRNAs. In HeLa cells, phosphorylation of hnRNP K by ERK stimulates nuclear export of hnRNP K mRNP complexes, raising the hypothesis that ERK's effects on axon outgrowth may, at least in part, be mediated via hnRNP K. To test this hypothesis, we used an *in vitro* phosphorylation assay to identify a phylogenetically conserved ERK1 phosphorylation site of *Xenopus* hnRNP K, and then tested this site's function *in vivo* by attempting to rescue hnRNP K morphants with phosphomimetic and phosphodeficient forms of hnRNP K at this site. Although neither form affected hnRNP K nuclear export, only the phosphomimetic form rescued both neurofilament protein expression and axon outgrowth. This finding represents a previously unidentified function of phosphorylation of hnRNP K at this conserved site and implicates hnRNP K as an intracellular molecular target of ERK-mediated signaling in axon outgrowth.
Supported by NSF IOS 1257449.

Program Abstract #347

Regulation of microtubule plus-end dynamics by TACC3 during axon outgrowth and guidance

Burcu Erdogan, Garrett Cammarata, Andrew Francl, Jessica Tiber, Laura Anne Lowery
Boston College, USA

Precise neuronal connection requires proper axon guidance. Microtubules (MTs) of neuronal growth cones are the driving force to navigate the growing ends of axons. Pioneering microtubules and their plus-end resident proteins, +TIPs, play integrative roles during this navigation. Recently, we introduced the protein TACC3 as a member of the +TIP family that regulates microtubule dynamics in growth cones, and we showed that manipulation of TACC3 levels affects axon outgrowth. Knowing that dynamic MT regulation is key to the growth cone's directional motility during axon outgrowth and guidance, we sought to further investigate the mechanism of TACC3-mediated MT regulation and the impact of this regulation in axon outgrowth and guidance. Here, we use quantitative analysis of high-resolution live imaging and show that TACC3 is required to promote axon outgrowth and prevent spontaneous retractions in cultured embryonic *Xenopus laevis* neurons. Furthermore, we find that TACC3 regulates the stability of microtubules within the growth cone. Additionally, we demonstrate that manipulation of TACC3 levels interferes with the growth cone response to axon guidance cues *ex vivo.* We also show that ablation of TACC3 cause pathfinding defects in axons of developing spinal cord motor neurons and retinal ganglion cells in *Xenopus laevis in vivo*. Together, our results suggest that by regulating MT behavior, the +TIP TACC3 is involved in axon outgrowth and pathfinding decision of neurons during embryonic development.

Program Abstract #348

The Role of Spontaneous Intracellular Calcium Transients in Neurotransmitter Phenotype Specification in *Xenopus laevis*

Eileen Ablondi, John Marken, Andrew Halleran, Sudip Paudel, Morgan Sehdev, Wendy Herbst, Margaret Saha
College of William and Mary, USA

Spontaneous intracellular calcium activity has been implicated in a host of processes related to nervous system development, including neurotransmitter phenotype specification. This process involves the acquisition of the correct balance and patterning of excitatory and inhibitory neurons, and its regulation is vital to proper nervous system functionality. While a high frequency of intracellular calcium transients in presumptive neurons during development has been correlated with an inhibitory fate, the persistence of this phenomenon in *in vitro* models has not been conclusively

demonstrated. Additionally, we believe that current methods of calcium activity analysis, which are limited to counting fluorescent indicator spikes above a particular threshold, are limiting. To this end, we employed *Xenopus laevis* presumptive neural tissue as an *in vitro* model system, imaging calcium activity in developing neural cells. This data was analyzed via a novel pipeline that uses fluorescence trace transition entropy as a comparative metric rather than relying on predetermined parameters to define particular features (i.e., spikes or waves). Use of this analysis method revealed differences in calcium activity across development, with cells dissected from younger embryos displaying more entropic calcium activity than cells dissected at older stages. Additionally, cells positive for different specific neural marker genes displayed significantly different levels of calcium activity transition entropy from one another. Additional experiments have been performed *in vivo* to better understand the role of spatial patterning of this calcium activity on phenotype specification. As a whole, these results provide support for the hypothesis that particular patterns of calcium dynamics are associated with the expression of particular genes involved in the neuronal differentiation process.

Program Abstract #349
Differential effects of Hes gene expression on trigeminal placode development.
Meyer Barembaum, Marianne E. Bronner
California Institute of Technology, USA
The trigeminal ganglion has dual origins from neural crest cells and ectodermal placodes. The neural crest component gives rise to the glia and the proximal neurons, while the distal neurons are derived from the placode cells. In the chick embryo, the placodes that give rise to the two branches of the trigeminal ganglion have different molecular signatures. For example, *Pax3* and *Ngn-2* are expressed in the anterior placode (ophthalmic branch--opV) whereas *Ngn-1* is expressed in the posterior placode (maxillomandibular branch--mmV). To further characterize molecular markers in the trigeminal placode, we have examined the expression of several Hes genes, members of the hairy and Enhancer of split family of basic Helix-loop-helix transcription factors involved in Notch signaling. We found that *Hes6* is expressed in the anterior part of the placode (opV) while *Hes5.2* and *Hes5.3* are present in the posterior half of the placode (mmV). Misexpression of *Hes6* represses *Hes5* genes. Conversely, misexpressing any of the Hes5 genes (*Hes5.1*, *Hes5.2* and *Hes5.3*) represses *Hes6*. Constitutively active Notch signaling by transfecting the intercellular domain (NICD) causes up regulation of the *Hes5* genes and repression of *Hes6*. Misexpression of *Hes6* causes down regulation of the *Ngn-1* that is normally expressed in the mmV but has no detectable effect on the *Ngn-2* gene expression. Over expression of any of the Hes5 genes causes repression of both *Ngn-1* and *Ngn-2* but induces *NeuroD* expression. These data implicate Notch signaling and Hes gene expression in determining the identity of the trigeminal placodes, with reciprocal cross-inhibition of *Hes5* family members and *Hes6*.
This work was supported by USPHS P01 HD037105 and DE16459.

Program Abstract #350
Annexin A6 controls the morphological maturation of sensory neurons during cranial gangliogenesis
Lisa Taneyhill, Ankita Shah
University of Maryland, USA
Cranial sensory ganglia are components of the peripheral nervous system that possess a significant somatosensory role and include neurons within the trigeminal and epibranchial nerve bundles. These ganglia arise from the intermixing and coalescence of two different cell populations, neural crest cells (NCCs) and neurogenic placode cells (PCs). Defects in the migration and interaction of these cells can cause abnormalities in craniofacial development and in the formation of the sensory nervous system. Both cell migration and intercellular interactions rely upon the rapid remodeling of the cytoskeleton, a process facilitated by Annexin proteins during sensory nervous system development in metazoans. Our prior work revealed that Annexin A6 modulates early chick cranial NCC migration. Intriguingly, Annexin A6 is expressed later in trigeminal and epibranchial PCs during cranial ganglia assembly, but its function has not been elucidated. To this end, we interrogated the role of Annexin A6 using gene perturbation studies in the chick embryo. Our data reveal that PCs lacking Annexin A6 still ingress and migrate normally to the ganglionic anlage, where NCC corridors correctly form around them. Strikingly, while Annexin A6-depleted PC-derived neurons still express mature neuronal markers, they fail to adopt a bipolar morphology with two neuronal projections, a feature thought to be associated with mature neurons. Accordingly, PC-derived neurons lacking Annexin A6 fail to innervate their designated targets due to this loss of neuronal processes. Collectively, these data suggest that the molecular program associated with neuronal maturation can be uncoupled from the required morphology change that occurs in chick trigeminal and geniculate PC-derived neurons. Importantly, our data provide insight into the molecular mechanisms underlying the cytoskeletal changes essential for interactions that occur during cranial gangliogenesis. This work is supported by NIH R01DE024217.

Program Abstract #351

Embryonic ceramide exposure alters tissue redox balance and increases the incidence of neural tube defects

Micah Ross, Jason M. Hansen, Michael R. Stark

Brigham Young University, USA

Ceramide, a modified sphingolipid, has been implicated in disease models of obesity and diabetes where increased circulating ceramide levels are well-documented, including in pregnant women. While the frequency of neural tube defects (NTDs) is increased in obese pregnancies, increased ceramide levels have not carefully been studied as a causative agent in NTDs. Interestingly, there is an established link between ceramide misregulation and NTDs, where exposure in pregnant women to the corn fungus toxin fumonisin, an inhibitor of the enzyme ceramide synthase, causes a significant increase in the incidence of NTDs. Studies also show that folate supplementation does not reduce the frequency of fumonisin-linked NTDs. We hypothesized that maintaining proper ceramide levels during embryonic development is critical, and that ceramide misregulation disrupts proper neurulation, resulting in an increase in NTDs. The preliminary data presented here are supportive of this hypothesis, showing that chick embryos exposed to C2-ceramide (100 uM) incur NTDs at a rate of 52% (n=51), compared to 13% in DMSO treated embryos (n=23). Because ceramides have been implicated in oxidative stress, we measured cellular redox state in treated embryos. We show differential responses to oxidative stress in embryonic tissue exposed to C2-ceramide or fumonisin. Preliminary studies also show a potential change in Pax3 expression in the dorsal neural folds. Our current studies aim to link ceramide, redox potential, and gene regulation in directing neural tube closure.

Program Abstract #352

The role of the Fzl1/2/7-PKC signaling pathway in the anterior neuroectoderm positioning mechanism of the sea urchin embryo

Marina Martinez-Bartolome, Ryan C. Range

Mississippi State University, USA

Anterior neuroectoderm (ANE) specification, positioning, and patterning is a crucial event in body plan formation in all deuterostomes. Studies from diverse metazoan embryos indicate that canonical Wnt/β-catenin signaling is essential for the specification of the primary axis. In early development of the sea urchin embryo, the positioning of the ANE around the anterior pole depends on integrated information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways, forming an interconnected network of Wnt signaling. We have previously shown that the Fzl1/2/7-PKC signaling pathway antagonizes the ANE positioning mechanism mediated by Fzl5/8-JNK signaling in the anterior ectoderm, allowing for the proper positioning of the ANE territory around the anterior pole. Yet, the exact mechanism by which Fzl1/2/7-PKC signaling antagonizes Fzl5/8-JNK signaling during this process is still unclear. Hence, our research aims to better characterize the Fzl1/2/7-PKC signaling pathway and the gene regulatory network (GRN) it activates to identify possible interactions between these different pathways. Using a candidate gene approach in combination with whole-transcriptome differential screens, we identified a potential transcriptional effector of the Fzl1/2/7-PKC signaling pathway, NFAT, an intracellular signal transduction modulator, Siah1, and several transcription factors in the GRN activated by Fzl1/2/7 signaling. Our preliminary loss-of-function data strongly suggest that most of these factors are necessary to antagonize the ANE positioning mechanism mediated by Fzl5/8-JNK signaling. Together, these data support the idea that Fzl1/2/7-PKC signaling antagonizes Fzl5/8-JNK signaling at both the intracellular transduction and transcriptional levels.

Program Abstract #353

Early evidence suggests neural patterning mechanisms vary during development and regeneration of *Nematostella vectensis*

Michael Layden

Lehigh University, USA

Recent studies argue that adult regeneration does not exactly recapitulate development. Thus, understanding how mechanisms that control development and regeneration vary would improve our fundamental understanding of regeneration. Our lab has been developing the sea anemone *Nematostella vectensis* as a novel model to investigate the relationship between developmental and regenerative neurogenesis. The *Nematostella* nervous system is comprised of endodermal and ectodermal nerve nets, which are patterned via a conserved neurogenic cascade during development. Here we focus on an *achaete-scute* homolog *NvashA* during neurogenesis. During development *NvashA* is both necessary and sufficient to promote development of *NvLWamide*+ neurons in both the endodermal and ectodermal nerve nets. Using transgenic reporter lines, we show that *NvLWamide* neurons are detected in regenerating oral regions by approximately 48 hours post amputation (hpa), and regenerate in both the endoderm and ectoderm. *NvashA* expression is detectable by ~28

hpa. However, its expression is restricted only to cells in the regenerating ectoderm, suggesting that endodermal *NvLWamide* neurons are patterned via an *NvashA* independent mechanism during regeneration. We also generated an *NvashA* transgenic reporter line using an enhancer region immediately upstream of *NvashA*. Surprisingly, the transgenic reporter only recapitulates developmental expression of *NvashA*. It fails to recapitulate regenerative *NvashA* expression. Our data argue regenerative and developmental neurogenesis occur via distinct mechanisms, and we are generating conditional alleles to confirm the putative differential requirement of *NvashA*. Future efforts will focus on identifying the regulatory inputs acting on *NvashA* during development and regeneration. This work will serve as a foundation to explore the mechanistic underpinning of variations between developmental and regenerative patterning.

Program Abstract #354

Identification and Characterization of Delta Targets during Neurogenesis in *Nematostella vectensis*

Suraj Pursnani

Lehigh University, USA

Notch-Delta signaling is a highly conserved signaling pathway that regulates cellular differentiation in animals. Notch and Delta are single-pass transmembrane proteins that have an extracellular and intracellular region. When Delta binds Notch, the Notch intracellular domain is cleaved and translocated to the nucleus, suppressing differentiation by inhibiting expression of neural genes. Our preliminary data suggests that the Delta intracellular domain (DICD) is cleaved, translocates to the nucleus, and promotes neural differentiation. We injected fluorescently targeted mRNA that codes for the DICD protein into *Nematostella* embryos. We found fluorescently targeted DICD protein in the nucleus of the cells. This suggested that the DICD translocates to the nucleus. We performed sufficiency and necessary experiments to determine the effects of Delta activity on the neural marker, *NvashA*, and cell proliferation. In the sufficiency experiment, overexpression of *NvdeltaICD* promoted *NvashA* expression and decreased the total number of cells without increasing cell death. In the necessary experiment, knocking down *NvdeltaICD* using *NvdeltaICD* Morpholino injections showed that *NvdeltaICD* is necessary for normal *NvashA* expression. We used RNAseq to identify putative target genes differentially expressed in *Nematostella* that received *NvdeltaICD*:venus injections in comparison to control-injected animals. We have confirmed two targets by performing qPCR in morphant animals lacking the *NvdeltaICD,* and we are currently screening the remaining putative targets. We will use the data from these experiments to generate preliminary mechanistic models for the function of *Nvdelta* during cellular differentiation, which we will test in future functional experiments. Our ultimate goal is to use these mechanistic models to better treat aggressive tumors by developing therapeutic targets for genomic therapy. This work is supported by Lehigh University and the Robert Langer-Neal Simon Award.

Program Abstract #355

Characterization of the *Nematostella vectensis NvLwamide* transgenic line reveals stereotypy in the cnidarian nerve net

Dylan Faltine-Gonzalez, Jamie A. Havrilak, Michael J. Layden

Lehigh University, USA

The cnidarian nervous system is often described as a diffuse nerve net lacking true organization. Given the phylogenetic position of cnidarians as the sister taxa to bilaterians improved characterization of the cnidarian nerve nets will improve our understanding of the origin and evolution of bilaterian nervous systems. We previously identified a neurogenic role for *NvashA,* an *achaete-scute* homolog, in embryonic ectodermal neurogenesis. Downstream targets of *NvashA* were identified in order to further characterize the nervous system. This work focuses on characterizing the downstream target *NvLwamide*-like. *NvLWamide* expression is first detected in the typical neurogenic salt and pepper pattern within the gastrula ectoderm. By planula larval stages it becomes detected in the forming endoderm, and expression persists in ectoderm and endoderm throughout polyp stages. To visualize the architecture of *NvLWamide* neurites, we generated a *NvLWamide::mCherry* transgenic reporter line. We then confirmed mCherry efficiency at colabeling with endogenous *NvLwamide* mRNA, finding that co-labeling increased as development occurred, with 80% colocaltization occurring by the juvenile polyp stage. We observed a surprising stereotypy in neurite projections described by our transgene. Sensory neurons in the ectoderm show consistence in that they appear to posses tripolar like projections, and occur once per radial segment, though exact location varies among individuals. Endodermal ganglion-type neural cells were consistently found immediately above the mesenteries and project the length of the oral aboral axis. Functional analysis showed that *NvashA* is required for both endodermal and ectodermal expression of *NvLWamide::mCherry.* This work demonstrates 1) that cnidarian nerve nets are likely highly organized, and 2) that *NvashA* also regulates endodermal neurogenesis. This work is funded by the GAANN fellowship (P200A120139) from the US Dept of Education.

Program Abstract #356

Identification of novel neurogenic transcription factors during development and regeneration of *Nematostella vectensis*

Jamie Havrilak, Nisha Singh, Caitlin Tedeschi, Michael Layden
Lehigh University, USA

Our lab is interested in understanding neural development and regeneration in the startlet sea anemone *Nematostella vectensis*. The inherent similarities and differences in the molecular regulation of neural development and regeneration remain poorly defined, but utilizing *Nematostella* as a model allows us to directly compare the molecular mechanisms that drive neural development to those that regulate regeneration within the same model organism. *Nematostella* is a diploblastic animal with a nervous system comprised of both ectodermal and endoderm "nerve nets". *Nematostella* neurogenesis occurs via a conserved cascade present in other current model systems, and two major families of neural bHLH transcription factors, *achaete-scute* (*Nvash*) and *atonal*-related proteins (*Nvarp/Ath-like*), are known to drive neurogenesis and also have conserved proneural functions. However, the expression pattern and function of many of these genes remains poorly defined. To identify novel proneural genes we performed an *in situ* hybridization screen to identify expression patterns of *Nvash* and *Nvarp* genes consistent with that of a neural regulatory factor. To date 5 genes display the characteristic "salt and pepper" pattern indicative of neural expression. We were particularly interested in *Nvarp1*, because its expression is restricted to the endodermal layer during early development. Functional analysis of *Nvarp1* morphant animals showed reduced expression of neural markers *Nvelav* and *NvRFamide*. Conversely, global misexpresisson of *Nvarp1* mRNA resulted in increased neural marker expression. These data support a neurogenic role for *Nvarp1*, and likely represents the first endoderm specific neurogenic transcription factor in *Nematostella*. We are currently characterizing the expression patterns of each novel and known neural gene during regeneration to provide a landscape of likely patterning cues acting to promote neural regeneration.

Program Abstract #357

MicroRNAs and regulation of retinoic acid induced growth cone turning in snail neurons

Sarah Walker, Gaynor Spencer, Robert Carlone
Brock University, CA

During development and regeneration, neurons navigate through a changing and highly complex environment and ultimately establish remarkably accurate connections with their target cells. The tips of these growing axons, growth cones, rapidly respond to various environmental cues, including classical chemotactic proteins such as netrins and semaphorins. Recent studies have led to the identification of other, non-traditional guidance cues, including the Vitamin A metabolite, retinoic acid (RA). RA has been shown to act as a chemoattractant for both vertebrate and invertebrate neurons in vitro. Little is known, however, about the nature of underlying regulatory molecules or biochemical pathways involved in fine tuning of the growth cone turning response to a gradient of RA. MicroRNAs (miRNAs), a class of conserved non-coding RNA transcripts, have recently been proposed to regulate gene expression and local protein synthesis during growth cone guidance in response to traditional protein cues. Our goal is to determine the role that miRNAs play as mediators of axonal guidance in response to a non-traditional guidance cue, RA. We have previously established that growth cones from neurons of the pond snail, Lymnaea stagnalis, exhibit positive turning towards RA in a local protein synthesis-dependent manner. We now have evidence for the compartmentalization of miR-124 and miR-133 in these axons, as well as in cell bodies within the brain. We are currently performing microarray and RNA Seq analyses to identify other axonally localized miRNAs involved in this chemoattractive response to RA. We are utilizing LNA-FISH to precisely localize these miRNAs in the growth cone with the goal of identifying their putative target mRNAs. These studies will advance our knowledge of the fine tuning of growth cone dynamics, particularly with respect to the underlying mechanisms of RA-induced chemoattraction during development and regeneration.

Program Abstract #358

The genetic regulatory logic of establishing neuronal dimorphisms in *C. elegans*

Emily Bayer[1], Meital Oren-Suissa[1], Oliver Hobert[1,2]
[1]Columbia University, USA; [2]Howard Hughes Medical Institute, USA

Sexual dimorphism is a widely acknowledged biological phenomenon, yet the mechanisms underlying specific developmental dimorphisms are largely unknown. As a result of the defined connectome available for both *C. elegans* males and hermaphrodites, it is clear that there are dimorphic wiring differences in shared neurons between the adult animals of the two sexes (1). Several of these dimorphisms are found in the phasmid sensory neurons, whose chemical synapses onto the command interneurons are sexually dimorphic, suggesting that they have sex-specific

functions. Using GRASP transsynaptic labeling technology we have shown that these sexually dimorphic synaptic connections arise from a mixed juvenile state, with many eventually dimorphic adult connections present in both sexes initially and then restricted by sex-specific synaptic pruning (2). Sexual determination is regulated across many invertebrate and vertebrate species by the highly-conserved *doublesex/mab* (DM) domain genes. In *C. elegans*, the DM domain class contains 11 paralogs. I have identified *dmd-4* as being dimorphically expressed in the phasmid neurons. Two upstream pathways, the canonical sex determination pathway and a phasmid subtype specification factor, *ceh-14*, regulate *dmd-4* in the phasmid neurons. These genes are required for establishing adult phasmid dimorphism, including sex-specific synaptic pruning, and in their absence the neurons maintain their juvenile non-dimorphic state. Ultimately, I will seek to elucidate how neuronal subtype identity and sexual identity intersect to establish sexual dimorphisms in the nervous system.

Funded by the NIH and HHMI.

Jarrell TA, Wang Y, Bloniarz AE, Brittin CA, Xu M, Thomson JN, Albertson DG, Hall DH, Emmons SW. 2012. *Science* 337, 437-444.
Oren-Suissa M, Bayer EA, Hobert O. 2016. *Nature* early online doi:10.1038/nature17977

Program Abstract #359

Contactins function as ligands for Amyloid Precursor Proteins in regulating polarized neuronal growth

Philip Copenhaver, Tracy Swanson, Jenna Ramaker
Oregon Health & Science University, USA

Although Amyloid Precursor Protein (APP) is the source of beta-amyloid fragments that accumulate in Alzheimer's disease, APP also regulates neuronal growth and polarity in the developing nervous system, albeit via mechanisms that are still controversial. Studies in cell culture suggested that APP can interact with the heterotrimeric G protein Goα and function as an unconventional G protein-coupled receptor, but compensatory interactions by other related proteins have hindered an analysis of this process in the mammalian brain. Using *Manduca* (hawkmoth) and *Drosophila* (fruitfly) as simpler models, we have shown that the sole insect ortholog of APP (APPL) directly binds Goα in the leading processes and synaptic terminals of developing neurons, and that endogenous APPL-Goα interactions are regulated by Goα activation. In cultured *Manduca* embryos, we found that stimulating APPL-Goα signaling restricts the polarized outgrowth and migration of developing neurons, consistent with other evidence that APP family proteins function as neuronal guidance receptors. Recent studies have shown that GPI-linked Contactins can interact with APP, potentially acting as binding partners or co-receptors. We have now shown that *Manduca* Contactin (MsContactin) is selectively expressed by glial cells that ensheath migratory neurons (expressing APPL), and that Contactin-APPL signaling regulates neuronal-glial adhesive interactions. Short-term treatment with Contactin-Fc fusion proteins labeled the migratory neurons in an APPL-dependent manner, while more prolonged treatment inhibited their migration and outgrowth, an effect that was blocked by knocking down APPL expression or preventing Goα activation. These results support the model that Contactins function as authentic ligands for APP family proteins that regulate Goα-dependent polarized growth, providing a novel mechanism for controlling neuronal guidance during embryogenesis. Funding: NIH NS078363 (PFC); NIA T32 AG023477 (JMR).

Program Abstract #360

Eyeless promotes apoptosis of mushroom body neuroblasts

Matthew Pahl, Susan E. Doyle, Sarah E. Siegrist
Department of Biology, University of Virginia, USA

A remarkable number of molecularly and functionally distinct neurons are generated during central nervous system (CNS) development. In *Drosophila*, neuronal diversity is generated in part through the asymmetric cell divisions of populations of lineage-restricted neural stem cells, known as neuroblasts (NBs). As development nears completion, NB cell divisions terminate in a spatially and temporally defined manner and no new neurons are produced during adulthood. Our lab is interested in the molecular mechanism that leads to termination of neurogenesis once development is complete, which could provide insight into restoring neurogenesis in brains of adult animals. Previously, we determined that a subset of brain NBs, known as the mushroom body neuroblasts undergo apoptosis late in development, and that blocking pro-apoptotic regulators allows MBNBs to survive and continue proliferation in brains of adults (Siegrist et al., 2010). To identify genes that regulate MBNB apoptosis, we carried out a directed candidate based RNAi screen. We found that knockdown of the Pax6 ortholog, eyeless (ey), allows MBNBs to survive and maintain proliferation. Conversely, overexpression of ey leads to premature MBNB cell death. Currently we are investigating the mechanism regulating ey

dependent MBNB apoptosis.
Funding Source: This work was supported by an R00 career development award to S.E.S. from the NICHD.

Program Abstract #361

A Toll receptor-FoxO pathway represses Pavarotti/MKLP1 to promote axonal transport and structural plasticity

Colleen McLaughlin[1], Inna Nechipurenko[2], Nan Liu[1], Heather Broihier[1]
[1]Case Western Reserve University School of Medicine, USA; [2]Brandeis University, USA

Properly regulated microtubule dynamics are key to neuronal structure and function. We previously found that FoxO promotes dynamic microtubules in developing motorneurons, highlighting the importance of transcriptional regulation in controlling microtubule behavior. To elucidate FoxO function, we screened for its upstream regulators and downstream effectors. On the upstream side, we present genetic and molecular pathway analyses indicating that the Toll-6 receptor, the TIR adaptor dSARM, and FoxO function in a linear pathway to regulate axonal transport and structural plasticity. On the downstream side, we find that Toll-6 signaling represses the mitotic kinesin Pavarotti/MKLP1 (Pav-KLP), which acts as a brake on microtubule dynamics. We present evidence that Pav-KLP dysregulation underlies transport and plasticity phenotypes in mutant backgrounds, arguing that a core function of this novel pathway is regulating microtubule dynamics via Pav-KLP. In addition to describing a novel molecular pathway, our work reveals an unexpected function for dynamic microtubules in enabling rapid presynaptic structural plasticity.

Program Abstract #362

Wnt, The Spinal Frontier: The role of Wnt5b in radial glial proliferation and differentiation during zebrafish spinal cord development

Carla Velez[1], Catalina Sakai[1], Kimberly Johnson[2], Chelsea Moriarty[1], Julia Kim[1], Sophie Chase[1]
[1]Smith College, USA; [2]University of Massachusetts Amherst, USA

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

Program Abstract #363

CDX is a key component of the spinal cord gene regulatory network controlling neurogenesis

Isaac Skromne, Piyush Joshi
University of Miami, USA

Spinal cord neurogenesis is sustained at the caudal end of the embryo during development throughout the continuously addition of precursor cells by the caudal lateral epiblast (CLE). Stem cells in the CLE progressively become specified and eventually differentiate to become part of the spinal cord. While the signaling factors FGF, WNT, and RA are known to regulate aspects of the specification and differentiation process, the mechanism coordinating their activities remains elusive. We propose Cdx4 transcription factor as a candidate integrator of signal information, switching cells from a proliferative to a differentiation mode of development. Here we shows that Cdx4 represses the pluripotency factor *Sax1*, while activating the neurogenic factor *Pax6*. Significantly, Cdx4 blocks Pax6-dependent activation of *Neurogenin-2*, thus promoting specification but not differentiation. This novel neurogenic activity of Cdx4 is in addition to its known role in spinal cord's spatial patterning, and provides a mechanism for the sequential maturation of cells during spinal cord neurogenesis. We propose that Cdx4 is part of a novel gene regulatory network that coordinates spinal cord specification, maturation and patterning.

Program Abstract #364

Gfap-positive radial glial cells are an essential progenitor population for later born neurons and glia in the zebrafish spinal cord.

Michael Barresi[1], Kimberly Johnson[1,2], Jessica Barragan[1], Sarah Bashiruddin[1], Cody Smith[3], Chelsea Tyrrell[2], Michael Parsons[4], Rosemarie Doris[5], Sarah Kucenas[3], Gerald Downes[2], Carla Velez[1], Caitlin Schneider[1], Catalina Sakai[1], Narendra Pathak[1], Katrina Anderson[1], Rachael Stein[1], Stephen Devoto[5], Jeff Mumm[6]

[1]Smith College, USA; [2]University of Massachusetts, USA; [3]University of Virginia, USA; [4]Johns Hopkins University, USA; [5]Wesleyan University, USA; [6]Wilmer Eye Institute, USA

Radial glial cells are presumptive neural stem cells (NSCs) in the developing nervous system. The direct requirement of radial glia for the generation of a diverse array of neuronal and glial subtypes however, has not been tested. Employing two novel transgenic zebrafish lines and endogenous markers of neural stem cells and radial glia, we show for the first time that radial glia are essential for neurogenesis during development. By using the *gfap* promoter to drive expression of nuclear localized mCherry we discerned two distinct radial glial derived cell types: a major *nestin*+/ Sox2+ subtype with strong *gfap* promoter activity and a minor Sox2+ subtype lacking thisactivity. Fate mapping studies in this line indicate that *gfap*+ radial glia generate later-born CoSA interneurons, secondary motorneurons, and oligodendroglia.In another transgenic line using the *gfap* promoter driven expression of the Nitroreductase enzyme, we induced cell autonomous ablation of *gfap*+ radial glia and observed a reduction in their specific derived lineages, but not Blbp+ and Sox2+/*gfap* negative NSCs, which were retained and expanded at later larval stages. Moreover, we provide evidence supporting classical roles of radial glial in axon patterning, blood brain barrier formation, and locomotion. Our results suggest that *gfap*+ radial glia represent the major NSC during late neurogenesis for specific lineages, and possess diverse roles to sustain the structure and function of the spinal cord. These new tools will both corroborate the predicted roles of astroglia and reveal novel roles related to development, physiology, and regeneration in the vertebrate nervous system.

Program Abstract #365

The Retinoic Acid signaling pathway temporally influences neural crest migration and neuronal differentiation during zebrafish enteric nervous system formation in vivo

Rosa Uribe, Stephanie Hong, Marianne Bronner

California Institute of Technology, USA

The enteric nervous system (ENS), an autonomous network of thousands of interconnected ganglia that innervate the digestive tract and control its motility, is derived from vagal neural crest cells. During development, vagal neural crest cells emigrate from the caudal hindbrain and migrate ventrally. In response to patterning and guidance cues, they migrate toward the primitive foregut and then caudally along the gut tube. Once reaching their final positions along the gut, they differentiate into distinct enteric neuron types or glia. Although much research attention has focused on the migration of neural crest cells after they have reached the gut, much less is known about how extrinsic signaling factors regulate early vagal neural crest gut entry, patterning and migration. Using zebrafish as a model coupled with FACS sorting and transcriptome analysis, we show that various Retinoic Acid (RA) signaling pathway components are expressed in the gut microenvironment during the time when neural crest invade the gut. Temporal exogenous treatment with RA prior to and during vagal neural crest gut entry enhanced the migratory progress, increased the number of crest around the gut and increased the number of differentiated enteric neurons during later phases of ENS development. Conversely, temporal attenuation of the RA pathway using Tg(*hsp70*:dnzRAR) heat-shocked embryos led to delayed gut entry by neural crest cells and live imaging revealed altered migratory behavior and progress along the gut. Together, these results suggest that the RA signaling pathway plays a key temporal role during the early phases of ENS development and they enhance our understanding of the genesis of the ENS *in vivo*. Funding provided by NIH DE024157 to M.E.B, by NIH F32HD080343 to R.A.U., by a Burroughs Wellcome Fund PDEP award to R.A.U. and by a Caltech SURF award to S.H.

Program Abstract #366

Developmental origins of the pioneer neuron and its role in facial branchiomotor neuron migration

Anastasia Beiriger, Sarah Wanner, Gordon Kindlmann, Victoria Prince

University of Chicago, USA

Neuronal progenitors are born in the ventricular zone of the neural tube and migrate from their birthplaces to final destinations where they integrate into the surrounding neural circuitry. Two main types of neuronal migration have been described: radial, in which neurons migrate using glial fibers as a scaffold, and tangential, in which neurons migrate parallel to the surface of the neuroepithelium. We are investigating mechanisms of tangential migration in the facial branchiomotor neurons (FBMNs) of the VIIth cranial nerve. FBMNs are born in rhombomere (r)4 of the hindbrain and

perform a chain-like tangential migration to r6/r7 that is conserved in zebrafish, mice, and humans. Our previous studies in zebrafish have demonstrated that the first FBMN to migrate acts as pioneer, leaving behind a trailing axon that subsequent FBMNs use as a track to guide their migration. When a pioneer neuron or its trailing axon is ablated, migration is disrupted, indicating that other FBMNs cannot functionally replace the pioneer after migration has already begun. Here, we are investigating the developmental mechanisms which specify pioneer and follower FBMNs within the hindbrain. Specifically, we are testing whether the pioneers arise A) in a lineage-dependent manner, such as from a single "C-division" across the midline or B) in a stochastic manner, such as from lateral inhibition between FBMN progenitors or induction upon migration into r5. Using light sheet microscopy, we are imaging the double transgenic line Tg(*zCREST:mRFP; H2B-GFP*) between 12 hpf (hours post fertilization), when FBMN progenitors are still dividing, and 24 hpf, when the first FBMNs reach r6/r7. We are using Imaris software to reconstruct the lineage of the pioneers, and are developing new code to visualize their trajectory through the hindbrain.
This work is supported by NSF grant (1528911) to VEP and GK; AB is supported by NIH T32 HD055164 and NSF GRFP #DGE-1144082.

Program Abstract #367
Investigating Cellular and Molecular Mechanisms of Neuronal Layering During Retinal Development in Zebrafish
Megan Eldred, Leila Muresan, William Harris
University of Cambridge, United Kingdom
The central nervous system is a complex, yet well-organised tissue. This robust organisation is evident in the architecture of the retina: consisting of 5 different neuronal types organised into distinct layers: RGC, AC, BP, HC and PR layers. This remarkable organisation is evolutionarily conserved in vertebrates, but little is known about the mechanisms by which these cells form the correct layers. Live imaging has revealed overlapping periods of birth and extensive inter-digitation followed by cells sorting out into their appropriate positions, suggesting cell-cell interactions are important. Here we present an organoid culture system for zebrafish retinal cells to investigate possible cellular and molecular mechanisms responsible for the establishment of the tissue architecture. The Spectrum of Fates fish is a multiply transgenic line in which each retinal cell type can be identified based on expression of a combination of fluorescently tagged cell fate markers. By dissociating zebrafish retinal cells and culturing them in non-adhesive microwells we observe their inherent self-organising properties, which, interestingly, result in an inside-out layering. We have begun to investigate the cell-cell interactions in this system by genetically removing individual cell types and assaying the resulting organisation of the reaggregated, cell-type deficient, retinal organoids. We have also begun investigating the role of candidate molecules in this system, which is proving to be a simple and easy platform to potentially reveal some of the important players in neuronal patterning.
This work is supported in parts by the BBSRC and the Wellcome Trust.

Program Abstract #368
Defining the roles of Slit and Robo signaling necessary for axon-glial interactions during post optic commissure formation in forebrain development
Jake Schnabl[1,2], Morgan Schwartz[2], Jin Sook Park[2], Scott Gilman[3], Brittney Edens[2], Nina Wren[2], Jon Caris[3], Michael Barresi[1,2]
[1]Molecular and Cellular Biology Program, University of Massachusetts Amherst, USA; [2]Department of Biological Sciences, Smith College, USA; [3]Spatial Analysis Lab, Smith College, USA
Connectivity in the brain is first established during embryonic development, when neurons project axons over great distances using navigational cues in the ectodermal environment to locate their specific targets. Connections between the two sides of the central nervous system are initiated by pathfinding axons that make the important decision to cross the midline and form a commissure. Midline crossing axons of the post optic commissure (POC) are provided both adhesive support and directional guidance by an astroglial bridge in the zebrafish forebrain. Secreted Slit proteins and their associated Robo receptors on the cell surface mediate condensation of the glial bridge and midline crossing of POC axons. The process of condensation is mediated by the repellant guidance cues of *slit2/3* and *robo2/3* signals in the POC. In contrast to the canonical *slit* and *robo* cues, we have discovered novel positive guidance roles for slit1a and *robo4*. We are taking local and global misexpression approaches in *slit1a* and *robo4* loss of function backgrounds to probe their roles in POC formation. We also developed a computational algorithm that can quantify pathfinding and astroglial phenotypes. Returning *slit1a* expression in *yot* mutants can rescue commissure formation even in the context of expanded Slit2/3 expression. Moreover, POC axons will extend toward ectopic regions of Slit1a expression. These data support a role for slit1a in promoting midline crossing at the POC. *robo4* is expressed by diencephalic astroglia (not POC axons), and

analysis of POC formation in *robo4* mutants shows glial bridge spreading along with axon defasiculation. These data suggest a novel role for astroglia in mediating POC condensation in a Robo4-dependent manner. We are currently testing whether Slit1a-mediated POC guidance depends upon Robo4 function. Understanding how Slit-Robo signaling functions to regulate axon-glial interactions will be a critical step in developing a model for neural regeneration.

Program Abstract #369

The RES spliciosomal complex is required for brain development: molecular dissection using CRISPR/Cas9 in zebrafish.

Juan Fernandez[1], Miguel Moreno-Mateos[1], Manuel Irimia[2], Murat Gunel[1], Antonio Giraldez[1]
[1]*Yale University, USA;* [2]*Centre for Genomic Regulation, Spain*

Increasing evidence suggest that regulation of pre-mRNA splicing is critical for correct tissue specification and mutation in spliceosome components are recurrently being associated with perturbed brain development and disease pathogenesis. The pre-mRNA REtention and Splicing complex (RES-c) is a spliceosomal complex that is conserved from yeast to human and is important for RNA splicing and retention of unspliced pre-mRNA. Yet, the molecular determinants that select specific targets for regulation, and their function in vertebrate development are unknown. In fish, the three members of the RES-c (*bud13*, *rbmx2* and *snip1*) are maternally expressed and are specifically restricted to the central nervous system at 24 hpf, indicating that RES might have a specific function during brain development. Using an optimized CRISPR-Cas9 system, we have generated zebrafish loss-of-function mutants in all three members of the RES-c. The mutants display smaller brains with a significant increase in neuronal cell death. These phenotypes that were specifically rescued by, either human or zebrafish, mRNA encoding for the component of the RES-c. Transcriptome analysis by RNAseq reveals specific intron accumulation in a distinct subset of transcripts in the three KO mutant embryos, suggesting that RES is required for the proper splicing of specific mRNA targets. We are currently assessing how misregulation of particular targets contributes to observed neural defects, and the regulatory sequences that mediate recognition of these introns. Taken together, our results suggest that the RES-c facilitate the processing of a subset of brain specific transcripts during early zebrafish neurulation, and that human and zebrafish RES may have a conserved functions in this capacity. These results illustrate how our systematic approach can provide key insight into the molecular mechanisms that mediate vertebrate brain development and how disrupting these pathways leads to developmental disorders

Program Abstract #370

Atypical postnatal development of inhibition in cortical layer I in autism

Iris Trutzer, Basilis Zikopoulos
Boston University, USA

Layer I of the cortex, which appears early in development, is composed of glia and few neurons dispersed among dendritic and axonal processes. The neurons in layer I direct the patterning of cortical lamination in mammals, including humans. In adulthood, layer I contains neurons that are overwhelmingly inhibitory. The dendrites of excitatory pyramidal neurons from deep layers of the cortex terminate in this layer, where they receive synapses from feedback projections that modulate cortical function. Despite the unique role of layer I, little is known about the postnatal development of this layer, which likely regulates the structural and functional development of the cortex. To address this gap, we quantitatively studied the distribution of distinct classes of inhibitory neurons in layer I of prefrontal cortices (PFC) in typically developing children and adolescents. We compared our findings with data from children and adolescents with autism, a disorder characterized by an imbalance in excitation and inhibition in the brain. Inhibitory neurons are categorized by their expression of calcium binding proteins. Neurons that express calretinin (CR) and calbindin (CB) exert modulatory inhibition, while those that express parvalbumin (PV) are strongly inhibitory. CR-expressing neurons have been observed in layer I in prenatal and adult tissue. Neurons expressing CB and PV are not present in layer I in the adult brain. We report that in addition to CR-expressing neurons, PV- and CB-expressing neurons are found in layer I of PFC in neurotypical children and adolescents. This superficial distribution of inhibitory neurons is accentuated in autism, where children, adolescents, and adults have more CB, PV, and CR-expressing neurons in layer I. The increased density of inhibitory neurons in layer I, along with the appearance of strongly inhibitory PV neurons, likely has significant implications for the efficiency of cortical processing in these areas. Supported by NIMH.

Program Abstract #372

Role of a novel postmitotic factor in regulating neocortical migration and patterning

Jessica Clark, Emily Capaldo, Makiko Yamada, Di Shao, Angelo Iulianella

Dalhousie University, CA
The formation of the neocortex relies on the production of a diverse set of neuronal populations and their migration into specific layers. This process is dependent upon the ordered migration of neurons responding to cues from neurons in the cortical plate and from Cajal-Retzius cells that derive from the subpial regions of the brain. Understanding the mechanisms that control specification and migration within the neocortex is crucial as perturbations in brain cytoarchitecture are seen in developmental disorders. We previously characterized the expression pattern for a novel gene called Mixed-lineage leukemia; translocated to chromosome 11 (Mllt11) and found it to be exclusively expressed in developing post mitotic neurons. We now characterize the role of Mllt11 as a novel regulator of neocortical cytoarchitectural organization with implications for how migratory cues are established. We show that the loss of Mllt11 leads to an abnormal distribution of cells expressing Reelin as well as an inversion of neocortical patterning and a loss of specific populations of cortical projection neurons. These findings suggest that Mllt11 regulates neocortical patterning by establishing proper migration and positioning of Reelin-expressing cells.

Program Abstract #373

Six3 regulation of FoxG1 expression in the anterior neuroectoderm

Sandra Acosta-Verdugo[1], Xin Geng[2], Oleg Lagutin[3], Guillermo Oliver[1]

[1]Northwestern University, USA; [2]Oklahoma Medical Research Foundation, USA; [3]St Jude Children Research Hospital, USA

FoxG1 is a transcription factor expressed in the anterior neuroectoderm of mouse embryos as early as the 5- to 8-somites stage. Foxg1 plays a pivotal role during early telencephalic development, promoting ventral identity downstream of SHH signaling. Also, it controls dorsal forebrain differentiation through direct transcriptional repression of Wnt ligands. Six3 is expressed in the anterior neuroectoderm starting at around E7.0. In medaka fish, FoxG1 expression is directly regulated by Six3. In this study, we aim to understand how FoxG1 is regulated in the mammalian anterior neural tube. In our lab, we have generated a Six3 hypomorphic mouse strain by inserting an in frame neomycin/thymidine kinase cassette (Six3 $^{neo/neo}$). These mice exhibit defects in rostral midline formation. The transcription of the Six3 ^{neo} hypomorphic allele is approximately only 30% of the total amount of normal Six3 mRNA transcript. We found that in the Six3 $^{neo/-}$ embryos, the levels of FoxG1 expression are reduced at early stages of embryonic development; however, at later developmental stages, normal expression levels are detected. Then, we evaluated whether Six3 directly regulates the earliest FoxG1 expression as we identified a conserved Six3-binding site located in a FoxG1 enhancer 1.5 kb upstream of the 5' UTR. ChIP analysis demonstrated that Six3 binds to site in chromatin isolated from E8.5 embryos. Moreover, luciferase reporter assays confirmed that Six3 activates this enhancer in vitro. Altogether, we have shown that FoxG1 is a downstream target of Six3 at early stages of the forebrain specification in mouse embryos.

This work was supported by National Eye Institute

Program Abstract #374

Molecular and cellular mechanisms underlying the role of Insm1 in the generation of basal progenitors

Stefania Tavano[1], Judith T M L Paridaen[1], Elena Taverna[1], Nereo Kalebic[1], Michaela Wilsch-Bräuninger[1], Holger Brandl[1], Andreas Dahl[2], Wieland B Huttner[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, DE; [2]BIOTEC, TU Dresden, DE

During brain development neurons arise from neurogenic divisions of neural progenitors (NPs). In the mammalian neocortex there are two main classes of NPs: (i) apical progenitors (APs) that undergo mitosis at the ventricular (apical) surface of the ventricular zone (VZ); (ii) basal progenitors (BPs) that undergo mitosis basal to the VZ, typically in the subventricular zone (SVZ). One of the most striking differences between human and mouse neocortical development is the increased generation and proliferative capacity of human BPs, thought to underlie the massive expansion of the human neocortex. A crucial, yet unaccomplished, goal of developmental neuroscience is to identify the molecular mechanisms responsible for this evolutionary increase in BP generation. Here we investigate the role of Insulinoma-associated 1 (Insm1), a zinc-finger transcription factor implicated in the generation of BPs. We show that Insm1 is highly expressed in newborn BPs, both in the mouse and human developing neocortex. Insm1 overexpression in the mouse causes detachment of the APs apical endfoot from the ventricular surface, leading to delamination of BPs. Moreover, Insm1 overexpression changes the identity of NPs in the VZ, by inducing BP fate without affecting neuronal differentiation. Finally, we perform transcriptome analysis of Insm1-overexpressing cells to identify Insm1 downstream target genes. Insm1 overexpression alters the expression of genes associated with cell morphogenesis, cell adhesion and cell motility. Notably, Insm1 overexpression decreases the expression of genes important for the retention of the apical contact, suggesting that Insm1 is a key regulator of delamination. We are currently analyzing the role of the most interesting downstream targets. By

identifying a network of genes downstream of Insm1 and investigating their function during delamination, this work contributes to the dissection of the molecular mechanisms that lead to BP production.

Program Abstract #375

Genetic mapping of developmental noradrenergic neuron subpopulations in respiratory homeostasis

Jenny Sun, Megan Key, Russell Ray
Baylor College of Medicine, United States

Central noradrenergic (NA) neurons are a critical participant in the brainstem networks that maintain respiratory homeostasis. However, limitations in previous approaches to study the NA system have provided conflicting results and it is still not entirely clear how NA neurons are integrated into central respiratory circuits and how they may play a role in congenital respiratory pathophysiologies including Sudden Infant Death Syndrome. Previous studies suggest that proper partitioning of the developing hindbrain into transient, genetically-defined segments called rhombomeres (r) is required for normal respiratory function. As many features of neural circuits are determined during embryogenesis, we hypothesize that the genetic programs within individual rhombomeres play a role in functionally patterning the adult NA system to integrate into the broader central respiratory circuitry. To test our hypothesis, we used acute, non-invasive and reversible pharmaco-genetic DREADD receptors to either perturb (hM4D) or stimulate (hM3D) genetically-defined rhombomere-specific NA subpopulations in the adult brainstem. Whole-body barometric plethysmography with conscious and unrestrained mice was used to determine the effect of hM4D and hM3D DREADD activation in rhombomeric NA subtypes under room air, hypercapnic (5% CO_2) and hypoxic (10% O_2) conditions. Our data demonstrate that perturbation of the whole NA system results in reduced hypercapnic and hypoxic responses. Our data also show that perturbation of neurons derived from whole rhombomeres (r1, r2, r3&5, r4, and r7&8) results in a variety of respiratory phenotypes. Finally, preliminary data suggests that several developmental NA subpopulations are uniquely involved in the adult hypercapnic and hypoxic ventilatory responses. Cumulatively, our data supports the contribution of early embryonic patterning in defining the functional organization of the adult respiratory network.

SUPPORT: R01HL130249

Program Abstract #376

Regulation of embryonic neurogenesis by germinal zone vasculature

Mathew Tata[1], Ivan Wall[1,3], Andy Joyce[1], Joaquim Vieira[1,4], Nicoletta Kessaris[2], Christiana Ruhrberg[1]
[1]UCL Institute of Ophthalmology, University College London, 11-43 Bath Street, London, EC1V 9EL, United Kingdom; [2]Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, United Kingdom; [3]Department of Biochemical Engineering, University College London, Bernard Katz Building, Gordon Street, London, WC1H 0AH, United Kingdom; [4]Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford, OX1 3PT, United Kingdom

In the adult rodent brain, new neurons are born in two germinal regions that are associated with blood vessels, and both blood vessels and vessel-derived factors are thought to regulate the activity of adult neural stem cells. However, it is not known whether a vascular niche also regulates prenatal neurogenesis. Here, we identify the mouse embryo hindbrain as a powerful model to study neurogenesis and define the relationship between neural progenitor cell (NPC) behaviour and vessel growth. Using this model, we show that a subventricular vascular plexus (SVP) extends through a germinal zone populated by NPCs whose peak mitotic activity follows a surge in SVP growth. Unexpectedly, hindbrains genetically defective in SVP formation due to constitutive loss of NRP1 showed a precocious peak in NPC mitoses that was followed by a premature decline in NPC mitotic activity due to cell cycle exit and premature neuronal differentiation. Defective regulation of NPC mitosis was also seen in mice lacking NRP1 selectively in endothelial cells, but not in mice lacking NRP1 expression by NPCs. Germinal zone vascularisation therefore sustains NPC proliferation in the prenatal brain by delaying NPC cell cycle exit and terminal differentiation.

Program Abstract #377

Suppressor of Fused (Sufu) controls cerebellar granule neuron proliferation via GLI3R

Tayyaba Jiwani[1,2], Jinny J. Kim[2,3], Lianne Rotin[2], Norman D. Rosenblum[1,2,3]
[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; [2]Program in Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, Canada; [3]Department of Physiology, University of Toronto, Toronto, Canada

Sonic hedgehog (SHH) signaling tightly controls the proliferation of cerebellar granule cells (GCs), the most abundant neurons in the brain. Reduced SHH signaling leads to hypoplasia-related motor deficits, while over-activated signaling

can cause medulloblastoma, a malignant brain tumour. However, the intracellular mechanisms by which SHH signaling effectors – GLI activator and repressor proteins – establish the correct level of signaling in GCs have not been defined. We deleted Suppressor of Fused (Sufu), a known modulator of GLI protein levels, specifically from mouse GC precursors using Math1-Cre recombinase. Mutant cerebella contained increased GCs with higher rate of proliferation demonstrated using BrdU incorporation assays. Increased proliferation did not result from failure of cell cycle exit or differentiation as mutant GCs correctly stopped incorporating BrdU and expressed differentiation markers. Moreover, TUNEL staining showed no difference in GC apoptosis, while cell counting at embryonic stages revealed no increase in GC specification. Contrary to previous Sufu deletion models, qRT-PCRs on FACS-isolated GCs revealed that SHH targets –Ptc1, Gli1, Nmyc and Ccnd1– were *not* up-regulated in mutant cells, suggesting that increased proliferation may *not* be driven by conventional SHH targets. However, GLI3-repressor (GLI3R) protein levels were reduced by 60%. Indeed, constitutive transgenic GLI3R expression in mutants rescued GC proliferation (reduced GC number and cell cycle marker expression). Finally, RNA Sequencing identified new Sufu targets, including Fgf8. A 3.5-fold increase in Fgf8, and downstream target Etv5, expression was confirmed by qRT-PCR in GCs. We conclude that Sufu restrains the rate of cerebellar GC proliferation via GLI3R-mediated gene repression. Our results identify novel Sufu targets, and a new GLI3R-mediated mechanism by which SHH signaling is calibrated in GCs, suggesting GLI3R as a promising therapeutic avenue in medulloblastoma.

Program Abstract #378

Gene transcription regulation of anterior hypothalamic development in mouse

Abdullah Al Mahmud, Jacques L Michaud

CHU Ste Justine, University of Montreal, CA

The paraventricular nucleus (PVN) of the anterior hypothalamus regulates several processes that are critical for survival, including the regulation of energy balance and of blood pressure. SIM1 directs the terminal differentiation of at least five types of PVN neurons identifiable by the production of OT, AVP, CRH, SS and TRH. Whereas Sim1-/- mice die shortly after birth, Sim1+/- mice survive but develop hyperphagia and early-onset obesity. We have shown that Sim1 functions along a physiological pathway in the PVN for the control of food intake. Sim1 thus regulates the development of the PVN as well as its function. The objective of this project is to identify novel regulators of PVN development. We have identified a regulatory element that specifically directs expression in all cells of the developing PVN. Using this element, we have generated transgenic mice that express gfp in these cells. In this study we collected PVN of the E12.5 from the wild-type (Wt) and Sim1-/- mice.We next collected the domain expressing gfp at different developmental stages (E11.5, E12.5, E13.5 and E14.5) as well as the immediate posterior domain of the developing hypothalamus. We are currently comparing the transcriptomes from these samples by performing RNA-seq. By comparing the transcriptomes of these different sets of embryos, we have found different clusters of gene sets the goes up or down between Wt vs Sim1-/- embryos. Some of these genes regulate many important developmental pathways such as Wnt signaling, axon guidance, MAPK signaling, adipocytokine signaling pathway etc. Differential gene expression were also observed among different embryonic developmental stage. As shown by our work on Sim1, regulators of PVN development have the potential of influencing physiological processes. The factors that we have identified in the course of this project may thus play a role in the pathophysiology of common disorders of homeostasis.

Program Abstract #379

Cyclin D1 May Couple Mitochondrial Biogenesis and Retinal Progenitor Cell Proliferation

Anthony Barrasso[1], Elda Rueda[2], Xuefei Tong[1], Donald Fox[2], Ross Poche[1]

[1]Baylor College of Medicine, United States; [2]University of Houston, United States

Premature cell cycle exit of retinal progenitor cells (RPCs) disrupts retinogenesis and causes developmental defects that affect vision. Proliferating cells bypass the G1 cell cycle exit checkpoint and promote S phase progression via a Cyclin D-mediated pathway. Genetic ablation of *Cyclin D1*, the predominant D-type cyclin in the developing retina, causes premature cell cycle exit of RPCs, which results in thinner retinae with degenerative lesions in the photoreceptor cell layer[1–3]. This phenotype is mirrored in mice with RPC-specific loss of *Ronin*, a transcriptional regulator of mitochondrial genes[4]. However, there is no molecular or genetic interaction between Cyclin D1 and Ronin. Thus, we hypothesize that Cyclin D1 promotes mitochondrial activity to drive cell cycle progression of RPCs, independent of Ronin. One study reports that Cyclin D promotes mitochondrial biogenesis[5], but others contradict these findings[6,7]. Our preliminary analysis suggests that *Cyclin D1* loss decreased expression of *Nrf1* and other genes that promote mitochondrial biogenesis. Additionally, activity of Complex IV of the electron transport chain was reduced in *Cyclin D1* KO retinae when analyzed via histochemical staining. To further explore the link between mitochondrial activity and the cell cycle, we are

developing a transgenic mouse that expresses a mitochondrially-localized fluorescent protein. These mice will be bred with Fucci cell cycle reporter mice to track mitochondrial dynamics coincident with cell cycle stage. Finally, we have developed a live imaging technique to track mitochondrial dynamics and cell cycle kinetics in developing retinal tissue. This study suggests that Cyclin D1 may promote mitochondrial activity and describes novel tools and techniques that will be of value for understanding the role of mitochondria in cell cycle progression. This project was funded by the NIH (T32 EY07102 and R01 EY024906).

Program Abstract #380

Roles of Nuclear respiratory factor 1 (Nrf1) in the developing mouse retina

Takae Kiyama[1], Ping Pan[1], Shinako Takada[2], William Klein[2], Chai-An Mao[1]

[1]The University of Texas Health Science Center at Houston, USA; [2]The University of Texas M. D. Anderson Cancer Center, USA

In the developing mouse retina, an extensive reprogramming of metabolic pathways from glycolysis to oxidative phosphorylation is closely coupled to the progression of retina progenitor cells (RPCs) from the proliferated state to terminal differentiated neurons. Such transition is observed in many developmental systems, suggesting the regulatory mechanisms enabling the reconfiguration of metabolic pathways is likely intricately mapped onto the regulatory networks controlling the cell cycle progression and differentiation. It is unclear, however, whether and how transcription regulators enable progenitor cells to alter their metabolic program and advance to a committed neuronal fate. Here we provide new insight into this question by studying the roles of *Nuclear respiratory factor 1* (*Nrf1*) in proliferative RPCs and differentiated retina ganglion cells (RGCs). *Nrf1* encodes an evolutionarily conserved transcription activator that binds to GC-rich DNA elements in promoters of a large number of nuclear genes required for mitochondrial biogenesis and respiratory function, implicating its role in the energy production. Nrf1 has also been shown to play a role in cell growth and proliferation, and in the pathogenesis of neurodegenerative diseases. Here we showed that *Nrf1* is highly expressed in the proliferative RPCs. In developing and mature retinas, Nrf1 expression is enriched in RGCs and photoreceptors both of which consume large amounts of energy. By combining genetic and genomic approaches with functional assays, we demonstrate *Nrf1* is essential for the cell cycle progression in RPCs and the extension of axonal processes in developing retina neurons. In contrast, disruption of Nrf1 in RGCs does not affect mitochondrial oxidative metabolism, suggesting that Nrf1 is not required for mitochondrial biogenesis in terminal differentiated retinal neurons. Funding sources: National Institutes of Health grants NEI EY024376 (C.-A.M.), NEI EY011930 (W.H.K.), and AI057504 (S.T.).

Program Abstract #381

Defining the effects of the ciliary protein TTBK2 on neural development, cilia structure, and function.

Emily Bowie

Duke University, USA

Primary cilia play a critical role in mediating the Sonic hedgehog pathway (Shh), an essential developmental signaling pathway. We previously identified a serine-threonine kinase, Tau tubulin kinase 2 (TTBK2), as a key regulator of the initiation of ciliogenesis. Mouse embryos homozygous for a null allele of *Ttbk2*, *bartleby* (*Ttbk2*^{bby}), lack cilia and die at midgestation due to Shh-dependent patterning defects. Mutations in human *Ttbk2* are associated with the dominantly inherited neurodegenerative disorder spinocerebellar ataxia type 11 (SCA11). We show that WT TTBK2 is impaired in its ability to rescue cilia formation in *Ttbk2*^{Sca11} cells, often causing misshapen ciliary axonemes. In parallel, we find that the SCA11 truncation of TTBK2 forms a dimer with WT TTBK2, consistent with a dominant interfering function. To examine the consequences of reduced levels of functional TTBK2 on cilia structure and development, we generated a *Ttbk2* allelic series. We obtained a gene-trap allele of TTBK2 (*Ttbk2*^{gt}) that produces reduced levels of WT *Ttbk2* transcript. *Ttbk2*$^{gt/gt}$ animals survive to adulthood but exhibit a variety of phenotypes similar to those seen in ciliopathies. We use the *Ttbk2*^{gt} allele as a sensitized genetic background on which to compare the null *bby* and *SCA11*-associated alleles by generating compound heterozygotes. We find more severe phenotypes associated with Shh signaling deficits in*Ttbk2*$^{sca11/gt}$embryos compared to *Ttbk2*$^{bby/gt}$. This genetic evidence further supports our hypothesis that the SCA11 truncation impairs the function of WT TTBK2.Consistent with the increased phenotypic severity, we also find that the frequency of cilia formation is further reduced in cells from*Ttbk2*$^{sca11/gt}$ embryos. Future work will focus on understanding the mechanisms by which TTBK2^{SCA11} impairs WT protein function and ciliogenesis, and the consequences for neural function.

Funding Sources: This work is funded by grants from NIH (R00HD076444) and the National Ataxia Foundation.

Program Abstract #382

Interactions between spiral ganglion neurons and glia influence intrinsic neuronal programs during early targeting.

Noah Druckenbrod, Lisa Goodrich
Harvard Medical School, United States

Precise wiring between spiral ganglion neurons (SGNs) and hair cells (HCs) is critical for the integration sound information. Improper wiring results in deafness even though HCs may respond to stimuli correctly. Many animal models of deafness exhibit disorganized wiring indicative of defects in early axon outgrowth. Therefore the early extension of SGNs is a critical yet delicate stage of circuit development. As the cochlea forms, spiral ganglion neurons (SGNs) and their peripheral axons undergo a series of stereotyped behaviors to establish their final morphology and wiring pattern. What signals guide these morphological and targeting decisions through the three-dimensional, multicellular terrain of the cochlea? Most studies have focused on the roles of classically described guidance cues, which act over long distances to attract or repel axons. However, less is known about the role of transient homo- and heterotypic interactions between SGNs and glia in live intact cochlea. Using unique labeling strategies, advanced morphometry, and organotypic imaging, we reveal for the first time a critical role for contact dependent interactions on auditory circuit formation.

Program Abstract #383

Investigating the functional significance of a-tubulin gene Tuba1a during neuronal development

Jayne Aiken, Jeffrey Moore, Emily Bates
University of Colorado Anschutz Medical Campus, USA

Proper development and function of the nervous system depends on appropriate regulation of microtubules (MTs) and their structural components, tubulin proteins. MTs in the developing brain must form long, stable arrays that are unique to neuronal cells. How these special MT networks are properly formed and maintained remains unknown. One potential level of specification lies with the tubulin genes themselves. Vertebrates contain numerous tubulin genes that encode distinct tubulin isotypesthat are very similar in structure and amino acid sequence but are expressed differentially depending on cell type and developmental stage. The α-tubulin Tuba1a is the primarily-expressed alpha-tubulin isotype in the brain during late stages of development, when neurons must produce long, stable MT arrays that mediate neuronal migration and axon elongation. In this study, we investigate the specific cellular functions during neurodevelopment that require Tuba1a. Using a transgenic mouse line containing a mutation in Tuba1a ($Tuba1a^{ND}$) that severely disrupts neurodevelopment, we are investigating the role of Tuba1a in cell survival, proliferation, differentiation, and neuronal migration. Additionally, numerous mutations in Tuba1a have been linked to a wide spectrum of severe neurodevelopment defects in human patients. These disease-causing mutations in Tuba1a provide a unique opportunity to discover the cellular consequences of Tuba1a disruption. To investigate the molecular and cellular impact of these Tuba1a disease-mutations, we have modeled six mutations in the primary α-tubulin expressed in budding yeast. Our results in yeast reveal that distinct mutations alter different tubulin/microtubule functions, with some leading to haploinsufficiency and others leading to gain-of-function disruptions to the microtubule network.

Program Abstract #384

A Glo1-methylglyoxal pathway that is perturbed in maternal diabetes regulates embryonic and adult neural stem cell pools in offspring

Guang Yang, Axel Guskjolen, Gonzalo Cancino, Siraj Zahr, Anastassia Voronova, Denis Gallagher, Paul Frankland, David Kaplan, Freda Miller
The Hospital for Sick Children, CA

Both genetic alterations and prenatal environmental insults like gestational diabetes are associated with autism spectrum disorder (ASD), but the basis for these gene:environment interactions is largely unknown. Here, we asked about a potential metabolic mechanism, focusing on the glycolysis metabolite methylglyoxal, since genetic alterations in the methylglyoxal detoxifying enzyme Glyoxalase 1 (Glo1) are associated with ASD, and circulating methylglyoxal levels are increased in maternal diabetes. We show that decreasing Glo1 levels in neural precursor cells (NPCs) depleted their numbers as a result of premature neurogenesis, and that a similar phenotype was observed in embryonic offspring when methylglyoxal was increased in the maternal circulation or following exposure to a maternal diabetic environment. Transient embryonic exposure to these perturbed maternal environments also caused long-term depletion of adult NPCs and aberrant behavior in adult offspring. Thus, the Glo1-methylglyoxal pathway integrates maternal and NPC metabolism to regulate NPC pools and neurogenesis throughout life. Funding Resources: Canadian Institutes of Health Research/CIHR and the Three to Be Foundation.

Program Abstract #385

Investigation of KLF4 role in neural progenitors

Beatriz de Toledo, Maurício Rocha-Martins, Gabriel Matos-Rodrigues, Marcelo Santiago, Rodrigo Martins, Mariana Silveira

Federal University of Rio de Janeiro, BR

The transcription factor KLF4 is a well-known cell cycle regulator in normal development and cancer, acting as a tumor supressor or oncogene depending on the context. In 2010, KLF4 was described as cell cycle inhibitor in medulloblastoma, a cancer originated from cerebellar progenitors (Nakahara et al. 2010). A previous study showed an antiproliferative effect of Klf4 in cortical progenitors (Qin et al, 2009). Our previous data also suggested that an increase in KLF4 content in retinal progenitors induces cell cycle exit. (Njaine & Rocha-Martins et al, 2014; Rocha-Martins et al, unpublished data). These findings support the hypothesis that KLF4 is a cell cycle regulator in neural progenitors. In order to elucidate the role of endogenous KLF4 during retinal and cerebellar development, we used conditional knockout mice (cKO), in which the KLF4 gene is deleted with the Cre-loxP technology. The deletion occurs in neural progenitors from the central nervous system in Nestin-cre mice or only in the periphery of the retina in alpha-Cre mice. No difference was found in adult cKO eye size. Histological evaluation in cresyl stained slides of cKO adult retina and cerebellum did not reveal significant alterations. To access visual function and balance/motor coordination we performed in nestin-Cre mice optomotor response and rotarod tests, respectively. cKO and control mice behaved equally.Then, we accessed the consequences of Klf4 loss on cell proliferation in P0 retina. Klf4-inactivation did not affect density of pH3+ and Brdu+ cells in alpha-cre P0 retina and the mRNA levels of cyclin D1, p27kip1 e p21cip1. Also the density of Brn3a+ cells in P60 retinas was not affected. Moreover, preliminary results showed that KLF4 did not affect density of pH3+ cells in nestin-cre P7 granule cells progenitors. These results suggest that KLF4 doesn't have a major role in retinal and cerebellar development. We are currently investigating compensation by other KLF members.

Program Abstract #386

Regulation and role of polycomb repressor complex 2 component Jarid2 during neural differentiation

Adriana Amorim Torres, Kate G. Storey

Division of Cell & Developmental Biology, School of Life Sciences, University of Dundee, United Kingdom

Neural differentiation in the vertebrate embryonic body axis is regulated by a switch between Fibroblast Growth Factor (FGF) and Retinoid signalling, and one mechanism that might coordinate onset of differentiation genes is regulation of Polycomb Repressor Complex 2 (PRC2). *Jarid2*/*Jumonji* is a transcription factor component of PRC2 involved in priming and epigenetic silencing of differentiation genes in embryonic stem cells, and could be involved in the coordinated regulation of differentiation in the embryo. We analysed *Jarid2* expression pattern in chicken and mouse embryos. *Jarid2* mRNA is detected transiently in the early neural plate and expression is restricted to the isthmus region of the midbrain/hindbrain and the stem zone/preneural tube at the ten somites stage. Later, expression is confined to the tail end, and is lost from this region towards the end of body axis elongation. *Jarid2* expression is similar to that of Fibroblast growth factor (*Fgf*) *8* and of a readout of FGFR signalling, *Spry2*. Ectopic maintenance of FGF signalling after cells have left the stem zone/preneural tube of the chicken embryo elicited ectopic *Jarid2* transcription, while inhibition of FGFR signalling reduced endogenous expression of *Jarid2*. Consistent with this, *Jarid2* expression domain expands rostrally, along with that of *Fgf8*, in retinoid deficient (*Raldh2*$^{-/-}$) mouse embryos. We are investigating the role of Jarid2 in this context using a Nkx1.2-ERT2 *Jarid2* conditional knockout mouse line to analyse the consequences of *Jarid2* acute loss specifically in the stem zone/preneural tube. We are also using a gain of function model by *in ovo* electroporation of *Jarid2* into the chicken preneural tube. We aim to determine the mechanisms controlling coordinated regulation of genes during cell differentiation and we propose that *Jarid2* might be a link between FGF signalling and coordinated epigenetic regulation of differentiation genes.

Funding: CAPES (Brazil), University of Dundee (UK)

Program Abstract #387

***Drosophila* Smooth-like Testes Muscles Compensate Failure of Myoblast Fusion**

Katharina Fritzen, Jessica Kuckwa, Detlev Buttgereit, Silke Rothenbusch-Fender, Renate Renkawitz-Pohl

Philipps-Universität Marburg, DE

The inner male reproductive organs of *Drosophila melanogaster* comprise paired testes, seminal vesicles, paragonia, one ejaculatory duct and an annexed sperm pump. Individual organs of somatic origin are surrounded by different types of muscles: mono- and multinucleated striated muscles. In contrast, the testes are encircled by a multinucleated, smooth-like muscle sheath (Susic-Jung et al., 2012 Developmental Biology). During metamorphosis all muscles of the male

reproductive system build from myoblasts located on the genital disc. The myoblasts at the anterior part of the genital disc are the testes muscle precursors. Between 28 and 32 hours after puparium formation (APF) these myoblasts become multinucleated nascent myotubes and migrate from the prospective seminal vesicle onto the testes. To characterise the testis myoblasts we used RT-PCR and immunofluorescence analysis (IF). In isolated myoblasts at distinct time points we identified the transcripts of described myoblast-specific determination factors as well as components which are involved during embryonic myoblast fusion. In accordance with the myoblast-specific RT-PCR, we found these components in IF in the anteriorly localized testis myoblasts. The distinct protein distribution led us to classify the myoblasts as 'founder cell like myoblasts' and 'fusion-competent myoblast like cells'. Myoblast-specific attenuation of the molecules via RNAi resulted in a decreased number of nuclei per testis muscle. The lack of fusion did neither affect the migration of the nascent myotubes onto the testes, nor the muscle filament formation and orientation. The overall testis morphology and germ cell development was indistinguishable from the wildtype. We propose that testes muscles are able to compensate fusion defects to ensure male fertility as main precondition for survival of the species. This work supported by the Deutsche Forschungsgemeinschaft [RE 628/16-1 and GRK1216].

Program Abstract #388

Identifying Downstream Effectors of AMH Signaling During Male Reproductive Tract Differentiation in Mouse

Rachel Mullen, Richard Behringer

MD Anderson Cancer Center, USA

Most men wrongly assume they lack a uterus because it never formed. However in mammals the Müllerian duct (MD), which developments into the uterus, oviducts and upper vagina, forms in males and females. Initially the reproductive tract consists of the Wolffian duct and the MD, two epithelial tube pairs surrounded by mesenchyme. In males, MD regression requires binding and signal transduction from the transforming growth factor-β family member anti-Müllerian hormone (AMH) secreted from fetal testis and its type 1 and 2 receptors found in MD mesenchyme. The downstream effectors of the AMH-signaling pathway during MD regression are largely unknown. Transcriptome differences in the MD mesenchyme during regression between males (AMH signaling on) and females (AMH signaling off) were identified using RNA-Seq analysis. This identified BMP effectors: *Msx2*, *Dlx5*, and *Osterix (Osx)/ Sp7* as potential mediators of AMH signaling during regression. MSX2, DLX5 and *Osx* are expressed in a male-specific pattern in the MD mesenchyme during regression and this expression is lost in AMH signaling mutant males. Additionally, transgenic mice ectopically expressing AMH in females have a male pattern of *Osx* expression. Further, some $Amhr2^{Cre/+};Msx1^{flox/flox};Msx2^{flox/flox}$ conditional knockout and *Dlx5/6* null male mice retain uterine tissue. Additionally MD regression is delayed in functional null *Osx* males. Together this indicates *Msx2*, *Dlx5* and *Osx* are AMH signaling target genes and contribute to MD regression. Current work focuses on understanding how these transcription factors, up regulated in the MD mesenchyme, contribute to regression of the MD epithelial tube both molecularly and mechanically.

Program Abstract #389

Dissecting BMP4 downstream modules in ureter development

Tamrat Mamo

Hannover medical school (MHH), DE

The ureter is composed of two tissue compartments, an outer mesenchymal coat of fibroblasts and smooth muscle cells (SMC) and an inner multi-layered urothelium consisting of basal (B-cells), intermediate (I-cells) and superficial cells (S-cells). These highly differentiated cell types arise from undifferentiated progenitor pools in a precise temporal-spatial fashion. The signaling pathways that coordinate development of the two tissue compartments as well as the molecular mechanisms that control cellular differentiation are poorly understood. Mutations in the *Bmp4* gene have been shown to be a cause for human congenital anomalies of kidney and urinary tract (CAKUT) and mice with a heterozygous loss of *Bmp4* exhibit CAKUT phenotypes, many of which are linked to malformation of the ureter. However, the precise molecular functions of BMP4 in the developing ureter are not known. Here, we used a conditional gene targeting approach in combination with small molecule inhibitors in explant organ cultures to dissect SMAD dependent and independent functions of BMP4 in the developing ureter. Mice lacking *Bmp4* in the ureteric mesenchyme (UM) showed hydroureternephrosis, lack of SMC and of urothelial differentiation at birth. Analysis at earlier embryonic stages revealed severely reduced epithelial and mesenchymal proliferation. Conditional inactivation of *Smad4* in UM resulted in delayed SMC differentiation. Pharmacological inhibition of individual downstream pathway mediators ERK, P38 or AKT in ureter explant cultures resulted in reduced epithelial proliferation. However, a mesenchymal proliferation defect was only observed with AKT inhibition. Analysis of these pathways during differentiation revealed that BMP4 regulates B-cell, I-cell and S-cell development through individual downstream kinase pathways. Together, our result unveiled the role of

individual signaling entities acting downstream of BMP4 in mesenchymal and epithelial proliferation and differentiation of the ureter.

Program Abstract #390

Stromal Cells Control Nephron Formation in a Hedgehog-Dependent Manner

Chris Rowan[1,3], Winny Li[2,3], Hovhannes Martirosyan[1,3], Norman Rosenblum[1,2,3]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, CA; [2]Institute of Medical Science, University of Toronto, CA; [3]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, CA

The functional unit of the kidney, the nephron, develops as nephron progenitors differentiate to form mature glomerular and tubular structures. A full endowment of nephrons is crucial for normal kidney function as variations in total nephron number has been linked to kidney failure and cardiovascular disease. Emerging evidence has demonstrated the importance of Hedgehog (HH) signalling in adjacent stromal cells in nephrogenesis; however the molecular mechanisms that mediate crosstalk between the stroma and nephron progenitors remains undefined. Genetic elimination of HH signalling in the stromal lineage causes a reduction in nephron number, suggesting that stromal-HH signalling regulates nephron differentiation in a non-cell autonomous manner. Based on this data, we hypothesize that HH-induced stromal gene expression controls nephron differentiation. To address this question, we A) identify pathways and genes differentially regulated by stromal-HH signalling, and B) determine the functional contribution of HH-stimulated stromal genes on nephron formation using an *ex vivo* model of murine kidney development. In order to define the molecular pathways controlled by stromal-HH signalling, we performed RNA sequencing on whole kidneys from wild type and HH-deficient (mutant) mice, and identified differentially expressed candidate genes, *Tgfb2* and *Tgfb3*. Gene expression analyses on mutant kidneys shows that TGFβ signalling is perturbed in mutant mice. Functional contribution of TGFβ2/3 downregulation in mutant kidneys was tested in kidney explants. E12.5 wild type kidneys were cultured *ex vivo* in the presence of neutralizing antibodies and vivo-morpholinos against TGFβ2/3. Analysis of treated explants confirmed neutralization and ablation of TGFβ signalling resulted in decreased nephron number. Follow up analyses on mice that lack TGFβ signalling in nephron progenitors will be performed to confirm the importance of HH-mediated TGFβ signalling in nephrogenesis.

Program Abstract #391

An investigation of the role of Adamts-18 metalloproteinase during mammalian kidney morphogenesis

Shifaan Thowfeequ, Franklin Costantini

University of Columbia, USA

Branching morphogenesis of the ureteric bud (UB) is a hallmark of kidney organogenesis, and it gives rise to the elaborate renal collecting system. This branching of the ureteric epithelium is strictly regulated by Ret-GDNF signalling, via the ETS transcription factors Etv4 and Etv5, and it is thought to be facilitated by extensive remodelling of the surrounding extracellular environment. Adamts-18, is a member of the metcizin family of metalloproteinases, whose specific substrate(s) remains unknown. *Adamts-18* is expressed specifically in the UB tips throughout kidney development, and its expression is reduced or lost in *Ret51* hypomorphic or *Etv4 ;Etv5* compound mutant kidneys, respectively, suggesting its regulation via this pathway. *Adamts-18* homozygous mouse mutants have hypoplastic kidneys, reduced nephrogenic zones and roughly a third of them have a double papillae phenotype instead of the single papilla normally seen in mouse kidneys. Abnormalities in early branching events around E10.75-E11 were seen to underlie this double papillae phenotype, instead of the papillary remodelling mechanisms that are initiated later during development. Furthermore, defective UB branching during development also leads to low nephron endowment in adults, with possible implications to maintenance of normotensive blood pressure and proper physiological functioning of the kidney.

Program Abstract #392

Distal nephron tubule perturbation in Sim1 null mouse mutants

Michael Todd Valerius[1,3], Terry Gemelli[2], Andrew Zinn[2]

[1]Brigham and Women's Hospital, USA; [2]UT Southwestern Medical Center, USA; [3]Harvard Medical School, USA

We have examined the role of the transcription factor *Sim1* in patterning nephron segments using a loss-of-function mouse mutant. During development, transcriptional regulators and secreted signaling molecules coordinate to pattern the nephron into proximal-distal domains. We identified genes regionally expressed in the S-shaped body (SB) as potential regulators of patterning during nephron formation (GUDMAP.org). We found *Sim1* is expressed in middle and distal segments of the early SB. To determine the role of *Sim1* in the developing kidney, we examined *Sim1* loss-of-function mutants for kidney defects at E18.5 and P1. *Sim1* null mutants were compared with littermate controls histologically and with nephron

segment-specific markers. We performed immunofluorescence using the lectins Lotus tetragonolobus lectin (LTL, proximal tubule) and Dolichos biflorus agglutinin (DBA, collecting duct), and antibodies detecting uromodulin (Tamm-Horsfall protein, Loop-of-Henle), Aquaporin 2 (Aqp2, collecting duct) and neuronal Nitric Oxide Synthase (nNOS, macula densa). *Sim1* null mutants had normal sized kidneys but exhibited dilated nephron tubules with the tubular epithelial cells containing large vacuoles. Immunofluorescence indicated these were LTL+ proximal tubule segments that also contained dispersed cells ectopically expressing the collecting duct marker DBA. Uromodulin staining indicated aberrant loop-of-Henle formation with co-staining of LTL at the junctions with the proximal tubules. Controls exhibited no overlap of these markers, suggesting the proximal and distal boundaries of the loop-of-Henle are not distinct in the mutants. Furthermore, immunofluorescence staining revealed nNOS+ macula densa cells, part of the distal tubules, were drastically reduced in number with some glomeruli lacking an associated MD. Our interpretation is that the distal tubules require *Sim1* gene function to properly pattern boundaries and the distal tubule for proper macula densa formation.

Program Abstract #393

Mesenchymal microRNAs are involved in the expression of Dkk2 to control eyelid development

Takahiro Nagai[1,2], Momoko Watanabe[1,2], Katsushige Kawasaki[1], Maiko Kawasaki[1], Atsushi Kitamura[1,2], Yasumitsu Kodama[2], Ritsuo Takagi[2], Takeyasu Maeda[1], Paul Sharpe[3], Robert Hindges[4], Atsushi Ohazama[1,3]

[1]Division of Oral Anatomy, Niigata University, Japan; [2]Division of Oral and Maxillofacial Surgery, Niigata University, Japan; [3]Department of Craniofacial Development & Stem Cell Biology, King's College London, UK; [4]MRC Centre for Developmental Neurobiology, King's College London, UK

Eyelids play a crucial role in protecting and moistening the surface of the eye. Mammalian eyelids fuse and re-open during development and growth – both important events in eye formation. Failure of this process results in the eyelids open at birth (EOB) phenotype in mice. MicroRNAs (miRNAs) are noncoding small single-stranded RNAs, typically 19-25 nucleotides in length, that negatively regulate gene expression by binding target mRNAs. Dicer is an essential miRNA processing molecule. Although conditional deletion of mesenchymal *Dicer* (*Dicer$^{fl/fl}$/Wnt1Cre*) has been shown to lead to the EOB phenotype, the molecular mechanisms remain unclear. We identified downregulation of *Dkk2* and subsequent upregulation of canonical Wnt signaling in the developing eyelids of *Dicer$^{fl/fl}$/Wnt1Cre* mice. The loss of mesenchymal *Dicer* also led to extra hair follicle formation in the palpebral conjunctiva without transformation of palpebral conjunctiva into epidermis – a feature also seen in *Dkk2* mutants. DKK2 protein rescued the EOB phenotypes in mutant mice. Wnt activity, regulated by *Dkk2*, is thus controlled by mesenchymal miRNAs. Understanding the molecular basis of the EOB phenotype in mice can provide significant insights into the molecular mechanisms of eyelid formation.

Program Abstract #394

Myc transcription factors regulate mouse lens development and eye organogenesis

Gabriel R. Cavalheiro, Gabriel E. Matos-Rodrigues, Anielle L. Gomes, Rodrigo Martins

Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Brazil

Myc transcription factors regulate cell proliferation, growth and differentiation in various developing tissues, as its deregulation may lead to tumorigenesis or developmental malformations. The lens is composed of proliferative epithelial progenitor cells that after cell cycle exit undergo terminal differentiation to form fiber cells. The differentiation of these fiber cells includes the degradation of their organelles, including the nuclei (denucleation) resulting in a fully transparent organelle-free zone (OFZ) in the center of the lens. Nuclei degradation requires low levels of p27Kip1 expression and high Cdk1 activity in post-mitotic fiber cells to dismantle the nuclear envelope, and DNase II-beta activity for DNA degradation. Previously, we demonstrated that c-myc is required for cell proliferation in the developing lens, but not for OFZ formation. Targeted deletion of N-myc from mouse lens progenitors resulted in eye and lens growth impairment. Interestingly, we observed that N-myc inactivation did not affect cell proliferation or survival in embryonic lens. Remarkably, N-myc-inactivated lenses present a delay to form an OFZ, since their terminally differentiating fiber cells retain the nucleus for longer than control lenses. This is associated with a decreased DNase II-beta expression. The remnant nuclei failed to decrease $p27^{Kip1}$ expression, suggesting that Cdk1 activity may be downregulated in N-myc-inactivated lens fiber cells. Furthermore, simultaneous inactivation of N-myc and c-myc drmatically reduced eye and lens volume and also fail to form an OFZ. Our findings suggest that Myc transcription factors regulate distinct cellular events during lens development *in vivo*. These data contribute to a better comprehension of the molecular mechanisms that control of organelle degradation in the lens, and constitute a previously undescribed function for the N-myc proto-oncogene.

Program Abstract #395

The FGF pathway is important for proper neural crest cell migration into the *Xenopus* pharynx

Terri VanDyke, Emily Shifley

Northern Kentucky University, USA

During early stages of development neural crest cells migrate from the neural tube into different parts of the organism, including the pharyngeal arches. These cells later form the cartilage and bone of the face and neck. When neural crest cell migration is disrupted, craniofacial birth defects can arise. We hypothesized that the FGF genetic signaling pathway is required for the migration of the neural crest cells into the *Xenopus* pharynx. We inhibited the FGF pathway during different stages of development with small molecule inhibitors to determine when FGF signaling was required for neural crest cell migration. The neural crest marker *Twist* had reduced expression at Stage 35 when FGF signaling was inhibited during pharyngeal development compared to controls. Additionally, we saw changes in the craniofacial cartilage of FGF-inhibited Stage 45 embryos compared to controls. Overall this research will help determine the importance of FGF signaling during the development of the *Xenopus* pharynx and migration of the neural crest.

Program Abstract #396

Functional characterization of a minimal *dlx2b* tooth enhancer in zebrafish

Hana Littleford, Audrey DeFusco, Sarah Liu, Vy Nguyen, Elisabeth Carter, Andrea Jowdry, Elizabeth Richards, William Jackman

Bowdoin College, USA

We are interested in understanding the molecular signals that initiate the organogenesis of teeth both from ontogenetic and evolutionary perspectives. The transcription factor *dlx2b* is expressed relatively early and specifically in zebrafish tooth-forming tissues. We are investigating the regulation of this gene as a directed way to pinpoint factors closer to or at the top of the tooth initiation cascade. We have identified a ~200 bp minimal enhancer within 1 kilobase upstream of the *dlx2b* gene that is sufficient to drive gene expression in the developing dental epithelium. This enhancer region contains stretches of identifiable homology with other vertebrate genomic sequences, suggesting evolutionary significance, but also regions comprised of unique sequences that emphasize the importance of the empirical study of *cis*-regulation. We have mutated predicted Fgf/Pea3 transcription factor binding sites in this minimal enhancer and determined that these are not necessary for its function directing tooth expression. We are continuing to test other predicted binding sites in the hope of identifying novel regulators of tooth organogenesis. Identifying the transcription factors that regulate *dlx2b* during tooth development may facilitate future work in regenerative dentistry as well as increase our understanding of how developmental mechanisms have changed during vertebrate evolution.

Program Abstract #397

A ß-catenin Signaling-Dependent Network Mediates Islet1 in Sustaining the Midline Boundary of the Lower Jaw

Feixue Li, Guoquan Fu, Ying Liu, Xiaoping Miao, Xueqin Yang, Yan Li, Xiaoyun Zhang, Dongliang Yu, Zunyi Zhang

Institute of Developmental and Regenerative Biology, College of Life and Environmental Science, Hangzhou Normal University, China

The lower jaw in mice and many other vertebrate species is composed of two unfused mandibular bones. This is distinct from the chin in human, where the two mandibular bones fuse in the midline. Jaw patterning information initially resides in the epithelium during development. However, whether and how the transcriptional regulation of epithelial-derived signaling controls in sustaining the midline boundary to prevent the fusion of the mandibular bones remains unknown. We ablated transcription factor Islet1 which is exclusively expressed in the epithelial compartment, resulting in a truncated and midline fused mandible with premature ossification. Genetic and profiling analyses revealed that Islet-1 is required for the mesenchymal gene expression, the distal confinement of the *Bmp4/Fgf8* pathway critical for the early patterning of the mandibular arch; as well as for the activation of the epithelial β-catenin/Shh pathway to ensure proper development of the mesenchymal compartment by control of midline transcription regulators. Re-activation of either β-catenin signaling or hedgehog in the epithelium rescues mandibular morphogenesis. Together, we demonstrate that Islet-1 is an essential transcription regulator mediated by β-catenin and Shh signaling to ensure the lack of midline fusion of the lower jaw in the mouse.

Program Abstract #398

Repressive regulation of osteogenic differentiation in the frontal bone formation via the level of Hedgehog signaling in neural crest cell-derived mesenchyme

Jianying Li, Ying Cui, Qihui Wang, Xueqin Yang, Yan Li, Xiaoyun Zhang, Zunyi Zhang

Institute of Developmental and Regenerative Biology, College of Life and Environmental Science, Hangzhou Normal University, China
Malformation of neural crest cell-derived organs is associated with one third of congenital defects in humans, including clinical symptoms of the skull bone formation.Conditional knockout of Hh pathway inhibition factor *Sufu* allele in the cranial neural crest cells (CNCCs) using *Wnt1-Cre* mouse results in the failure of the CNCCs-derived skull bones including frontal bones in mutant mouse. We demonstrate that *Sufu* deletion does not perturb the migration of CNCCs, and cell survival of the osteogenic mesenchymal cells. However, the proliferation of these cells is significantly reduced. Moreover, the expression of crucial regulatory factors involved in osteoblast differentiation including *Runx2* and *Osterix* is inhibited in the frontal bone primordium. Expression of genes upstream of Runx2,including *Msx1, Msx2* and *Dlx5* is obviously down-regulated in Sufu mutants. Meanwhile, the expression of Hh pathway target molecule *Gli1* and Notch pathway molecule *Jag1* is up-regulated, but FGF and BMP pathway molecules including *Fgfr2* and *Bmp2* are down-regulated. RNA-seq analysis reveals that the genes with differential expression are enriched in many signaling pathways such as Hh and Notch pathways. Western blot analysis shows that the repressor form of Gli3 in mutant is significantly decreased, whereas the expression of full-length Gli2 is significantly increased. Furthermore, genetic study shows that the compound mutations of Sufu and Gli2 in CNCCs rescue the skull bone formation in Sufu mutation. These results suggest that during frontal bone development Sufu can repress the activity of Hh pathway by inhibiting the generation of full-length Gli2 protein and facilitating the processing of Gli3 protein. The interruption of these signaling pathways will lead to inhibition of proliferation and differentiation of the osteogenic mesenchyme cells, and ultimately to failed formation of the CNCCs-derived skull bones in Sufu mutant.

Program Abstract #399

Golgb1 regulates protein glycosylation and is crucial for mammalian palate development

Yu Lan, Nian Zhang, Han Liu, Jingxue Xu, Rulang Jiang
Cincinnati Children's Hospital Med Ctr, USA

Cleft palate is a common major birth defect for which currently known causes account for less than 30% of pathology in humans. In this study, we carried out mutagenesis screening in mice to identify new regulators of palatogenesis. Through genetic linkage mapping and whole exome sequencing, we identified a loss-of-function mutation in the *Golgb1* gene that co-segregated with cleft palate in a new mutant mouse line. *Golgb1* encodes a ubiquitously expressed large coiled-coil protein, known as giantin, that is localized at the Golgi membrane. Using CRISPR/Cas9-mediated genome editing, we generated and analyzed developmental defects in mice carrying additional *Golgb1* loss-of-function mutations, which validated a critical requirement for Golgb1 in palate development. Through maxillary explant culture assays, we demonstrate that the *Golgb1* mutant embryos have intrinsic defects in palatal shelf elevation. Just prior to the developmental stage of palatal shelf elevation in the wildtype littermates, *Golgb1* mutant embryos exhibit increased cell density, reduced hyaluronan accumulation, and impaired protein glycosylation in the palatal mesenchyme. Together, these results demonstrate that, although it is a ubiquitously expressed Golgi-associated protein, Golgb1 has specific functions in protein glycosylation and tissue morphogenesis.

Program Abstract #400

Ift88 is essential for downregulation of the palatal shelf through Shh signalling

Momoko Watanabe[1,2], Katsushige Kawasaki[1,3], Maiko Kawasaki[1,3], Takahiro Nagai[1,2], Atsushi Kitamura[1,2], Yasumitsu Kodama[2], Ritsuo Takagi[2], Takeyasu Maeda[1], Paul Sharpe[3], Atsushi Ohazama[1,3]
[1]Division of Oral Anatomy, Niigata University, Japan; [2]Division of Oral and Maxillofacial Surgery, Niigata University, Japan; [3]Department of Craniofacial Development & Stem Cell Biology, King's College London, UK

Ift88 is known to encode a protein that is required for intraflagellar transport and formation of primary cilia. The role of *Ift88* in palatogenesis remains unclear. We analyzed the role of *Ift88* in palate development using *Ift88* mutant mice. In order to investigate *Ift88* function, we generated mice with epithelial (*Ift88;K14Cre*) or mesenchymal (*Ift88;Wnt1Cre*) conditional deletion of *Ift88.* Molecular and morphological analyses were performed in these mice. Cleft palates were observed in all *Ift88;Wnt1Cre* mice, but not in *Ift88;K14Cre* mice. Cleft palates in *Ift88;Wnt1Cre* mice were found to be caused by the lack of palatal shelf downgrowth, which was accompanied by increased apoptosis and significantly reduced Shh signaling activity. In order to investigate the relationship between *Ift88* and the Shh signaling pathway in palate development, we generated mice with mesenchymal conditional deletion of an essential transducer of Shh signaling, *Smo* (*$Smo^{fl/fl}$;Wnt1Cre*). *$Smo^{fl/fl}$;Wnt1Cre* mice showed similar cleft palate phenotypes to those in *Ift88;Wnt1Cre* mice. Our results indicated that mesenchymal *Ift88* is essential for downgrowth of palatal shelves, and *Ift88* regulates palatogenesis through Shh signaling.

Program Abstract #401

PRMT5 is essential for the maintenance of chondrogenic progenitor cells in the limb bud

Jacqueline Norrie[1], Qiang Li[1], Swanie Co[1], Bau-Lin Huang[2], Susan Mackem[2], Ding Ding[3], Zhicheng Ji[2], Mark Bedford[4], Antonella Galli[5], Hongkai Ji[3], Steven Vokes[1]

[1]University of Texas at Austin, USA; [2]NCI, Frederick MD USA, USA; [3]Johns Hopkins Bloomberg School of Public Health, USA; [4]M.D. Anderson Cancer Center, USA; [5]Sanger Institute, UK

Vertebrate skeletal elements exhibit dramatic morphological diversity but originate as homogenous aggregations of nascent chondrocytes. These aggregates subsequently expand by recruiting cells from a pool of mesodermal progenitor cells. Though the extrinsic signals regulating the proliferation and differentiation of progenitor cells are well-characterized, the intrinsic mechanisms by which these cells maintain an undifferentiated state are largely unknown. Here, we report that the stem cell pluripotency factor *Prmt5* is essential for the maintenance of progenitor cells in the developing mouse limb. *Prmt5*-deficient forelimbs have severely truncated skeletal elements, including wispy digits that lack joints. The forelimb phenotype is preceded by the widespread apoptosis of progenitor cells undergoing precocious differentiation in response to elevated levels of non-canonical BMP signaling. We conclude that *Prmt5* is an intrinsic factor that is essential for the maintenance of mesodermal progenitor cells that give rise to chondrocytes.

Program Abstract #402

Kdf1 inhibits p63 in the limb to regulate AER size and regression

Priya Date, Sunjin Lee, Scott Weatherbee

Yale University School of Medicine, USA

More than half of all human birth defects involve limb malformations and >700 human syndromes have associated limb anomalies, yet we still know very little about the genetic network governing limb development. We isolated a mouse line carrying a recessive mutation in the *Keratinocyte Differentiation Factor 1* (*Kdf1*) gene. Homozygous *Kdf1* mutants have short, fused digits while the rest of the limbs appear normal, modeling symbrachydactyly in humans. Limb outgrowth requires Fgfs in the apical ectodermal ridge (AER). Although *Kdf1* mutant limbs are shortened, they show increased Fgf signaling, progressive dorsal-ventral broadening of the AER, and delayed AER regression. This suggests that Kdf1, which is expressed in limb epithelium and the AER, is required to regulate Fgfs and/or their signaling.

Proliferation in stratified epithelia, including the AER, requires the p63 transcription factor. *p63* mutants fail to form an AER and show a complete loss of elements distal to the shoulder blades/pelvis. We found that Kdf1 reduces p63 protein levels when overexpressed and *Kdf1* mutant limbs show increased and expanded p63 expression. Kdf1 homozygotes lacking one copy of p63 have a partial rescue of the digit phenotype suggesting that Kdf1 normally acts to curb p63 levels in the AER. In addition we have shown that Kdf1 physically interacts and colocalizes with components of the IKK complex, which regulates the levels of p63 isoforms in several tissues. Mutations in complex component Ikkα cause symbrachydactyly and delayed AER regression, similar to *Kdf1* mutants. Our data suggest that Kdf1 encodes a novel Ikk complex-interacting protein, which is a critical regulator of limb development. Our model is that Kdf1 acts in conjunction with the Ikk complex to limit p63 levels and regulate Fgf signaling.

This work was supported by NIH grant R01AR059687.

Program Abstract #403

Speckle-type POZ Protein promotes skeletal development by upregulating Indian Hedgehog signaling

Hongchen Cai, Noriaki Okita, Neil Sharkey, Aimin Liu

Pennsylvania State University, USA

Skeletal disorders such as osteoporosis and brachydactyly (shortness of fingers and toes) affect over ten millions of people in the United States, but the genetic basis for their predisposition remains poorly understood. Here, we report the identification of Speckle-type POZ Protein (Spop) as an important regulator of skeletal development. Spop is an E3 ubiquitin ligase component, and previous in vitro studies suggested that it inhibits the Hedgehog (Hh) pathway by targeting the pathway effectors Gli2 and Gli3 for degradation. To determine the roles of Spop in mouse development, we characterized two apparent *Spop* null mouse strains. These mutants exhibit systemic reduction of bone mineralization resulting from defects in both chondrocyte maturation and osteoblast differentiation. We find a two-fold increase in the level of Gli3 transcriptional repressor protein, leading to compromised Indian Hedgehog (Ihh) pathway activity. Importantly, removing one copy of *Gli3* significantly rescued skeletal development in *Spop* null mutants. Biochemical studies indicate that Spop targets both the full-length and repressor forms of Gli3 for ubiquitination and degradation. To overcome the neonatal lethality of *Spop* null mutant, we generated a limb-specific *Spop* conditional mutant strain. Interestingly, loss of Spop caused reduction of bone mass (osteoporosis or osteopenia) and brachydactyly in adult limbs,

which were both rescued by reducing *Gli3* genetically. In conclusion, we show that Spop is an important regulator of vertebrate skeletal development, and surprisingly, a positive regulator of Ihh pathway. Our results reveal Spop as a novel factor/target of predisposition for common skeletal disorders like osteoporosis and brachydactyly.
This research was supported by NIH grant HD083625, a Penn State Start-up Fund for A.L. and a HUCK Graduate Dissertation Research Award for H.C.

Program Abstract #404

Sall4 regulates the Tbx6-Msgn1-Hes7 system for segmentation of presomitic mesoderm in the posterior body in mouse embryos

Shinichi Hayashi[1], Yasukazu Nakahata[2], Malina Peterson[1], Kenji Kohno[2], Hiroko Kawakami[1], Ryuichi Nishinakamura[3], Takaaki Matsui[2], Yasumasa Bessho[2], Yasuhiko Kawakami[1]
[1]University of Minnesota, USA; [2]Nara Institute of Science and Technology, Japan; [3]Kumamoto University, Japan

Somites are transient embryonic tissue consisting of multipotent progenitors for vertebrae, trunk muscles and dermis. A paired somite is periodically formed as spherical cell masses in the pre-somitic mesoderm (PSM) and is regulated by the coordinated action of "clock genes" that show oscillatory gene expression patterns in PSM. We have recently analyzed limb skeletal phenotypes of *Tcre; Sall4* conditional knockout (*Sall4* cKO), in which recombination occurs as early as E7.5 in the mesoendoderm. The mutants exhibited disorganized vertebrae, posterior to lumber vertebrae, which is similar to mice lacking *Hes7*, a clock gene that regulates oscillatory expression of other clock genes. These observations led to a hypothesis that *Sall4* regulates segmentation of PSM through clock genes. By *in situ* hybridization, strong *Sall4* expression is detected in the PSM until E13.5 in wild type embryos. In *Sall4* cKO embryos, genes involved in somitogenesis, such as *Hes7*, *Mesogenin1* (*Msgn1*), *Tbx6*, *Ripply2*, are downregulated. By chromatin immunoprecipitation, we detected enrichment of SALL4 in the promoter regions of *Tbx6* and *Msgn1*, but not in *Hes7*, suggesting that *Sall4* directly regulates *Tbx6* and *Msgn1* in PSM. To understand mechanisms of *Hes7* regulation, we identified a PSM-specific *Hes7* enhancer by LacZ transgenesis in mouse embryos. We found that TBX6 and MSGN1 bind the *Hes7* enhancer, and synergistically activate the enhancer *in vitro*. We also found that TBX6 is enriched in the *Hes7* enhancer in vivo, and LacZ transgenesis indicated that a TBX6 site and a MSGN1 site in the *Hes7* enhancer are necessary for LacZ reporter activation. These results support a model in which *Sall4* directly regulates *Tbx6* and *Msgn1* expression, which directly control *Hes7* and segmentation of PSM. The results also suggest *Sall4* regulates somitogenesis in the posterior part of the body, while different mechanisms may regulate the *Tbx6-Msgn1-Hes7* axis in the anterior part of the body. Grant: NIAMS (R01AR064195).

Program Abstract #405

Osr1 regulates lung specification and branching morphogenesis

Lu Han
Cincinnati Children's Hospital Med Ctr, USA

Respiratory system derives from ventral foregut and undergoes stereotypical branching to form complex tree-like structures for vital air exchange. The molecular mechanisms that control early lung development, including specification and early lobe formation, are only partially understood and defects in these processes can lead to severe congenital defects, such as lung hypoplasia and branching anomalies, the etiology of which remains unclear. Recent work in *Xenopus* has implicated zinc-finger transcription factors Osr1 and Osr2 as important developmental regulators of the primitive lung in this species. Using mouse genetics we have tested the hypothesis that the functions of Osr factors are conserved for mammalian fetal lung development. We found that during respiratory specification, *Osr1* is expressed in both endoderm and mesenchyme, but during branching phase, *Osr1* is lost in epithelial and distal mesenchyme, concentrating only in medial mesenchyme around trachea and main bronchi. *Osr1-/-* mutant mice exhibit lung hypoplasia, while Osr2 appears dispensable for lung development, even in the absence of Osr1. Tissue specific conditional knockouts in combination with molecular analysis indicate that Osr1 is primarily required in the foregut mesenchyme, to maintain expression of critical lung inducing signals, such as Wnt2/2b and BMP4. As a result *Osr1-/-* mutants exhibit a delayed respiratory specification with fewer Nkx2-1+ progenitors. During branching stages, the unique medial expression patterning modulates branching signals such as FGF10 and BMP4, especially for cranial lobe to form only distal to the bronchi bifurcation, which otherwise results in tracheal bronchus, a bronchial lesion identified in humans. Based on these evidence, we conclude that Osr1 is a novel transcription factor that is required for lung specification and branching morphogenesis, through modulating mesenchymal signals such as Wnt2/2b, FGF10 and BMP4.

Program Abstract #406

HOXA5 plays tissue-specific roles in the developing respiratory system

Lucie Jeannotte[1], Kim Landry-Truchon[1], Olivier Boucherat[1], Nicolas Houde[1], Polyxeni Philippidou[3], Stéphanie Fournier[1], Richard Kinkead[1], Jeremy Dasen[2]

[1]Université Laval, CA; [2]New York University, USA; [3]Case Western Reserve University, USA

The transcription factor HOXA5 is important for the development of several organs and tissues. In the respiratory system, the role of *Hoxa5* is critical since the loss of *Hoxa5* gene function causes death at birth of a high proportion of mutant mouse pups due to respiratory distress. The HOXA5 protein expression in the mesenchyme of the respiratory tract and in the phrenic motor neurons of the central nervous system led us to address the tissue-specific contribution of *Hoxa5* to lung development. Using a conditional gene targeting approach, we demonstrated that the genetic ablation of *Hoxa5* function in the mesenchyme established the importance of *Hoxa5* for trachea development, lung epithelial cell differentiation and lung growth. The targeted deletion of *Hoxa5* in motor neurons resulted in abnormal innervation of the diaphragm, altered diaphragm musculature and lung hypoplasia, a phenotype reminiscent to that observed in patients with congenital diaphragmatic hernia. Moreover, the neonatal lethality and the impaired respiratory function, as seen in *Hoxa5* null mutants, were reproduced in *Hoxa5* motor neuron mutants indicating that the defective diaphragm was the main cause of lethality at birth and of breathing defects in surviving adult animals. Thus, *Hoxa5* possesses tissue-specific functions that differentially contribute to the morphogenesis of the respiratory tract.

(Supported by NSERC)

Program Abstract #407

Epithelial cells dynamically localize molecular activities and generate time-varying interfacial tension gradients that drive radial intercalation

Neil Neumann[1], Mathew Perrone[2], Jim Veldhuis[2], Robert Huebner[1], G. Wayne Brodland[2], Andrew Ewald[1]

[1]Johns Hopkins University School of Medicine, Department of Cell Biology, Center for Cell Dynamics, USA; [2]University of Waterloo, Department of Civil Engineering, Canada

Collective cell migration in the mammary gland is regulated by RTKs. At puberty, a low-polarity, stratified, and highly motile cell population elongates the tissue and transitions to a polarized, simple duct. Aggregate molecular analysis has revealed key pathways in morphogenesis. However, the single-cell behaviors, molecular activities, and force mechanics coordinated in time and space to build epithelial ducts have remained underexplored. The fatty stromal matrix of the mammary gland makes *in vivo* single cell imaging difficult. Therefore, to observe single cell events, we generate multicellular organoids from mouse mammary glands, explant them into 3D gels, and induce morphogenesis by adding FGF2. We then observe molecular events in real-time using 4D time-lapse microscopy. We tracked single cells and observed them radially intercalate in the transition to a simple duct. Then, using fluorescent molecular biosensors, we visualized the downstream RTK signaling pathway. We observed individual epithelial cells enriching Ras activity, PIP3, and F-actin in protrusions. In the protrusions, PIP3 enriched at the site of, but prior to, F-actin enrichment. Furthermore, inhibiting actin dynamics prevented protrusion formation and morphogenesis. Additionally, we used force inference analysis and finite element modeling to analyze interfacial tension equations within an organoid. We found that cell migration through a tissue environment requires specific ratios of protrusion tension and posterior interfacial tension gradients. For radial intercalation, the duct requires high basal tension and posterior interfacial tension gradients that vary with time as protrusions are initiated. Here, we demonstrate that transition to a simple duct results from an epithelial motility program that leads to altered cellular force mechanics, and thereby morphogenesis. Finally, we would like to understand how individual cells exchange cellular contacts and transition to polarized cells.

Program Abstract #408

Systematic large-scale dominant screens in zebrafish to uncover unique genetic regulation affecting post-embryonic development

Katrin Henke, Michael Brent Hawkins, Matthew P. Harris

Harvard Medical School/Boston Children's Hospital, USA

Forward genetic screens have been key to uncovering genes involved in development. However, many genes are indispensable during early development, thus loss-of function alleles often lead to early lethality and hinder the study of gene function during post-embryonic development. To investigate genetic regulation of late development we chose to focus on the identification of dominant mutations that override, or fail to maintain, normal development resulting in altered adult phenotypes. Using the zebrafish, we screened 10.000 F1 progeny of mutagenized founders at >10 wpf for changes in morphology. We identified over 150 mutants in which 70 were re-identified and characterized. These mutants

had a wide range of phenotypes including changes in pigmentation, eye morphology as well as changes in the shape of different components of the skeletal system. One of the largest group of mutants identified in the screen, affects the development of the vertebral column. Existing mapping strategies however are not applicable for efficient identification of dominant mutations. Thus, we devised a new mapping strategy using association and identification of unique SNPs through exome capture and next-generation sequencing (NGS) to permit identification of mutations in a high-throughput manner. We used this method to systematically identify genetic changes linked to mutant phenotypes, in a number of classes of mutants from our screen. We identified candidate mutations in known disease genes, like for example *collagen1a1* and *collagen1a2*, as well as novel genes like *calymmin*, a notochord sheath associated extracellular matrix protein with previously unknown function. Taken together, these results show that is feasible to perform large-scale screens for dominant phenotypes in zebrafish to quickly identify causative mutations. These tools allow interrogation of genetic pathways regulating post-embryonic development and will help to further our understanding of gene function.

Program Abstract #409

Epithelial folds affect cell proliferation patterns during development of the *Drosophila* wing

Maria del Carmen Andrés[1], Matheus Viana[2], Susanne Rafelski[2], Marcos Nahmad[1]

[1]CINVESTAV-IPN, México; [2]University of California Irvine, United states

Mechanical interactions between cells play an important role in determining organ size and shape during development. The wing disc of *Drosophila* has long been studied as a model to understand the feedback between tissue mechanics and growth control. During patterning and growth of the wing disc, deep epithelial folds arise at the boundaries of different cell-fate territories. These folds should affect mechanical interactions among cells and the global pattern of tissue stress, but their possible role on cell proliferation and growth control has not been studied. Using confocal microscopy and quantitative image processing tools, we examined the spatio-temporal patterns of cell proliferation in the wing blade as epithelial folds at the blade/hinge boundary form. We found that proliferation rates are significantly higher near the epithelial folds for much of normal third-instar development, except for late-third instar discs with fully-formed folds where proliferation rates decrease and become uniform throughout the disc. These results suggest that the formation of epithelial folds relieves mechanical stress that allows cells near the folds to proliferate at higher rates during normal wing disc development. We suggest that this effect allows the disc to transiently expand before mechanical forces balance with growth factor signaling.

Program Abstract #410

The Auxin Response Factor MONOPTEROS controls numerous plant patterning processes through the direct regulation of auxin-inducible gene expression

Naden Krogan[1], Danielle Marcos[2], Aaron Weiner[1], Thomas Berleth[2]

[1]American University, USA; [2]University of Toronto, Canada

The phytohormone auxin plays a crucial regulatory role in all stages of plant growth and development. This regulation is mediated by the canonical auxin signal transduction pathway involving the Auxin Response Factor (ARF) family of transcription factors. ARF5/MONOPTEROS (MP), a functionally well-characterized ARF, controls organ formation and auxin-inducible responses throughout development of the model plant *Arabidopsis*. To better interrogate the role of MP in patterning processes and auxin-responsive gene expression, we generated a steroid-inducible MP transgenic background that allows conditional MP activation. We used this genetic tool to identify members of the *Aux/IAA* gene family of transcriptional repressors that are directly targeted by MP in different tissue types. We also investigated MP-dependent regulation of factors that facilitate the polar transport of auxin, a property that is critically important in many plant patterning events. This work demonstrates that MP influences many developmental processes by regulating the transcription of multiple auxin-related genes.

Program Abstract #411

Generation of human pluripotent stem-cell derived mesothelial progenitors for tissue engineering and regenerative medicine applications

Miranda Hayworth[1], David Reynolds[1], Thomas Colunga[1], Michael Kulik[1], Laura Menendez[1], Luoman Chen[1], Ben Li[2], Zhaohui Qin[2], Tatiana Yatkievych[3], Parker Antin[3], Kit Nazor[4], Jeanne Loring[4], Stephen Dalton[1]

[1]University of Georgia, USA; [2]Emory University, USA; [3]University of Arizona, USA; [4]Scripps Research Institute, USA

The mesothelium, which constitutes the outermost layer of the coelomic organs including the heart, lung, liver and gut, plays a critical role in the development, homeostasis and potentially in repair of the internal organs following injury or disease. Here, we describe methods for the efficient differentiation of human pluripotent stem cells (hPSCs) into

mesothelial progenitor cells (MPCs) and define their developmental potential in both *in vitro* and *in vivo* models. Differential gene expression analysis of freshly isolated murine embryonic mesothelium was used to validate the characterization of our hPSC-derived MPCs as mesothelial in origin. Clonogenic assays were used to determine the in vitro differentiation potential of hPSC-derived MPCs into fibroblast, smooth muscle and endothelial lineages and the multipotency of hPSC-derived MPCs was evaluated in vivo by assessing integration of hPSC-derived MPCs into embryonic chick hearts and mechanically-damaged neonatal mouse hearts. At the molecular level, hPSC-derived MPCs are indistinguishable from their in vivo counterparts and respond to signaling molecules that are known to impact mesothelial cell fate decisions during development as shown by their in vitro differentiation into fibroblasts, smooth muscle cells and endothelium in response to PDGF-alpha, PDGF-beta and Vegf signaling, respectively. When transplanted onto developing chick hearts, MPCs incorporate into the host mesothelium and invade the underlying myocardium. MPCs transplanted into mechanically-damaged neonatal mouse hearts migrate into damaged tissue along with endogenous epicardium-derived cells and assemble into coronary vessels in the repair zone. In addition to the utility of these cells for modeling mesothelial development and disease, this study opens up new avenues for tissue engineering and regeneration/repair of coelomic organs.

Program Abstract #412

Dormant Pluripotent Cells Emerge during Neural Differentiation of Embryonic Stem Cells

Megumi Ikeda[1,2]

[1]Graduate School of Biostudies, Kyoto University, JP; [2]Institute for Virus Research, Kyoto University, JP

One major concern for clinical applications of embryonic stem cell (ESC)-derived cells is residual undifferentiated cells potentiating latent tumorigenicity. Despite intensive methodological approaches to eliminate residual undifferentiated cells, properties of these cells remain elusive. Here, we show that in a serum-free neural differentiation condition, residual undifferentiated cells markedly delay cell cycle progression without compromising their pluripotency. This dormancy is reversible, as cells exit the dormant state to undergo proliferation and differentiation into all three germ layers upon serum stimulation. Microarray analysis revealed a set of genes that are significantly upregulated in the dormant ESCs compared with proliferating ESCs. Among them, we identified the transcription factor forkhead box O3 (FoxO3) as an essential regulator for maintenance of pluripotency in the dormant ESCs. Our study demonstrates that transition into the dormant state endows residual undifferentiated cells with FoxO3-dependent and leukemia inhibitory factor/serum-independent pluripotency.

Program Abstract #413

Derivation and differentiation of haploid human embryonic stem cells

Nissim Benvenisty

The Azrieli Center for Stem Cells and Genetic Research, Department of Genetics, The Hebrew University, Jerusalem, Israel

Diploidy is a fundamental genetic feature in mammals, in which haploid cells normally arise only as post-meiotic germ cells that serve to insure a diploid genome upon fertilization. However, haploid cells provide valuable tools for delineating genome function through loss-of-function genetic screening. We have generated and analyzed a collection of human parthenogenetic ES cell lines originating from haploid oocytes, leading to the successful isolation of human ES cell lines with a normal haploid karyotype. Haploid human ES cells exhibited typical pluripotent stem cell characteristics. Although haploid human ES cells resembled their diploid counterparts by several aspects, they also displayed distinct properties including differential regulation of X chromosome inactivation and genes involved in oxidative phosphorylation, alongside reduction in absolute gene expression levels and cell size. Most surprisingly, while studies on mouse haploid ES cells showed that haploidy is lost upon differentiation, we found that a haploid human genome is compatible not only with the undifferentiated pluripotent state, but also with differentiated somatic fates representing all three embryonic germ layers both *in vitro* and *in vivo*. Importantly, differentiation occurred despite persistent dosage imbalance between the autosomes and X chromosome. The surprising differentiation potential of haploid human genomes suggests that diploidy-dependent adaptations, rather than haploidy, pose the predominant barriers for human development. Finally, we demonstrated the utility of haploid human ES cells for loss-of-function genetic screening by analyzing a haploid gene-trap mutant library for genes conferring resistance to the purine analog 6-thioguanine. Thus, haploid human ES cells hold a great potential for biomedically-relevant functional genomics by forward genetic screening, and will provide novel means for studying human genetics and development.

Nature 532:107-111 (2016)

Program Abstract #414
In vitro culture of *Monodelphis domestica* epiblast stem cells
Lisa-Qiao MacDonald, Jeremy Morrison
Oberlin College, USA
Stem cell lines are useful for understanding the various differentiative events that comprise embryonic development. To supplement our investigations regarding embryonic development in marsupial embryos, we attempted to obtain epiblast stem cells from embryos of the lab opossum. We first confirmed that the pluripotency genes *Pou5f1*, *Nanog* and *Sox2* are present in the opossum genome, established the expression patterns of these genes during opossum epiblast specification, and used these patterns as indicators of pluripotency in our cultured cells. Epiblasts were explanted from day-9 opossum embryos. Each epiblast was microsurgically bisected prior to culture. Each epiblast piece was then grown on one of four types of extracellular matrices (laminin, E-cadherin, vitronectin, Matrigel) and in one of two kinds of defined culture media, TeSR2 (STEMCELL Technologies, Vancouver, Canada) and CDM (completely defined medium, Brons *et. al.* 2007, Nature 448:191). We confirmed the identity of the cells we succeeded in growing *in vitro* by immunostaining them for the aforementioned pluripotency factors. Our results indicate that opossum epiblast stem cells grew best in TeSR2 on Matrigel-coated surfaces. We successfully passaged 35/160 colonies of opossum epiblast cells once, but were hampered in further efforts by the necessity to use clumps of, instead of disaggregated, epiblast stem cells in starting new cultures. Current efforts are directed at continuing to improve our *in vitro* culture techniques. Funding: Oberlin College.

Program Abstract #415
Glucosamine Transported by Glucose Transporter-2 Stimulates Embryonic Stem Cell Proliferation Through Altered Glucose and Glutamine Metabolism Without Affecting Pluripotency
Jin Hyuk Jung, Mary Loeken
Joslin Diabetes Ctr, USA
The hexose transporter, GLUT2 (SLC2A2), which is expressed by mouse embryos, is important for survival before embryonic day 10.5, but its function in embryos is unknown. Although GLUT2 can transport glucose, its K_M for glucose (~16 mM) is significantly higher than glucose concentrations surrounding embryos (3.4-5.5 mM). GLUT2 can transport the amino sugar glucosamine (GlcN) (K_M=0.8 mM), although GLUT2 has not been shown to be a physiological GlcN transporter. GlcN can enter the hexosamine biosynthetic pathway (HBSP) that produces substrate for O-linked N-acetylglucosamine modification (O-GlcNAcylation) of proteins, supplementing HBSP intermediates synthesized from fructose-6-PO_4 + glutamine. Increased protein O-GlcNAcylation has been shown to stimulate proliferation and pluripotency of embryonic stem cells (ESC). We hypothesized that GLUT2-transport GlcN increases substrate for the HBSP, thereby stimulating proliferation and/or pluripotency. Glut2 expression is lost upon isolation of ESC in high glucose (25 mM) media, however, a novel ESC line derived in low glucose (5.5 mM) media retains functional GLUT2 transporters. Here we show that GlcN stimulates ESC proliferation in a GLUT2-dependent manner but neither stimulates pluripotency nor inhibits differentiation. GlcN stimulation of proliferation was not due to increased O-GlcNAcylation. Instead, glucose- and glutamine-derived intermediates needed for anabolic metabolism and biomass accumulation were increased. Thus, ESC that express GLUT2 can be used to model embryo metabolism utilizing the HBSP more authentically than ESC that lack functional GLUT2 transporters. A further therapeutic implication of these findings is that responsiveness to graft site GlcN by GLUT2-expressing stem cells may engraphment potential and influence cell fate commitment.

Program Abstract #416
Lgr6 marks nail stem cells and is required for digit tip regeneration
Jessica Lehoczky[1,2], Clifford Tabin[1]
[1]*Harvard Medical School, USA;* [2]*Brigham and Women's Hospital, USA*
The tips of the digits of some mammals, including human infants and mice, are capable of complete regeneration following injury. This process is reliant on the presence of the overlaying nail organ and mediated by a proliferative blastema. Epithelial Wnt/β-catenin signaling has been shown to be necessary for mouse digit tip regeneration. Here, we report on Lgr5 and Lgr6, two important agonists of the Wnt pathway that are known to be markers of several epithelial stem cell populations. We find that Lgr5 is expressed in a dermal population of cells adjacent to the specialized epithelia surrounding the keratinized nail plate. Moreover, Lgr5-expressing cells contribute to this dermis, but not the blastema during digit tip regeneration. In contrast, we find that Lgr6 is expressed within cells of the nail matrix portion of the nail epithelium, as well as in a subset of cells in the bone and eccrine sweat glands. Genetic lineage analysis reveals that Lgr6-expressing cells give rise to the nail during homeostatic growth, demonstrating that Lgr6 is a marker of nail stem cells.

Moreover, Lgr6-expressing cells contribute to the blastema, suggesting a potential direct role for Lgr6-expressing cells during digit tip regeneration. This is confirmed by analysis of Lgr6 deficient mice which have both a nail and bone regeneration defect.

Program Abstract #417
Epidermal stratification in zebrafish: the role of Transit Amplifying Cells
Emma Rangel-Huerta, Ernesto Maldonado
Unidad Academica de Sistemas Arrecifales, Instituto de Ciencias del Mar y Limnologia, Universidad Nacional Autonoma de Mexico, UNAM., Mexico
Postembryonic zebrafish development is a period of sustained growth, while this happen, the skin must keep pace in order to maintain all the fish covered and protected from the environment. Epidermis stratification occurs at the same time, thickening from 5 to 30 micrometers, by the time that the fish larvae reaches about 10 mm of standard length (SL). The basal epidermis layer is the source of stem cells that will form the different epidermis cell types. Based on the current model for mammalian epidermis formation we look for the participation of Transit Amplifying Cells (TACs), these originate by asymmetric divisions of basal stem cells, they are highly proliferative and divide several rounds before differentiation occurs. We detected TACs during zebrafish epidermal stratification at 7 – 10 mm SL larvae (that is around 25 – 30 days of age), these were located in suprabasal layers, both TACs and basal stem cells express the transcriptional factor p63, that is the bona fide marker for stem cells in epidermis. TACs, but not basal stem cells, express the phosphorylated form of p63 (Pp63), however, while P63 labeling was found in the nuclei, Pp63 was located in the cytoplasm, which is different from what has been observed in mammalian epidermis, where both p63 and Pp63 are located in the nuclei. Since p63 keep the TACs in a pluripotent state, its degradation must be required for cell differentiation to occur, and it is known that p63 phosphorylation is required for its degradation in the proteasome. Once p63 is phosphorylated is then ubiquitinated and labeled for proteosomal degradation. In mammalian model systems; It has been proposed that the TGF-B receptor is the Pp63 kinase, at the same time there are many E3 ubiquitin ligases that are candidates to be responsible for Pp63 ubiquitination. We are in the process of studying the regulatory mechanisms of p63 degradation leading to the the transition of TACs to a differentiated epidermis.

Program Abstract #418
Preferential Propagation of Six2+ Stem Cells from Rodent Metanephroi and Human Wilms Tumors
Shunsuke Tanigawa[1,2], Nirmala Sharma[1], John Lee[1], Ryuichi Nishinakamura[2], Harold Lovvorn[3], Alan Perantoni[1]
[1]NCI-Frederick, USA; [2]Kumamoto University, JAPAN; [3]Vanderbilt University, USA
Understanding the mechanisms responsible for nephrogenic stem cell preservation, self renewal, and commitment is fundamental to harnessing the potential of metanephric mesenchyme (MM) for nephron regeneration. We have established a serum-free culture model that preferentially expands both the rat and mouse MM progenitor pool and sustains the expression of key stem cell regulators, Six2 and Pax2, over several passages. Moreover, these progenitors remain competent to respond to Wnt4 induction and to form mature tubular epithelia and glomeruli. Using a differentiation protocol for mouse embryonic stem cells, we were also able to differentiate the ES cells to a Six2+ lineage, which could then be stabilized and induced to form the various segments of nephron. We have applied similar niche conditions to propagate cells from Wilms tumors, which caricature nephrogenesis and are driven by a blastemal population that resembles the nephronic progenitor. These conditions also selected for Six2+ cells from primary Wilms tumors, suggesting that we are expanding the putative cancer stem cell in these tumors. Thus, these conditions may not only stabilize normal progenitors for tissue regeneration studies but also facilitate the expansion of the critical therapeutic target population in these tumors, allowing real-time testing of tumor cells from patients in the development of personalized therapies.

Program Abstract #419
Regenerative activation of wingless drives neoplastic transformation of epithelia
Adrian Halme, Rajan Bhandari, Rebecca Jaszczak, Thu Tran
University of Virginia School of Medicine, USA
Tumor progression depends on the accumulation of mutations that alter cellular growth and differentiation. However, the cellular environment where these mutations arise is also important for determining phenotypic outcome. Here we report that constitutive activation of regenerative signaling is an essential for epithelial neoplastic transformation, and that developmental context determines the phenotypic outcome of transforming mutations. Mutation of the neoplastic tumor suppressor *lethal(2) giant larvae (lgl)* in *Drosophila melanogaster* imaginal disc epithelia produces large neoplastic

tumors that elicit systemic responses, developmental delay and growth inhibition, similar to those produced by regenerating imaginal discs. Critical regenerative signaling pathways are activated in *lgl*⁻ mutant tumors including expression of Dilp8, which is responsible for developmental delay and growth control during regeneration, and *wingless*, which regulates growth and patterning in the wing during regeneration and normal development. *wingless* activation during regeneration is mediated by a downstream regulatory region, the regenerative regulatory element (RRE), which is also active in *lgl*⁻ tumors. Removal of the RRE has little effect on *wingless* expression during normal disc development, but severely reduces the regenerative capacity of imaginal tissues and prevents neoplastic transformation as well as neoplasia-mediated developmental delay. Therefore, regenerative activation of *wingless* promotes neoplastic transformation. Consistent with this, inducing regeneration in imaginal discs strongly enhances the neoplastic potential of *lgl*⁻ clones, which is blocked by removal of the RRE. Finally, during our analysis of *lgl*⁻ clones in the wing imaginal disc, we found unexpected heterogeneity between clones, in both their neoplastic potential and RRE dependence, that may reflect differences in regenerative pathways and potential found in different regions of the developing tissue.

Program Abstract #420

Transcriptional regulation of *Drosophila* intestinal stem cells

David Doupe[1], Owen Marshall[2], Andrea Brand[2], Norbert Perrimon[1,3]

[1]Harvard Medical School Department of Genetics, USA; [2]Gurdon Institute and Department of Physiology Development and Neuroscience, University of Cambridge, UK; [3]Howard Hughes Medical Institute, USA

Intestinal stem cells (ISCs) in the *Drosophila* midgut have emerged as an excellent model for the study of epithelial stem cells and homeostasis. The modes of ISC fate, their ability to respond to tissue damage and the signaling pathways involved in their regulation are broadly conserved with mammalian epithelia of the intestine, epidermis and esophagus. While the extrinsic signaling pathways regulating *Drosophila* ISCs have been extensively studied over the last several years, less is known about the intrinsic determinants of stem cell fate. We have used targeted DamID to profile gene expression in undisturbed stem/progenitor cells and the major differentiated cell type (enterocytes). This allowed us to identify 53 transcription factors (TFs) specifically expressed or enriched in the stem/progenitor population. Characterization of the most highly enriched TFs identified critical regulators of proliferation and differentiation with mammalian orthologs implicated in epithelial homeostasis or cancer. Target profiling of these TFs by DamID reveals binding to the regulatory regions of cell cycle genes and signaling pathway components, providing possible links between intrinsic and extrinsic regulators of ISC fate.

Acknowledgments: DD is supported by the Human Frontier Science Program and a Dana-Farber / Harvard Cancer Center Gastrointestinal SPORE Career Development Award. Work in the Perrimon lab is supported by the National Institutes of Health and the Howard Hughes Medical Institute, work in the Brand lab is supported by the Wellcome Trust.

Program Abstract #421

Regulation of ribosome biogenesis and protein synthesis controls germline stem cell differentiation in *Drosophila*

Felipe Karam Teixeira, Carlos Sanchez, Andrea Zamparini, Jessica Seifert, Malone Colin, Ruth Lehmann

Skirball Institute - NYU School of Medicine, USA

Complex regulatory networks regulate stem cell behavior and contributions to tissue growth, repair, and homeostasis. A full understanding of the networks controlling stem cell self-renewal and differentiation, however, has not yet been realized. To systematically dissect these networks and identify their components, we performed an unbiased, transcriptome-wide in vivo RNAi screen in female *Drosophila* germline stem cells (GSCs). Of >8100 genes screened (comprising >97% of genes expressed in *Drosophila* ovaries), 646 genes were identified as autonomously required for GSC development, making up the largest set of germline genes described so far. Based on characterized cellular defects, we classified the identified genes into phenotypic and functional groups, and unveiled a comprehensive set of networks regulating GSC maintenance, survival, and differentiation. In addition to uncovering energy metabolism-independent roles for mitochondria cristae maturation during stem cell differentiation, our analysis revealed an unexpected role for ribosomal assembly factors in controlling stem cell cytokinesis. Moreover, our data show that the transition from self-renewal to differentiation relies on enhanced ribosome biogenesis accompanied by increased protein synthesis. Collectively, our results detail the extensive genetic networks that control stem cell homeostasis and highlights intricate regulation of protein synthesis during differentiation.

Program Abstract #422

Innexins mediate cellular enwrapping behavior in the *C. elegans* germ stem cell niche

Lara Linden, Kacy Gordon, Qiuyi Chi, David Sherwood

Duke University, USA
The stem cell niche is a specialized microenvironment composed of stem cells, support cells and extracellular matrix. While considerable work has focused on how niches maintain stem cells, little is known about how stem cells influence their niche. In *C. elegans*, the germ stem cell niche is composed of a single somatic cell, the distal tip cell (DTC). The DTC expresses Notch ligands required for germ stem cell maintenance and extends a dense network of cytoplasmic processes that enwrap adjacent stem cells. This enwrapping behavior is thought to anchor stem cells in the niche and possibly regulate niche signaling. Somatic support cells also surround germ stem cells in flies and mice and hematopoietic stem cells in zebrafish, suggesting that enwrapping is a conserved feature of stem cell niches. Despite its common appearance in stem cell niches, the mechanism regulating cellular enwrapment is not understood. Through laser ablation studies, we have observed that DTC niche processes are reduced in germline-ablated animals, suggesting that germ cells may signal to the niche. Strikingly, germ cells that were forced to escape into the body cavity were enwrapped by muscle cells. This muscle cell-germ cell interaction further supports the idea that a germline-expressed cue induces enwrapping behavior by somatic cells. Innexin protein family members are expressed in the germline, DTC, and muscle cells, where they form gap junctions. Innexin knockdown resulted in loss of niche enwrapping behavior and reduced Notch signaling, leading to a reduced stem cell population. Preliminary data suggests that innexins are also required for escaped germ cell enwrapment by muscle. Vertebrate gap junction proteins also link mouse hematopoietic and cardiac stem cells to their niches. We expect that by defining the role of innexins in cellular enwrapment, we will provide a mechanistic understanding of a common feature of stem cell niches.
Funding: NIH, and an NSF-GRFP grant to LL.

Program Abstract #423
Characterization of the planarian SWI/SNF-related chromatin remodeling complexes
Toria Trost, Peyton Crowe, Brittany Mersman, Mallory Robbins, Austin Dillon, Amy Hubert
Southern Illinois University Edwardsville, USA
Epigenetic regulation of gene expression through modification and rearrangement of the histone proteins associated with DNA is important for controlling stem cell pluripotency and differentiation in many species. However, many of the proteins that mediate this type of regulation have yet to be fully characterized in planarians, an emerging model for the study of stem cells and regeneration. We identified sixteen planarian proteins (*ARID1A/B*, *ARID2-1*, *ARID2-2*, *ACT6A/B*, *SMARCA2/4-1*, *SMARCA2/4-2*, *SMARCB1*, *SMARCC2-1*, *SMARCD1/2/3*, *SMARCE1*, *polybromo*, *BAZ1B*, *SUPT16H-2*, *TOP2B*, *PHF10*, and *DPF1/3*) homologous to members of the mammalian SWI/SNF-related chromatin remodeling complexes and have begun characterizing their roles in planarian regeneration and stem cell function by whole-mount in situ hybridization and RNAi. The majority of the genes are expressed in the central nervous system and throughout the mesenchyme of the worm where the stem cells reside. Knockdown of fourteen of the genes (all except *SMARCA2/4-1* and *PHF10*) resulted in regeneration defects, including delayed/absent photoreceptors and reduced blastema size. Phenotypes typically associated with stem cell loss (head regression, ventral curling and lysis) were also observed following knockdown of several of the genes. Staining to label mitotic cells revealed a reduction in their number following knockdown of *SUPT16H-2* and *TOP2B*, whereas there was an apparent increase in the number of mitotic cells following knockdown of some of the core complex proteins. Future directions for this project include quantifying the mitotic cell number in each knockdown group and performing fluorescent in situ hybridization to each gene in combination with a stem cell marker to look for co-expression.

Program Abstract #424
Analysis of retinoic acid signaling effects on planarian regeneration.
Betsy Dobbs-McAuliffe, Michael Speziale
Central Connecticut State University, USA
For over 200 years biologists have used planaria as a laboratory to investigate tissue regeneration. Planaria are bilaterally symmetrical, triploblast flatworms with regenerative capabilities seen in few other animals. Planaria have the capacity to replace all cell types and all body systems. Previous studies revealed that classical developmental signals, such as Wnt, Hedgehog, and BMP, have conserved functions in planaria. Thus, many pathways required to establish chordate embryonic axes also function during planarian regeneration. Until recently retinoic acid signaling (RA) pathways were identified only in chordates, suggesting that RA signaling evolved more recently than other pathways. However, genomic studies have identified homologs of RA receptors and RA metabolic and catabolic enzymes in echinoderm and hemichordate deuterostomes and in lophotrochozoans. A planarian retinoid X receptor sequence can be found in GenBank (JF912376.1, AEH84412.1). Other studies have documented physiological effects of exogenous RA in non-chordate

animals. We are investigating possible RA signaling functions in planaria by testing effects of exogenous RA and diethylaminobenzaldehyde (DEAB), an RA synthesis inhibitor, on planarian regeneration. To follow both anterior and posterior regeneration in a single worm we cut above and below the pharynx. We found that treating trunk fragments with RA delayed regeneration of eye spots from the anterior blastema. To refine these data we are using synapsin antibodies to visualize the nervous system. We expect that RA treated worms have deficits in regeneration of the cephalic ganglia. Some trunk fragments treated with DEAB regenerated normally at the anterior cut site, but did not progress beyond wound healing at the posterior cut site. We are currently assessing mitosis and blastema size using immunofluorescence. We expect reduced numbers of mitotic neoblasts and smaller resulting blastemas in the posterior of DEAB treated worm fragments.

Program Abstract #425

A functional genomics screen in planarians reveals regulators of whole-brain regeneration

Rachel Roberts-Galbraith[1], John Brubacher[2], Phillip Newmark[1]

[1]University of Illinois at Urbana-Champaign and Howard Hughes Medical Institute, USA; [2]Canadian Mennonite University, Canada

After injury, an organism faces numerous challenges to heal and replace missing tissue. New cells must be made in the appropriate number and ratios. New tissue must be patterned and integrated in the context of existing tissues; in the central nervous system (CNS), new and old neurons must be reconnected with their targets. Regeneration also requires tight spatial and temporal control to prevent hyperproliferation or damage to healthy tissue. Based on their regeneration capacity, planarians provide an *in vivo* model for understanding how an organism overcomes the challenges of tissue replacement. In this study, we employed an unbiased screen to identify regulators of CNS regeneration in planarians. We screened >300 genes upregulated after head amputation and identified dozens of genes that function in regeneration. These genes include transcriptional regulators that affect neural fate (*soxB-2a*, *runt*), brain patterning (*hesl-3*), reestablishment of a key commissure (*arrowhead*), and restoration of chemosensory function (*FLI-1*). We also identified several potential signaling molecules, some of which are expressed by an unknown parenchymal cell type (*heparan sulfate proteoglycans*, *F-spondin*). We are currently investigating the mechanisms by which several of these genes function during regeneration. We also examined genes downregulated after amputation in an attempt to identify factors or cells that limit the extent or timing of planarian regeneration. One downregulated gene, *intermediate filament-1*, revealed putative glial cells in the planarian CNS that respond to amputation by downregulating several transcripts.
Taken together, our work reveals cellular and molecular players in planarian CNS regeneration and provides useful starting points for investigating mechanistic themes underlying the regenerative process.
(Funding: HHMI to PAN & Jane Coffin Childs Memorial Fund to RRG)

Program Abstract #426

Mechanisms of Pattern Control in Regeneration

Eric Hill, Rachel Lander, Constanza Vasquez-Doorman, Christian Petersen

Northwestern University, USA

Regeneration relies critically on robust regulation of tissue patterning to define with precision the appropriate content of the regeneration blastema, yet the underlying mechanisms remain enigmatic. Planarian flatworms have emerged as a model to study whole-body regeneration mediated by adult pluripotent stem cells, and our studies have used this organism to identify the cell signaling and regulatory principles that allow restoration of an axis truncated by amputation. We identified a canonical Wnt/beta-catenin signaling pathway mediated by asymmetric expression of the Wnt inhibitor *notum* that responds to tissue orientation at the wound site and polarizes the identity of the axis termini in regeneration. Downstream of this early decision step, a stem-cell-dependent pathway activated by injury-induced expression of Zic-family transcription factors forms a Wnt inhibitory signaling center needed for head outgrowth after decapitation. Furthermore, we find a cohort of regionally expressed genes as candidates that define the identity of pre-existing tissues independent of stem cell activity. Among these, a pathway involving three genes expressed in overlapping body-wide gradients regulates tissue identity within the trunk: *wntP-2*, a noncanoncial Wnt signaling co-receptor ptk7, and a conserved FGFR-like tyrosine kinase-deficient cell-surface protein. These analyses suggest that natural mechanisms of pattern restoration in adulthood involve early injury-induced directional cues used in axis polarization, the use of stem cells to create tissue organizing centers needed for blastema outgrowth, and constitutive expression gradients of signaling molecules used to restore regional identity along an amputated axis. Together, these analyses seek to uncover the regulatory logic underlying regenerative growth. Funding sources: NRSA F32GM108395-01A1, Ellison Medical Foundation, NIDCR 1DP2OD017280-01.

Program Abstract #427

Neoblast specialization during regeneration of the planarian *S. mediterranea* nervous system

Kellie Kravarik[1,2,3], M. Lucila Scimone[1,2], Peter Reddien[1,2,3]

[1]Whitehead Institute, MIT, USA; [2]HHMI, USA; [3]Biology Department, MIT, USA

Planarians are well known for their ability to regenerate entire animals from small tissue fragments. Planarian regeneration requires a population of dividing cells called neoblasts that are distributed throughout the body. Historically, neoblasts have largely been considered a homogeneous population of stem cells capable of differentiating into all cell types. Most studies, however, involved analysis of neoblasts at the population rather than the single cell level, making it difficult to determine how heterogeneous this population is. Here, we use the planarian nervous system, comprised of hundreds of different cell types, to study how neoblasts specialize into specific cell fates. We recently showed that 41 transcription factors expressed in distinct regions of the nervous system are also enriched in subsets of neoblasts during regeneration, marking putative progenitor cell types. Together with previously known transcription factors required for additional neuronal cell types, we infer that these genes specify a diversity of neuronal lineages within neoblasts, and we hypothesize that there may be one or more multipotent neural progenitor populations within neobasts, from which known neural sub-types are specified. To test this hypothesis, we isolated single cells in the G2/M phase from intact head-pieces and sequenced the mRNA of 188 individual cells. Analysis of these data indicate that there are several unexplored populations of neoblasts that express transcription factors and other genes associated with neural development, and mark distinct populations of differentiated neurons in uninjured animals. We are currently exploring the requirements of these transcription factors during regeneration.

This work was supported by NSF GRFP 1122374, NIH R01GM080639, NIH T32GM007287. PWR is an HHMI Investigator.

Program Abstract #428

Searching for the Planarian Stem Cell Niche

Christopher Fincher[1,2,3], Irving Wang[1,2,3], Josien van Wolfswinkel[1,2], Peter Reddien[1,2,3]

[1]Whitehead Institute, United States; [2]Howard Hughes Medical Institute, United States; [3]Massachusetts Institute of Technology, United States

Planarians possess a population of dividing cells, known as neoblasts, that contain pluripotent stem cells and are required for regeneration of the animal. Following injury or feeding, neoblast numbers sharply spike, with a gradual return to basal levels after 7 days. The mechanism underlying this regulation of neoblast numbers is unclear, however. Neoblasts reside in a mesenchymal space called the parenchyma. The identity and functions of cells that reside in the parenchyma are poorly characterized. Here we have initiated cellular and molecular characterization of cell populations identified from in situ hybridization screens with apparent parenchymal localization. We have found at least four populations of cells with different gene expression that reside in close proximity with the neoblasts, with multiple genes expressed in these cell types being found. Some cells may be mucous-secreting gland cells, but we have not as yet defined the functions of identified cells. Preliminary data indicates preferential proximity of two of these parenchymal cell types towards zeta- and gamma- neoblasts, over sigma (non-zeta/gamma) neoblasts. zeta-neoblasts are progenitors for the planarian epidermis, and gamma neoblasts are progenitors for the planarian intestine. We are interested in whether these cells might play any regulatory functions in neoblast maintenance or differentiation. Future work will investigate this possibility.

Program Abstract #429

Two FGFRL-Wnt circuits organize the planarian anteroposterior axis

Lauren Cote[1,2,3], M. Lucila Scimone[1,2,3], Travis Rogers[1,2,3], Peter Reddien[1,2,3]

[1]Whitehead Institute for Biomedical Research, USA; [2]MIT, USA; [3]HHMI, USA

The molecular basis for positional information is well studied during embryonic development, but the mechanisms regulating patterning in adult tissues are largely unknown. Planarians provide an excellent model to study such mechanisms because they undergo whole-body regeneration and replace all aging tissues during homeostasis, all while faithfully maintaining the adult body plan. Genes associated with key developmental pathways such as Wnt, Bmp, and Fgf are expressed in various regions along the different body axes in planarians. Recently, these genes were shown to be predominantly expressed in muscle cells. Planarian body-wall muscle surrounds the animal, indicating that these discrete expression domains do not simply reflect the distribution of different cell types but may provide positional information through a body-wide coordinate system. To identify regionally expressed genes, we performed single cell RNA-sequencing analysis on 115 muscle cells from distinct regions along the anteroposterior axis. The set of muscle regionally expressed genes found through differential expression analysis and *in situ* hybridization screening showed striking

enrichment in genes involved in Wnt signaling and *Fibroblast growth factor receptor like* (*Fgfrl*) homologs, termed *nou-darake-like* (*ndk*/*ndl*) in planarians. We found two Wnt-Fgfrl circuits controlling trunk and head tissue patterning, both consisting of an anterior *ndk/ndl* expression domain juxtaposed to a posterior *Wnt* expression domain. Inhibition of *wntP-2* and *ndl-3* caused expansion of trunk identity, with development of functional ectopic pharynges and mouths posterior to the original tissues. *fz5/8-4* inhibition, like that of *ndk* and *wntA*, caused posterior brain expansion and ectopic eye formation. Our results suggest that FGFRL-Wnt circuits operate within a body-wide coordinate system to control adult axial positioning.
We acknowledge the Howard Hughes Medical Institute and R01GM080639.

Program Abstract #430
Pattern formation relies on positional information in pre-existing tissue during planarian regeneration
Isaac Oderberg[1,2,3], Peter Reddien[1,2,3]
[1]Massachusetts Institute of Technology, USA; [2]Whitehead Institute for Biomedical Research, USA; [3]Howard Hughes Medical Institute, USA
Planarians are free-living flatworms and a classic model system for studying regeneration. They possess three distinct body axes; an anterior-posterior (AP) axis, a dorsal-ventral (DV) axis and a medial-lateral (ML) axis, and they are capable of fully regenerating each axis. Axial specification during development can occur by a number of mechanisms in different species, including deposition of maternal mRNAs or signaling events initiated by sperm entry. One of the challenges of regeneration is that axial specification must occur in the absence of external cues, using only positional information that is present in pre-existing tissues. Furthermore, in order for the animal to regenerate with the correct shape, the regeneration of structures along each axis must be coordinated. How the axes coordinate with each other during regeneration, resulting in orthogonal AP, ML and DV axes, is unclear. The anterior pole is a cluster of cells that reappears early in anterior regeneration, is important for patterning the anterior blastema, and importantly, occupies a specific position in 3-dimensional space. Given the role of the anterior pole in patterning, placing the pole in the correct location is likely essential for the proper restoration of form following injury. We examined the regeneration of the anterior pole in trunk fragments and observed that pole progenitors appear at the midline of the animal early in regeneration, and then migrate along the DV axis to their final position. We found that by perturbing the system, with either surgical strategies or RNAi, we can affect the location of pole progenitors and the final destination of the anterior pole. These results suggest positional cues encoded in the old tissue guide the formation of the anterior pole, which in turn patterns the newly formed tissue, resulting in an animal with an appropriate body plan.
This work was funded by grants from the National Institutes of Health and Howard Hughes Medical Institute.

Program Abstract #431
The wound response of the acoel *Hofstenia miamia*
Emily Neverett
Harvard University, USA
Regeneration is a remarkable phenomenon where an adult animal can make new cells and organs, which is a process that is usually limited to a developing embryo. Strikingly, there are species in most animal phyla that are capable of "whole-body regeneration," meaning they can regenerate virtually any missing tissue. Studies of *Schimidtea mediterranea*, a planarian flatworm that is an established model organism, have uncovered multiple genes required for regeneration. However, it is unknown whether the mechanisms required for regeneration in planarians represent conserved biological pathways. To compare regenerative mechanisms between animal phyla, we are using a new acoel model organism, *Hofstenia miamia*, in addition to *Schimidtea*. Acoels represent the earliest lineage of bilaterally symmetric animals (bilaterians), which last shared a common ancestor with planarians 550mya. The aim of this project is to discover how *Hofstenia* may detect wounds and how signals from the wound site launch processes necessary for the regeneration of missing tissue. We have identified wound-induced genes in *Hofstenia* using RNAseq data collected from animals at various time points after transverse amputation. We are currently using Fluorescent In Situ Hybridization (FISH) to visualize expression of candidate wound-induced genes. We are also using RNAi to study the functionality of the identified genes. With the identification of these wound-induced genes, we hope to contribute to furthering the development of *Hofstenia* as a model organism, as well as to provide insight for possible mechanisms underlying regeneration across animal phyla.

Program Abstract #432

Characterization of the stem cell population in the acoel *Hofstenia miamia*

Andreé Franco-Vasquez, Vincent Pham

Harvard University, USA

Regeneration is a fundamental feature of animal biology. Studies of whole body regeneration in the planarian *Schmidtea mediterranea* have revealed some mechanisms underlying regeneration. *Hofstenia miamia*, commonly known as the three-banded panther worm, is an acoel species capable of regeneration and has been recently established as a new model system. Several parallels have been discovered between the two species in terms of regeneration mechanisms, including the presence of pluripotent stem cells, called neoblasts. Neoblasts have been characterized extensively in planarians but have been studied minimally in *Hofstenia*. We aim to identify additional neoblast markers by characterizing the expression profile of genes enriched in a neoblast-specific RNAseq dataset in the three-banded panther worm by using fluorescent *in situ* hybridization. Our preliminary studies have revealed neoblast markers, some that are shared with *Schmidtea* and some that appear unique to *Hofstenia*, which we are investigating functionally.

Program Abstract #433

Sustained tapeworm regeneration requires contributions from both the scolex and germinative region.

Tania Rozario[1], Jennifer R. McDonald[1], Phillip A. Newmark[1,2]

[1]University of Illinois at Urbana-Champaign, USA; [2]Howard Hughes Medical Institute, USA

Tapeworms are notorious for their impressive feats of growth. However, the length of a tapeworm at any given time is merely a snapshot of their growth potential because tapeworms continuously shed and regenerate their body. In this study, we use the rat intestinal tapeworm, *Hymenolepis diminuta*, to ascertain how tapeworm regeneration is regulated. *H. diminuta* consists of an anterior "head," or scolex, which attaches to the intestinal wall of the host, a "neck" known as the germinative region (GR), and a series of body segments known as proglottids that are generated at the base of the GR. In the intestine, adult *H. diminuta* tapeworms continuously shed posterior proglottids while simultaneously regenerating new anterior proglottids. We amputated *H. diminuta* and cultured the fragments *in vitro* to assess their regenerative potential while bypassing the necessity of the scolex for attachment to the intestinal wall. We confirmed previous observations that the scolex alone cannot regenerate presumably because it is devoid of stem cells. Fragments of proglottids that are posterior to the GR also cannot regenerate. The GR is necessary and sufficient for proglottid regeneration, however, regenerative potential is not uniform across the GR: anterior GR fragments are more prolific in proglottid regeneration than posterior GR fragments. We are harnessing this asymmetry to perform RNA-sequencing to determine if there are factors across the GR that facilitate a position-dependent regenerative output. Additionally, though amputated GRs alone can regenerate proglottids, this regeneration is finite and will eventually cease. However, when *H. diminuta* is amputated to include both the scolex and GR, proglottid regeneration is seemingly infinite and proceeds over multiple rounds of serial amputation. We propose that the scolex provides hitherto unknown signals necessary to maintain stemness in the GR, which is required for sustained regeneration. (NIH R21 AI119960-01).

Program Abstract #434

Conserved transcriptional changes during metazoan whole-body regeneration

Gregory Cary, Andrew Wolff, Minyan Zheng, Katherine Huang, Veronica Hinman

Canegie Mellon University, USA

Regeneration is pervasive among the metazoa, but to vastly varying degrees. Platyhelminthes, Cnidaria, and Echinodermata are examples of phyla whose members are capable of whole-body regeneration. The molecular details of echinoderm regeneration are largely unknown, as is the degree to which conserved genes and mechanisms are involved. To address this we characterize the mechanism of whole-body regeneration in the larval sea star, *Patiria miniata*. Through examination of cell proliferation we find that normal larval proliferation is largely down-regulated following bisection and that wound-proximal cells exhibit increased rates of proliferation within 7 days post bisection. This blastema-like proliferation is preceded by the restoration of appropriate axis specification genes. We next measure the transcriptome dynamics across a time course of larval sea star regeneration in both regenerating anterior and posterior segments. While some expression changes are found to be segment-specific, there are many changes that are shared between regenerating anterior and posterior scenarios. Using hierarchical clustering of gene expression profiles, we identify expression responses that indicate the activity of regenerative processes such as wound healing and cellular proliferation. Finally, orthologous genes with similar temporal profiles were identified in published Platyhelminth and Cnidarian regeneration datasets. Despite the divergence over more than 580 Ma, we find evidence for conserved temporal dynamics of broad functional classes as well as direct gene orthologs in these species. These analyses show that sea star larvae undergo

regeneration through a trajectory including wound response, axis respecification, and blastemal proliferation. Comparing this Deuterostome model with Platyhelminth and Cnidarian models highlights commonalities between these three models and suggests a deep conservation of whole-body regeneration among the metazoa. Funding: Kauffman Foundation.

Program Abstract #435
Regeneration rates in North American brittle stars
Amy McElhinney
University of Mount Union, USA
An understanding of regeneration in brittle stars has steadily grown in recent years, but many species remain unstudied limiting an overall comprehension of regenerative processes in these organisms. Two species of brittle star species with little representation in the literature will be utilized in the proposed studies. In addition to furthering regenerative biology, this work also focuses on the effects of projected temperature increase and reduced pH in the ocean. These processes are happening in saltwater environments around the globe and are sure to impact survival of numerous species in marine habitats. A better understanding of the impact of global warming is necessary. Two species of brittle stars will be purchased from Gulf Specimen Marine Lab and maintained in aquaria at the University of Mount Union. These specimens will have rays amputated and regeneration rates will be determined. Once basal rates are known, the effects of increased temperature and decreased pH on regeneration rates can be determined. Various biomolecules (DNA, RNA, protein) will be collected for future analyses.

Program Abstract #436
Regulation of injury-induced ovarian regeneration by activation of oogonial stem cells.
Piril Erler[1], Alexandra Sweeney[2], James R. Monaghan[1]
[1]Northeastern University, United States; [2]University of Nottingham, United Kingdom
For well over a century, the Mexican axolotl (*Ambystoma mexicanum*) has provided us with tremendous insight into limb, skin, tail and spinal cord regeneration. Surprisingly, regeneration of many internal organs of the axolotl such as ovarian regeneration has never been studied. Here, we present the first proof of functional oocyte stem cells (OSCs) in adult axolotl ovaries that are capable of regulating a regenerative response. It is invaluable to understand OSC function in the context of ovarian regeneration in proposing new treatment strategies to reduced fertility. First, we performed histological analysis to identify cells in the developing and adult ovary having morphological similarities to germ cells. We next show that ovarian injury induces OSC activation and functional regeneration of the ovaries to reproductive capability. Adult axolotl ovaries expressed germ cell markers *Vasa*, *Oct4*, *Sox2*, *Nanog*, *Bmp15*, *PiwiL1*, *PiwiL2*, *DazL*, and *Lhx8*, supporting our claim of the presence of adult OSCs. Furthermore, we identified and quantified mitotically active oogonial and spermatogonial adult stem cells with Vasa-H3 co-expression. Providing evidence of stemness and viability of adult OSCs, GFP adult OSCs grafted into white juvenile host ovaries gave rise to GFP gonadal cells. Lastly, we showed that during regeneration, Vasa/Brdu positive OSCs become active, rapidly differentiate into new oocytes, and follicle cells proliferate to promote follicle maturation. Overall, these results show that adult oogenesis occurs via proliferation of endogenous OSCs in a tetrapod and mediates ovarian regeneration utilizing endogenous OSC population. Our novel results lay the foundations to elucidate mechanisms of ovarian regeneration that will assist regenerative medicine in treating pre-mature ovarian failure and reduced fertility.

Program Abstract #437
Compensatory Regeneration of the Amputated Axolotl Lung
Tyler Jensen, Peter Giunta, Natalie Schulz, Piril Erler, James Monaghan
Northeastern University, USA
Lung injury, through both disease and trauma, affects many people worldwide. Efforts to elucidate novel mechanisms of lung regeneration have the potential to change lives and transform health care. While there are many who would benefit from a breakthrough in lung regeneration research, there are few models of lung regeneration that may be studied. The axolotl salamander, *Ambystoma mexicanum*, has long been held as a model organism of vertebrate regeneration, particularly in limbs. It is thought that they regenerate all of their tissues, but exploration of lung regeneration has not been performed until now. In this study, we have conducted experiments demonstrating that axolotls are capable of significant lung regeneration after amputation of the distal third of the lung. BrdU cell proliferation analysis showed that a proliferative response was observed throughout the regenerating lung, peaking at three weeks post amputation. Our finding that widespread proliferation occurs throughout the injured lung and contralateral lung suggests that lung regeneration utilizes a compensatory regenerative mechanism rather than an epimorphic process as seen during limb

regeneration. In order to identify molecular factors that may regulate regeneration, we found that mRNA for the epidermal growth factor receptor, HER2, is up-regulated during regeneration. Inhibition of HER2 decreases regenerative cell proliferation suggesting an important role of these receptors in lung regeneration. This novel characterization is the first example of regeneration from a lung amputation in any organism, which demonstrates the unique regenerative abilities of the axolotl. This study lays the foundation for further examination of the underlying molecular basis of lung regeneration and may provide insights into the induction of mammalian lung regeneration.

Program Abstract #438

Cartilage and bone cells do not participate in skeletal regeneration in *Ambystoma mexicanum* limbs.

Catherine McCusker[1], Carlos Diaz-Castillo[2], Julian Sosnik[3], Anne Phan[2], David M. Gardiner[2]

[1]University of Massachusetts, Boston, USA; [2]University of California, Irvine, USA; [3]Wentworth Institute of Technology, USA

The Mexican Axolotl is one of the few tetrapod species that is capable of regenerating complete skeletal elements in injured adult limbs. Whether the skeleton (bone and cartilage) plays a role in the patterning and contribution to the skeletal regenerate is currently unresolved. Cells in the limb connective tissues are known to play a primary role in the establishment of the pattern in the regenerating limb, including the skeletal pattern. Additionally, connective tissue cells of dermal origin were shown to contribute to the regenerated skeletal tissues. Thus, the skeleton is derived at least in part by cells of non-skeletal origin. In the current study we tested the induction of pattern formation, the effect on mesenchymal cell proliferation, and contributions of skeletal tissues (cartilage, bone, and peri-skeletal) to the regenerating axolotl limb. We found that skeletal tissues fail to induce *de novo* pattern formation, and do not contribute to the newly regenerated skeleton. Peri-skeletal tissue, on the other hand, has robust pattern inducing activity and, similar to cells from dermal origin, contribute to the regenerating skeletal structures. These observations reveal that skeletal tissue does not contribute to the regeneration of skeletal elements; rather, these structures are patterned by and derived from cells of non-skeletal connective tissue origin. Lastly, we show that the depletion of intact heparan sulfate chains throughout the peri-skeletal tissue decreases its ability to induce pattern formation and promote cell proliferation in the regenerating mesenchyme. Future studies will be required to determine whether heparan sulfate plays a primary or secondary role in patterning the limb regenerate.

Program Abstract #439

The Role of AP-1 during Functional Spinal Cord Regeneration in Axolotl

Keith Sabin[1,2], Jaclyn Essig[3], Tiago Santos-Ferreira[4], Karen Echeverri[1,2]

[1]Dept. of Genetics Cell Biology and Genetics, University of Minnesota-Twin Cities, USA; [2]Stem Cell Institute, University of Minnesota-Twin Cities, USA; [3]Dept. of Physiology and Biophysics, University of Colorado, Anschutz Medical Campus, USA; [4]CRTD/DFG-Center for Regenerative Therapies, Dresden, Germany

Humans have very limited regenerative capacity especially in the central nervous system. In contrast, salamanders like the axolotl can functionally regenerate injured nervous tissue throughout their life. After spinal cord injury (SCI) in the axolotl a population of astrocyte-like cells, called ependymoglial cells, adjacent to the injury site proliferates and migrates to reconnect the injured spinal cord and direct subsequent axon regeneration. This is in stark contrast to the mammalian response to SCI whereby injured astrocytes become reactive and contribute to glial scar formation and inhibition of axon regeneration. A major gap in knowledge exists regarding the molecular mechanism promoting a pro-regenerative ependymoglial cell response to injury. Previously we identified the transcription factor c-Fos as an essential regulator of the pro-regenerative ependymoglial cell response to injury. However, c-Fos functions as an obligate heterodimer with JUN family members to regulate gene expression. In mammalian astrocytes, up-regulation of c-Fos and c-Jun after injury promotes reactive astrogliosis. Interestingly, immunohistochemical staining of injured axolotl spinal cords show that c-Jun is only expressed in neurons, not ependymoglial cells; suggesting c-Fos plays a different role to promote functional spinal cord regeneration in axolotl. Here I will present molecular data that identifies the molecular circuitry that must be precisely regulated to promote axolotl ependymoglial cells towards a regenerative response. Our data demonstrate an important role for the differential regulation of FOS and JUN family members in promoting functional spinal cord regeneration.

Funding Source: K.S. NIH Training Grant T32 GM113846

Program Abstract #440

The role of NRG1-ErbB2 signaling pathway in promoting spinal cord regeneration in the Mexican axolotl.

Polina Freitas, James Monaghan

Northeastern University, USA
Salamanders such as the Mexican axolotl (*Ambystoma mexicanum*) are the only known tetrapod vertebrates capable of full spinal cord regeneration throughout adulthood. Unfortunately, similar injury in mammals is completely debilitating, as it permanently forms a glial scar which prevents regeneration. Therefore, the axolotl is an indispensable model for the study of the molecular pathways underlying regenerative abilities. This work focuses on discovering the functional role of Neuregulin 1 (NRG1) during adult neurogenesis and spinal cord regeneration after injury. Neuregulin 1 is a growth factor particularly important to the nervous system. NRG1 signaling is associated with the promotion of remyelination and functional recovery after nerve injury, and the loss of this signaling has been shown to lead to schizophrenia and epilepsy. We have shown in preliminary studies that pharmacological inhibition of NRG1 signaling prevents axolotl tail and spinal cord regeneration. Animals treated with the NRG1 receptor inhibitor TAK 165 did not fully regenerate their tails and had impaired morphology compared to control animals. We hypothesize that Neuregulin 1 signaling promotes spinal cord regeneration by increasing neurogenesis and cell survival as well as improving the rate of remyelination. We have started testing this hypothesis by localizing and characterizing NRG1 positive cells in the spinal cord of injured and uninjured animals. We have also performed electroporation of gene constructs into the spinal canal of axolotls to elucidate NRG1 function during axolotl spinal cord regeneration. Overall, our preliminary results highlight the role of NRG1-ErbB2 signaling pathway in promoting spinal cord regeneration in the Mexican axolotl.

Program Abstract #441

Peripheral nerves have both a mitogenic and permissive role during axolotl limb regeneration

Johanna Farkas, James Monaghan
Northeastern University, USA
The Mexican axolotl (*Ambystoma mexicanum*) possesses the astonishing ability to fully regenerate adult limbs, but this regeneration does not take place if the limb is axotomized prior to or shortly after amputation. Denervation blocks the formation of the post-injury proliferative mass called the blastema, but the reason for this nerve-dependency remains unknown. While previous studies have suggested that intact nerve axons supply growth factors which support blastemal proliferation, others have indicated that the Schwann cells of denervated peripheral nerves may instead inhibit regeneration. Here we provide evidence for both hypotheses by showing that supplementation with the axon-derived growth factor Neureugulin-1 (NRG1) rescues regeneration to digits in denervated axolotl limbs, while implantation of axotomized peripheral nerve bundles blocks blastema formation in an accessory limb model of regeneration. Axolotl forelimbs that were axotomized and implanted with NRG1-soaked beads at 19 days post amputation regenerated to the point of digit formation, and immunohistochemical analysis and Alcian blue staining confirmed that the NRG1-treated limbs regenerated to digits despite the absence of nerves. Meanwhile, implantation of denervated peripheral nerve grafts inhibited blastema formation, attracted inflammatory cells, and induced aberrant wound closure in axolotls which underwent accessory limb surgery. Decellularization of the peripheral nerve grafts did not block blastema formation, suggesting that axotomized nerves do not mechanically inhibit blastema formation but may instead do so via the secretion of inhibitory factors into the wound environment. Taken together, our results suggest that NRG1 is a critical promoter of proliferation in the regenerating axolotl limb, and denervation may block limb regeneration both by depriving the limb of NRG1 and by inducing inflammatory signals which inhibit cellular proliferation and blastema formation.

Program Abstract #442

The regenerating limb blastema as a niche supported by AxRBP-1

Tae Lee[1], Brian Hass[2], Kimberly Johnson[1], Donald Bryant[1], Nicholas Leigh[1], Tia DiTommaso[1], Tzu-Hsing Kuo[1], John Trombetta[2], Benjamin Lewis[3], Orit Rozenblatt-Rosen[2], Rahul Satija[2], Alexander Shalek[2], Aviv Regev[2], Clifford Tabin[4], Jessica Whited[1]
[1]Brigham Regenerative Medicine Center and the Department of Orthopedic Surgery, Brigham and Women's Hospital, Harvard Medical School, and the Harvard Stem Cell Institute, U.S.A.; [2]Broad Institute of M.I.T. and Harvard, U.S.A.; [3]Department of BIology, M.I.T., U.S.A.; [4]Department of Genetics, Harvard Medical School, U.S.A.
Total appendage regeneration in vertebrates relies upon the creation of a specialized structure for accumulating and proliferating progenitor cells, the blastema. Cells throughout the blastema undergo common biological processes such as proliferation while remaining undifferentiated, regardless of their ancestries. The underlying molecular forces for such collective behaviors remain largely unknown but are likely to provide important clues for understanding the mechanisms of regeneration. To address this, we interrogated the transcriptional profiles of individual cells from the regenerating tip of axolotl limbs. Computational analysis revealed genes enriched in largely three cell types: blastema cells, wound epidermal cells, and blood-related cells. We found a gene encoding the axolotl ortholog of cold-inducible RNA-binding protein,

AxRBP-1, to be expressed highly in all profiled blastema cells, implying this gene may perform essential functions regardless of their varied lineages. Over-expressing AxRBP-1 impaired regeneration, implying that AxRBP-1 must be tightly controlled during limb regeneration. Further transcriptional profiling showed that AxRBP-1 overexpression can sustain blastema-enriched and wound epidermis-enriched genes in regenerating blastemas. Importantly, we tested a subset of these genes to show their induction in non-regenerating intact limbs when AxRBP-1 is ectopically over-expressed. These results together suggest that AxRBP-1 can support a regenerative niche. We are currently performing experimentation to further elucidate the mechanism of AxRBP-1 action during limb regeneration.

Program Abstract #443
Reference transcriptome during limb regeneration in plethodontid salamander
Claudia Arenas, Andrea Gómez, Jean Paul Delgado
University of Antioquia, CO
Salamanders have been extensively used as model organisms in regeneration research, *Ambystoma mexicanum* (Axolotl) and *Notophthalmus viridescens* (newt) have been so far the model organisms in this field. Contrary to Ambystomatidae and Salamandridae that have paedomorphic and larval stages respectively, salamanders of the Phetodontidae family have direct development, nonetheless there are few studies regarding the regenerative capabilities in this family and, to the best of our knowledge, there are no transcriptome analysis. Here we present a preliminary result about the limb regeneration process in a wild plethodontid (*Bolitoglossa ramosi*) and their reference transcriptome, which will be help us to understand how this process take place in other families of salamanders. Our result shows that the process take longer time than reported for Axolotl and newt. The preliminary analysis of the novo transcriptome allowed to identify 78.104 transcripts with functional annotation using different data bases performing reciprocal best-hits BLAST. Comparative analysis on differential expression in the blastema showed important genes related to mechanotrasduction process during the limb regeneration process in this species. These results reflect the need to study regenerative processes in non-model salamanders, which will allow future comparative gene expression analysis with axolotl and newt.
Acknowledge: COLCIENCIAS (Departamento Administrativo de Ciencia, Tecnología e Innovación de Colombia), University of Antioquia (CODI-Estrataegia de sostenibilidad)

Program Abstract #444
Understanding urodele limb regeneration in the context of tetrapod limb development
Sruthi Purushothaman, Ashley W Seifert
University of Kentucky, United States
Pattern formation during embryonic development is an intriguing phenomenon wherein undifferentiated cells assemble into higher order tissue assemblages within a spatiotemporal framework. While the tetrapod limb has been an important model for understanding the cellular and molecular basis for pattern formation during development, the control of pattern formation during limb regeneration is poorly understood. The urodele *Ambystoma mexicanum* depicts close to perfect limb regeneration via epimorphosis and pattern formation is a key aspect of this process. Interestingly, gene expression patterns suggest that limb development in urodeles may differ substantially compared to other tetrapods. Additionally, urodele limb development appears to follow an alternate route compared to other tetrapods in terms of ossification pattern, presence of an ectodermal signaling center and some gene expression topology. This begs the question as to whether similar developmental mechanisms are operating in urodeles and other tetrapods during limb development and whether these differences can partly explain regenerative ability in salamanders and newts? Using developing and regenerating axolotl limbs, this study examines the spatiotemporal expression pattern of key genes known to control patterning and growth during mouse and chick limb development. Whole mount *in-situ* hybridizations (WISH) were done on developing larval limb buds at stages 40, 42-43, 46, 48 and 53 and on regenerating forelimbs at early blastema, late blastema, cone/palette, early digit condensation and late digit condensation stages. Cryosections of the WISH limbs were used to define compartmentalization in ectodermal and mesenchymal compartments along with regionalization along anteroposterior and dorsoventral axes. We also used real time PCR to substantiate and quantify the observed spatial expression patterns in regenerating limbs.

Program Abstract #445
SOCS1 and SOCS3 expression in developing and regenerating limb buds of *Xenopus laevis*
Lubna Abu-Niaaj
Central State University, USA
Suppressor of cytokine signaling (SOCS) proteins play critical roles in cell proliferation, development and homeostasis.

Wound healing is a dynamic process involving inflammation, proliferation and tissue remodeling. Successful healing is compromised in prolonged chronic inflammation due to a decreased tolerance of microenvironment to newly differentiating cells reconstructing the wounded structure. Understanding the connection between SOCS proteins as immune-modulators and wound healing might lead to therapeutic uses in regenerative medicine. The African clawed frog, *Xenopus laevis,* regenerates amputated limb completely at early developmental stages (48 through 53), however, an amputated limb in froglet forms a cartilage-spike like structure that is deficient in pattern and structure. SOCS1 and SOCS3 have been recognized in the SOCS family as the most effective regulators of the innate and adaptive immune responses. We report for the first time the isolation of a full-length SOCS1 cDNA in *X. laevis* in addition *to* isolation of SOCS3 cDNA. The expression ofSOCS1 and SOCS3 mRNA transcripts is active at maternal and early embryonic development. In response to amputation, SOCS1 and SOCS3 expression is up-regulated in severed hindlimbs at regeneration-competent (st53) and incompetent stages (st57). Cultures of selected three parts of blastemas at the competent stage (st53) show induction of SOCS1 and SOCS3 transcripts that persists up to seven days post-amputation. This indicates that induction of SOCS1 and SOCS3 expression is a localized response. We show that SOCS1 expression is induced in beryllium-treated blastemas within 24 hr post-amputation, sustained until 5-days with no detection at 7-day post amputation. This study indicates a potential role of immune system especially SOCS1 and SOCS3 in the success of wound healing. Identifying the types of cells expressing SOCS genes in severed limbs in *X. laevis* will highlight their potential role in tissue regeneration.

Program Abstract #446

Analysis of cleaved caspase-3 and cell proliferation in *Xenopus laevis*' tail regeneration, comparing regenerative and refractoty period

Geyse Gomes

Federal University of Rio de Janeiro, Brazil

Regeneration is the reactivation of development in post-embryonic life to restore lost or damaged tissues. *Xenopus laevis*' tail is one of vertebrate model that is employed to understand regeneration. At the regenerative period, stage 40 (St.40), *X. laevis* larvae can regenerate tail. However, at st.46, tadpoles cannot regenerate tail (refractory period). The difference of these two stages has been well described histologically and morphologically, but the molecular mechanism is not clear. Thus, the aim of our work is to understand molecular difference between regenerative and refractory periods. We investigated the role of cleaved caspase-3 (cle-Casp3) and the correlation of apoptotic versus proliferative areas in tail regeneration. We have sectioned larvae (St.40) and tadpoles (St.46) tails about half of their original size and followed the analysis of cle-Casp3 and proliferation assay with Click-iT EdU at 12, 24, 48, 72 and 96 hours post-amputation (hpa). At regenerative period, cle-Casp3 was expressed at regenerative bud, followed by a reduction of its expression until 96 hpa. At refractory period, we noticed cle-Casp3 continuous expression at the healing bud until 96 hpa. Our preliminary data showed the tail regeneration is inhibited when apoptosis was blocked by Z-VAD (pan-apoptotic blocker) administration at regenerative period. The regenerative period also showed noticeable enhancing of proliferative areas starting at 48 hpa, suggesting that apoptosis is followed by a proliferative event. In summary, our data suggest that differences of apoptosis and proliferation regulation of these two periods may explain their regenerative capacities. Therefore, our next steps are to understand how apoptosis is regulated to allow to *X. laevis* tail regeneration.

Program Abstract #447

Evolution of the maintenance and loss of regeneration in lizards: comparative analysis of functional anatomy and genetic mechanisms

Mariana Grizante[1], Dale F. DeNardo[1], Tiana Kohlsdorf[2], Melissa A. Wilson Sayres[1], Rebecca E. Fisher[1,3], Kenro Kusumi[1,3]

[1]Arizona State University, USA; [2]University of Sao Paulo, Brazil; [3]University of Arizona, USA

Most species of lizards are able to lose and regrow their tails throughout adulthood. This ability is dependent on fracture planes in the caudal vertebrae and associated segmental axial muscles that allow for controlled loss, as well as on genetic mechanisms coordinating the regeneration process. We have previously shown in the green anole lizard, *Anolis carolinensis*, that the regenerated tail is structurally quite distinct from the original tail and that differentially expressed genes identified in transcriptomic analysis include both developmental pathways, including Wnt and FGF genes, as well as distinct adult wound healing pathways. While likely an ancestral trait, lizard species vary in their ability to autotomize and regenerate the tail, and we hypothesize that i.) distinct morphological adaptations such as decreased relative tail length are predictors of the loss of tail autotomy and regeneration, and ii.) genetic pathways we identified in transcriptomic analysis of regeneration will show reduced levels of purifying selection in species that have lost the ability to regenerate.

We are expanding our morphological analysis beyond the green anole to encompass 5 species in 3 families, focusing on changes in vertebral number and morphology and the distribution of fracture planes. These data are being correlated with species differences in tail function and ecological niche. Genome and transcriptome data are now available for a number of squamate species, and we are working to analyze rates of purifying and positive selection for the regeneration genetic pathways, comparing species that have maintained and lost regenerative ability.
Research supported by funding from the College of Liberal Arts and Sciences at Arizona State University to KK and a postdoctoral fellow from the Brazilian National Council for Scientific and Technological Development (CNPq) to MBG.

Program Abstract #448
The role of Sal-like 4 during scar-free wound healing
Jami Erickson
University of Minnesota, USA
Although mammals form scars upon skin wound healing, the Mexican "Axolotl" salamander has the extraordinary ability to heal wounds scar-free. While axolotl skin histologically resembles mammalian skin, molecular details that prevent scar formation during axolotl wound healing are largely unknown**.** To address this knowledge gap, we performed an mRNA microarray comparing the transcriptional profile of uninjured axolotl skin to axolotl skin acquired at 2, 14 and 21 days post injury. We analyzed genes that displayed differential gene expression during axolotl wound healing compared to previously published human array data. We found that Sal-like 4 (*Sall4*) expression was increased early during axolotl skin regeneration, but did not increase in humans until later time points. **We hypothesize that early up-regulation of SALL4 after injury is required for scar-free wound healing.** To test this hypothesis, we've depleted Sall4 *in vivo* during wound healing. We found that when Sall4 is depleted, we see excessive Collagen XII deposition that occurs earlier and is not fully remodeled, resulting in a scar-like phenotype. Here I will present data on the effect of down-regulating SALL4 in axolotl during wound healing and on upstream regulation that may be responsible for the differential regulation of SALL4 in axolotl versus humans after injury.

Program Abstract #449
Role of ARID3a in the adult medaka kidney
Tomoko Obara
OUHSC, USA
In mammals, kidney units (nephrons) once lost to environmental damage or disease will not be replaced. The ability to regenerate new nephrons in a manipulatable *in vivo* model is a major advance that will allow identification of key stages that we predict to cure kidney disease. In the past few years, it has been possible to use adult tissues to "reprogram" cells into adult stem for many organs. To date, no one had identified early adult renal stem cells capable of giving rise to the multiple cell types that construct adult kidney, which is an essential requirement to generate fully functional nephrons with vascularized glomerulus filtration barrier. Thus there is an urgent need for additional approaches such as adult renal stem/progenitor cells. Fifteen members of the ARID family of proteins act as a component of large epigenetic regulatory complexes; the molecular mechanisms by which ARID affects transcription at pluripotency promoters are still under investigation. It has been reported that tissues from the ARID3a knockout mice formed developmentally plastic cell lines from adult organs, including kidneys. We previously showed that an adult mouse ARID3a knockout kidney cell like (KKPS5) spontaneously generates multipotent adult renal stem cells in 2D cultures. Flow cytometric analyses revealed expression of high levels of renal stem cell surface markers CD133 and CD24. To further study the role of ARID3a in adult stem cells, we have developed a novel methodology to eliminate ARID3a in an adult animal model. ARID3a knockout in adult medaka induced and increased renal progenitors within 5-7 days, leading to the formation of a mature vascularized glomerulus filtration barrier and tubular structures within 28 days. By understanding the role of ARID3a signaling in adult renal stem cell, we hope to develop new approaches for addressing CKD.

Program Abstract #450
Visualizing Cell Turnover During Epithelial Tissue Homeostasis and Repair Using Developing Zebrafish
Oscar Ruiz[1], Elizabeth Sumner[1], Jonathon Hill[2], George Eisenhoffer[1]
[1]MD Anderson Cancer Center, USA; [2]BYU, USA
Epithelial tissues provide an essential barrier for the organs they encase, and are also the primary sites of solid tumor formation. Changes in the balance between cell loss and division have been implicated in numerous human diseases, yet the mechanisms that regulate overall cell numbers within epithelia remain poorly understood. We have found that cell extrusion, a process used to eliminate cells from epithelia without disrupting barrier function, is key in driving cellular

turnover. To investigate extrusion in a living epithelium, we identified a set of GAL4 enhancer trap lines that are expressed in discrete epithelial cell types in the developing zebrafish. When combined with UAS effector lines, our epithelial GAL4 lines provide the opportunity to visualize specific cells for imaging, overexpress genes of interest, and genetically target cells for ablation. Here we have used time-lapse imaging to characterize cell turnover under normal physiological conditions and after damage. For these studies, we created an epithelial wounding assay that allows induction of death specifically in a subset of the surface keratinocytes. Live imaging revealed that damaged cells were rapidly eliminated by extrusion and undergo apoptosis. Importantly, we found that increased cell extrusion drives compensatory proliferation to replace the lost cells and maintain a functional barrier. Transcriptional profiling at defined times during the repair process uncovered distinct molecular pathways associated with the observed cellular behaviors. We are now investigating the changes that occur when extrusion is perturbed and damaged cells are not properly eliminated. Together, this study provides a high-resolution *in vivo* characterization of epithelial cell turnover and creates a system to rapidly identify mechanisms controlling tissue homoeostasis and the specific alterations in these that may lead to pathologies and cancer.

Program Abstract #451

Apoptotic Cells Promote Epidermal Stem Cell Maintenance and Regeneration in Developing Zebrafish

Courtney Brock, Elizabeth Sumner, Stephen Wallin, George Eisenhoffer

The University of Texas MD Anderson Cancer Center, USA

In order to maintain barrier function and homeostatic cell numbers, epithelial tissues require both the removal of excess or defective cells and the replacement of lost cells. In bilayered epithelia, stem cells reside in the basal layer and divide to generate new cells that replenish the tissue. However, little is known about how dying basal cells are eliminated from bilayered epithelial tissues or the signals produced to induce their renewal. Here we report that apoptotic basal cells produce WNT to stimulate proliferation of neighboring stem cells in order to maintain population numbers. Time-lapse imaging and electron microscopy experiments indicate that apoptotic basal cells are rapidly engulfed by the surrounding basal cells. The remaining stem cells then undergo division to compensate for the cell loss and thus maintain tissue integrity and function. Inhibition of either cell death or WNT signaling eliminated the apoptosis-induced division and resulted in failed regeneration. Conversely, genetic overexpression of WNT signaling in the context of a damage response led to an increase in overall cell numbers. Together, our data suggest a novel regulatory mechanism guiding epithelial stem cell turnover during tissue homeostasis and demonstrate the ability to visualize and study individual cellular behaviors and population dynamics in a living vertebrate epithelial tissue.

Program Abstract #452

dnmt1 is required for stem cell maintenance in the ciliary marginal zone of the zebrafish retina

Krista Angileri

University of Pittsburgh, USA

The ciliary marginal zone (CMZ) consists of retinal stem cells (RSCs) which generate retinal neurons throughout the life of the zebrafish. DNA methyltransferase 1 (dnmt1) is expressed within the CMZ and copies the DNA methylation pattern from parent to daughter strands during replication. Loss of dnmt1 function causes disorganization of the CMZ by 4 days post fertilization (dpf) and its loss by 6dpf. We hypothesize that dnmt1 activity is required to maintain RSCs in the CMZ. To more accurately assess onset of the CMZ phenotype, cell numbers were quantified between 3-5dpf. The *dnmt1*$^{-/-}$ CMZ was expanded at 3dpf and reduced by 5dpf. The fates of retinal cell types produced by the *dnmt1*$^{-/-}$ CMZ are biased toward the inner and outer nuclear layers of the retina. Analyses of TUNEL+ cells indicated that there was no significant change in apoptosis within the *dnmt1*$^{-/-}$ CMZ between 4-5dpf. The *dnmt1*$^{-/-}$ CMZ displays an 85% reduction in the proportion of BrdU^{+} cells at 5dpf. Cellular birth-dating experiments demonstrate an inability of RSCs to exit the CMZ between 3-5dpf. Preliminary gene expression analyses at 4dpf demonstrate *dnmt1*$^{-/-}$ RSCs maintain their progenitor-like gene expression domains (*pax6a* and *dnmt1*)within the CMZ while genes required for cell cycle progression and differentiation (*cyclinD1 and atoh7* respectively) are reduced in their expression domains. Additionally, at 4dpf the cell cycle inhibitor, *cdkn1c*, was present within the *dnmt1*$^{-/-}$ RSC population indicating cells are exiting the cell cycle prematurely. These results support a model in which dnmt1 function is required to maintain RSC stemness to promote self-renewal within the CMZ; without dnmt1 activity, RSCs exit the cell cycle and differentiate prematurely. Ongoing experiments will identify gene regulatory networks required for RSC maintenance through comparative transcriptome and DNA methylation analyses of RSCs in the CMZ of wild-type and *dnmt1*$^{-/-}$retinae.

Program Abstract #453

Regeneration of the retinal pigment epithelium in a novel zebrafish model of macular degeneration

Nicholas Hanovice[1], Ross Collery[2], Brian Link[2], Jeffrey Gross[1]

[1]University of Pittsburgh School of Medicine, USA; [2]Medical College of Wisconsin, USA

Geographic Age-Related Macular Degeneration (AMD) results from progressive atrophy of the retinal pigment epithelium (RPE), resulting in photoreceptor degeneration and blindness. While much has been learned about putative RPE stem cells and stem cell-based RPE transplant therapies, our understanding of RPE regeneration and the process by which new RPE integrates with damaged retinal tissue is incomplete. Zebrafish display a robust regenerative response to injury; thus we hypothesized that the RPE would successfully regenerate. Following ablation of the central RPE, we characterized the recovery and regeneration of the RPE by utilizing immunohistochemistry markers for cell death, photoreceptor cells, and the RPE at varying time points following ablation. These analyses indicate that ablation of the RPE rapidly leads to apoptosis and degeneration within the photoreceptor layer. At 1 week post injury, the photoreceptor becomes more organized, and by 2 weeks post injury, a morphologically normal RPE and photoreceptor layer is detectable. In mammals, proliferation of neighboring RPE cells has been associated with limited endogenous repair. We hypothesized that unaffected RPE proliferates to repair RPE injury. BrdU incorporation assays reveal the presence of proliferative cells in the RPE following ablation. BrdU-positive cells are most concentrated in the periphery soon after ablation, but appear more centrally as regeneration proceeds, strongly suggesting that proliferation of uninjured RPE cells in the periphery contribute to the regenerative response. These results establish a novel zebrafish paradigm through which the RPE can be specifically ablated, and are the first to demonstrate that the zebrafish RPE has the capacity to regenerate. This model enables the study of the molecular and cellular mechanisms underlying the regenerative response of the RPE in zebrafish, and will augment further development of AMD therapies.

Program Abstract #454

atf5a CRISPR/Cas9 generated zebrafish mutants analysis

Luis Colón-Cruz[1], Victor Román-Rivera[2], Gaurav Varshney[3], Shawn Burgess[3], Martine Behra[1]

[1]School of Medicine,Anatomy and Neurobiology Department, University of Puerto Rico, San Juan, PR; [2]University of Puerto Rico, Bayamón Campus, Bayamón, PR; [3]National Human Genome Research Institute, NHGRI/NIH, Bethesda, Maryland, USA

Our laboratory is interested in the genetics of regeneration of hair cells (HCs) which are mechanosensory receptors in sensory epithelia of the vertebrate inner ear that detect sound and head motion [4]. Loss of HCs is the leading cause of hearing impairment in humans who like all mammals lack the capacity to regenerate them. By comparison, lower vertebrates like fish can regenerate HCs of the inner ear but also of an evolutionary linked sensory organ that has structural and molecular similarities which is called the lateral line (LL). This superficial organ informs fish of movements in the surrounding waters through stereotypically distributed sensory patches called neuromasts (Ns). Each N is composed of centrally located HCs surrounded by supporting cells (SCs) which divide and differentiate to replace lost HCs [3]. Our laboratory has performed a transcriptional profiling of SCs of the LL and nasal placodes [1]. One of the ESTs (AW184269) that was strongly expressed has now been mapped to the *activating transcription factor 5a* gene (= *atf5a*). In mouse, *Atf5a* is exclusively expressed in olfactory sensory (OSNs). *Atf5a* KO pups are postnatal lethal because totally deprived of OSNs incapacitating their suckling reflex and jeopardizing their survival, therefore demonstrating the importance of this gene in differentiation and survival of OSNs[5]. Furthermore, differential microarrays performed in regenerating vs. non regenerating avian inner ear sensory tissues showed strong up-regulation of *Atfa5* [2], suggesting that this gene had an important role in regeneration of HCs in those tissues. To investigate *atf5a* role in regeneration further in HCs and/or OSNs we targeted this gene in zebrafish using the CRISPR/Cas9 genome editing tool. We will present some of the null alleles that we are growing to homozygosity and in which we plan to examine how regeneration of the LL and the nasal placodes are specifically affected.

Program Abstract #455

Zebrafish as a model of adult tendon injury, repair and regeneration.

Rishita Shah[1,2,3,5], Jenna Galloway[1,2,3,4,5]

[1]Harvard Medical School, USA; [2]Center for Regenerative Medicine, USA; [3]Harvard Stem Cell Institute, USA; [4]Department of Orthopaedic Surgery, USA; [5]Massachusetts General Hospital, USA

Tendon injuries are a significant concern for active adults and aging individuals, due in large part to their poor intrinsic healing capacity. Treatments for tendon injuries often require surgery and have a limited success rate. In the adult zebrafish, we identified a population of craniofacial tendons that share molecular and morphological characteristics with

mammalian tendons. In addition, using a custom microtensile testing device, we determined that these tendons are biomechanically equivalent to mammalian tendons. Importantly, tendons in the zebrafish are hypocellular and hypovascular, mirroring two of the key attributes thought to underlie poor healing capacity in mammals. Given the robust regenerative response of other tissues in the zebrafish, we developed an injury model to assess whether the zebrafish tendon also has healing potential. Using multiphoton microscopy to perform *in vivo* and real time imaging, we observed scarless healing of the tendon within a matter of weeks. This is in stark contrast to mammals, where injured tendons develop scar tissue (misaligned collagen). Ongoing work is aimed at measuring tensile properties following injury to determine whether healing at an ultrastructural level translates to recovery of mechanical function.
Supported by: NIDCR R03DE024771

Program Abstract #456
Leukocyte Itga4 Signaling Regulates Heart Regeneration in Zebrafish
Jing-Wei Xiong
Institute of Molecular Medicine, Peking University, China
Different from the human heart, zebrafish are capable of fully regenerating its damaged heart after ventricular resection, cryoinjury or genetic ablation of cardiomyocytes, but the underlying mechanisms are not completely understood. In this work, we report that Itga4, a subunit of integrin heterodimer, is necessary for adult zebrafish heart regeneration. Both mRNA and protein of Itga4 were induced during heart regeneration. Homozygous Itga4 mutants showed deficiency in the recruitment of leucocytes and coronary angiogenesis in the injury site, thus leading to severe cardiac fibrosis and compromised myocardial regeneration. Itga4 was mainly co-localized with Lcp1-positive and coronin1a-EGFP-positive inflammatory cells, suggesting Itga4 regulates heart regeneration primarily through its function in leucocytes. Taken together, these findings have gained novel insights of Itga4-mediated leukocyte signaling into zebrafish heart regeneration.

Program Abstract #457
Regenerative markers in the ROS-stressed *Xenopus laevis* heart indicate a conserved repair response
Kyle Jewhurst, Kelly McLaughlin
Tufts University, USA
The human heart is a fragile organ, incapable of dealing with extensive damage or extended periods of stress without permanent scarring and reduction of function. In contrast, the hearts of amphibians are superior at healing, showing both an improved stress response and robust repair capabilities. Our research aims to understand the means by which *Xenopus laevis*, the African clawed frog, can repair damage resulting from oxidative stress, one of the most common effectors of heart disease. In order to mimic the effects of heart disease in the *Xenopus* tadpole heart, we have developed an optogenetic tool that utilizes the fluorescent protein KillerRed to produce superoxide radicals and induce tissue damage upon activation with light. We have examined a number of common indicators of both stress and repair in the post-damage heart, characterizing the response on a molecular, morphological, and functional level. Our research is currently focused on the re-expression of early cardiac progenitor genes following oxidative damage, indicative of a repair response similar to the more extensive cardiac regeneration seen in urodele amphibians and many species of fish. We have observed a simultaneous increase in both these progenitors and in cell proliferation, suggesting that a program of partial dedifferentiation may be activated in cardiomyocytes after damage. We have also seen that the location of gene expression changes, with progenitor genes being activated throughout several tissue layers in the heart. We intend to further characterize these processes to better understand how the heart repairs itself following oxidative damage. With this research, we wish to elucidate the cardiac repair processes that are conserved across species, in the hopes that this will improve our understanding of how to reduce the dangers of heart disease in humans.
Funding: NSF, AHA (K. McLaughlin)

Program Abstract #458
Regeneration in the Nervous System: Insights from Chicken Embryos
Barbara Murdoch
Eastern Connecticut State University, USA
Traumatic brain injury (TBI) results in damage to brain tissue and the death of neurons - the cells responsible for communication within the brain. Neurons in the brain are not readily replaced after death, thus resulting in the permanent loss of functions associated with the damaged area. There are however, regions of the nervous system that do produce new neurons (ie. regenerate) to replace those lost. For example, when neurons die in the olfactory epithelium, progenitors

divide and differentiate to produce new neurons that allow for a continued sense of smell. This process termed neurogenesis, is governed by signals found in the environment close to progenitors; but precisely how these signals induce the maturation of progenitor cells into neurons is largely unknown. Using chick embryos, this study aims to identify the location of neuronal progenitors, their patterning within the chick olfactory epithelium and how this patterning changes during development. A longterm goal is to study the signals that surround and affect progenitor proliferation and use this information to develop *in vitro* assays for chick neural progenitors. We use molecular techniques such as fluorescence immunohistochemistry and laser scanning confocal microscopy to visualize and quantitate progenitors in the developing chick olfactory epithelium. Our results have identified progenitor domains within the chick olfactory epithelium, and show how this changes during development. These findings will contribute to our knowledge of neurogenesis and to the overall field of stem and progenitor cell biology.
Funding was provided by the CSU-AAUP and the Eastern Connecticut State University Biology Department.

Program Abstract #459
A system for remodeling the oviductal epithelium by stem-like cells
Yoshihiko Kobayashi[1], Yuki Yamamoto[1], Koji Kimura[1], Kiyoshi Okuda[1,2]
[1]Okayama University, Japan; [2]Obihiro University of Agriculture and Veterinary Medicine, Japan
In mammals, the epithelium of the oviduct is remodeled with the progression of the reproductive cycle in order to function in various events within the oviduct such as gamete/embryo transport, fertilization and embryo development. The oviductal epithelium has been considered to be composed by two-kinds of cells, ciliated and secretory. Recently, $CD44^+$ oviductal epithelial cells ($CD44^+$OECs) were identified as stem-like cells in the human oviduct, but their role remains unclear. To understand their functions, we investigated 1) immunolocalization of CD44 and several other oviduct-related proteins in the bovine oviduct and 2) invasion ability of $CD44^+$ cells isolated from bovine oviductal stroma ($CD44^+$OSCs). 1) Surprisingly, CD44 proteins were expressed in several cells of not only the epithelium but also the stroma of the oviduct. $CD44^+$OECs expressed neither FOXJ1 (a marker of ciliated cells) nor PAX8 (a marker of secretory cells). Some of the $CD44^+$OECs and $CD44^+$OSCs expressed Ki67 (a marker of mitotic cells). Several $CD44^+$OECs expressed either vimentin (a marker of stromal cells) or cytokeratin-7 (a marker of epithelial cells), the others of $CD44^+$OECs expressed both of these proteins. All of the $CD44^+$ OSCs expressed vimentin but not cytokeratin-7. Both $CD44^+$OECs and $CD44^+$OSCs expressed matrix metalloproteinase-2 (MMP2) and MMP14, which play roles in degrading components of the basement membrane. 2) Active MMP2 was detected in the supernatant of cultured $CD44^+$OSCs using by gelatin zymography. $CD44^+$OSCs passed through Matrigel-coated Transwell, indicating their invasion ability. The passage was inhibited by an antibody for MMP2. The overall findings suggest that $CD44^+$OSCs invade the epithelial layer by passing through the basement membrane. These cells could provide source of epithelial cells for remodeling the epithelium of the oviduct.

Program Abstract #460
Lineage tracing of the hair follicle mesenchymal niche during cycling regression and regeneration
Efrat Avigad Laron, Wisal Asaad, David Enshell-Seijffers
Bar-Ilan University, Faculty of Medicine, Israel
Hair follicle formation during embryogenesis initiates when follicular epithelial cells are molecularly distinguished from interfollicular keratinocytes. Subsequently, this molecular distinction resolves into morphological changes resulting in the formation of regularly spaced placodes. Signals generated by the nascent placode instruct the underlying dermal fibroblasts to aggregate and form the dermal condensate (DC). In turn, the DC provides cues that allow the keratinocytes of the placode to proliferate and extend into the dermis. Concomitantly, the proliferating follicular keratinocytes engulf the DC that differentiates to form the dermal papilla (DP). Postnatally, during the hair cycle, hair follicles undergo cycles of growth (anagen), destruction (catagen) and quiescence (telogen). These transitions through the hair cycle involve dramatic morphological alterations that require the coordination of multiple biological pathways. While the DP plays important role in regulating many aspects of the hair cycle, the fate of DP cells during the hair cycle, the mechanisms that maintain the DP compartment and the relationship of DP cells with other dermal fibroblasts of the skin remain poorly understood. We have generated a novel mouse line that expresses the tamoxifen-inducible cre recombinase specifically in the DP during the anagen phase. Using different tamoxifen injection protocols, we have labeled DP cells with GFP and performed cell lineage analysis of DP cells during the hair cycle. This study revealed that the DP remains restricted from the rest of the dermal fibroblast populations throughout the hair cycle. Furthermore, in contrast to previous reports, DP cells are committed to the DP compartment and they do not leave this compartment to transdifferentiate to other type of

mesenchymal cells. This analysis provides novel insights to the mechanisms mesenchymal niches are regulated and maintained within the context of cycling regression and regeneration.

Program Abstract #461

Mammalian Blastema Formation in the African spiny mouse, *Acomys cahirinus*

Shishir Biswas, Ashley Seifert

University of Kentucky, Department of Biology, USA

Although regeneration is widespread across metazoan taxa, it is severely curtailed in most mammals that have been examined. However, our recent discovery that spiny mice can regenerate complex musculoskeletal tissue raises the prospect that we can uncover the genetic mechanisms regulating tissue regeneration. To this end, our lab has been developing the African spiny mouse (*Acomys cahirinus*) as a novel model of mammalian regeneration. Spiny mice are evolutionarily separated from lab mice (*Mus musculus*) by only 30 million years, and unlike lab mice, can regenerate skin, cartilage, nerves and adipose tissue in the ear following injury. Thus, *Acomys* provides us a model with which we can use comparative methods to investigate regeneration biology. Our recent transcriptomic and immunohistological data show that *Acomys* create a pro-regenerative extracellular matrix, initiate a peripheral nerve response and form a specialized wound epidermis in response to injury, all of which are characteristics of epimorphic regeneration. This suggests that ear regeneration in Acomys is accomplished through formation of a blastema, which has not been previously observed in mammals. In this study, we examine cellular proliferation within the context of blastema formation. A 4mm ear punch assay was performed in mice of both species and proliferating cells were tracked at D10, D15, D20 and D30 post-injury. Multi-label immunohistochemistry was performed to 1) quantify the proliferative index in both species across time and 2) identify progenitor cell types and fibroblast subtypes actively proliferating and contributing to the blastema. In addition, primary cells from healing tissue of both species were cultured to examine cellular senescence. Taken together, these data help elucidate the role of proliferation during blastema formation in *Acomys cahirinus*.

Funding: NSF and OISE (IOS-1353713), University of Kentucky Graduate School and University of Kentucky Department of Biology

Program Abstract #462

Inflammation: setting the stage for tissue regeneration

Jennifer Simkin, Thomas Gawriluk, Shishir Biswas, John Gensel, Ashley Seifert

University of Kentucky, USA

Animals respond to physical injury in diverse ways. Some animals regenerate (e.g. urodele amphibians and zebrafish), while other animals form scars (e.g. mice, rats, humans, etc.). However, the initial response to injury, in both regenerating and non-regenerating animals, is the same: mobilization of a non-specific immune response and a burst of inflammation. Because regeneration in mammals is a rare event, we make use of a newly characterized epimorphic system: the African Spiny Mouse (*Acomys cahirinus*). *A. cahirinus* is able to regenerate tissues of the ear pinnae, i.e. elastic cartilage, adipose tissue, dermis, epidermis, hair follicles and sebaceous glands. The same injury in the common house mouse (*M. musculus)* results in scar formation. Using this comparative system, our study details the initial inflammatory and immune cell response to injury to determine how these processes unfold in a regenerating mammal. With a combination of immunohistochemical, genetic, and *in vitro* analysis, we find the acute inflammatory response in both *A. cahirinus* and *M. musculus* is characterized by an initial cytokine storm and a high macrophage presence. However, we find the spatial and temporal infiltration of macrophages differs between the two species. Futhermore, we present data to show that macrophage phenotypes are distinctly different during regeneration and scar-formation and suggest that a regeneration-specific macrophage is present during epimorphic regeneration. These studies create a foundation to understand how a regeneration-competent environment is composed and highlight specific differences in key inflammatory genes.

Program Abstract #463

Formation of Neuromuscular Junctions in the Regenerating Lizard Tail Recapitulates Developmental Processes

Minami Tokuyama[1], Cindy Xu[1], Rebecca Fisher[1,2], Jeanne Wilson-Rawls[1], Kenro Kusumi[1,2], Jason Newbern[1]

[1]School of Life Sciences, Arizona State University, Tempe AZ 85287, USA; [2]Dept. of Basic Medical Sciences, University of Arizona College of Medicine-Phoenix, Phoenix AZ 85004, USA

Formation and regeneration of the neuromuscular junction (NMJ) requires coordinated interactions between motor neurons, Schwann cells, and skeletal muscle. Under normal circumstances the NMJ is maintained throughout life after development. Mammals, following injury, cannot regenerate entire muscle groups but exhibit significant outgrowth of peripheral nerves. In contrast, lizards are capable of regenerating their tails following autotomy. The regenerate tail is

anatomically distinct from the original, including *de novo* skeletal muscle groups and peripheral nerves. However it remains poorly understood whether NMJ regeneration recapitulates developmental processes. To study nervous system regeneration of an amniote vertebrate, we have carried out an analysis of NMJ reformation in the lizard, *Anolis carolinensis*, by immunofluorescence and DiI retrograde labeling. We found extensive axonal outgrowth occurs early in regeneration and precedes remyelination. The appearance of alpha-bungarotoxin-labeled NMJs is found on newly formed muscle but interestingly are not morphologically mature until 120-250 days. At 120 days NMJ density in regenerate tails is statistically increased relative to non-injured tails, but subsequently diminished by 250 days. The presumptive loss of regenerated NMJs is consistent with developmental axon pruning. Activation of caspase-6, a marker of axon degeneration, was utilized to study timing of synaptic elimination. Our findings from transcriptomic analyses of tail regeneration in lizards indicate a complex process with activation of pathways that reiterate development and adult repair. Nonetheless, our results show that NMJ regeneration in the adult lizard tail recapitulates many aspects of development. These studies provide mechanistic insight into lizard tail regeneration and may advance the development of therapeutic strategies to promote mammalian neuromuscular repair. This work was supported by NIH grants R21 RR031305 and R21 AR064935 to KK.

Program Abstract #464

Homeobox transcription factor Six4 and Six5 regulates muscle regeneration and life span of Duchenne Muscular Dystrophy model mouse mdx.

Kiyoshi Kawakami, Hiroshi Yajima
Jichi Medical University, JP

Muscle regeneration is intensively programmed by mechanisms intrinsic to muscle satellite cells. We previously demonstrated that homeobox transcription factors, SIX1, SIX4 and SIX5, are involved in the coordinated proliferation and differentiation of isolated satellite cells *in vitro*. However, their roles in adult muscle regeneration *in vivo* remain to be elucidated. To investigate SIX4 and SIX5 functions in muscle regeneration, we introduced knockout alleles of *Six4* and *Six5* into an animal model of Duchenne Muscular Dystrophy (DMD), *mdx* mice, in which frequent degeneration-regeneration cycles occur in skeletal muscles. We compared phenotypes of skeletal muscle, serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH), grip strength and life span of $Six4^{+/-}5^{+/-}mdx$ with those of *mdx*. Increased diameter of myofibers and lower serum CK and LDH activities were observed in 50-week-old $Six4^{+/-}5^{+/-}mdx$ mice compared with those in *mdx*, indicating that of dystrophic phenotypes of *mdx* were improved. Number of regenerating myoblasts whose myonuclei were positive for MYOD1 and MYOG, and myonuclei positive for SIX1 (a marker of regenerating myoblasts and newly regenerated myofibers) were increased in 12-week-old $Six4^{+/-}5^{+/-}mdx$, suggesting enhanced regeneration compared with *mdx*. Although grip strength was similar in $Six4^{+/-}5^{+/-}mdx$ and *mdx*, treadmill exercisedid not induce muscle weakness in $Six4^{+/-}5^{+/-}mdx$, suggesting higher regeneration capacity. In addition, $Six4^{+/-}5^{+/-}mdx$ showed 33.8% extension of life span, probably due to systematic muscle recovery. The results indicated that low dosage od *Six4* and *Six5* improved dystrophic phenotypes of *mdx* by enhancing muscle regeneration, and suggested that SIX4 and SIX5 are potentially useful *de novo* targets in therapeutic applications against skeletal muscle disorders, including DMD.

Program Abstract #465

The mesonephros is dispensable for formation and early development of the chicken indifferent gonad

Itzik Sibony, Ronit Yelin, Tom Schultheiss
Technion – Israel Institute of Technology, IL

Vertebrate gonads are comprised of two components, the somatic gonad, which forms the bulk of the gonad, and the primordial germ cells (PGC's), which migrate into the somatic gonad from the outside. The earliest phase in somatic gonad formation is the indifferent gonad phase, in which the gonads are largely indistinguishable between male and female. Although much research has been conducted into sex determination and differentiation of the indifferent gonad into testis or ovary, relatively little is known regarding the specification of the indifferent gonad itself. In particular, the mesonephros (the embryonic kidney), which lies adjacent to the indifferent gonad, plays an important role in development of the testis, but it is not clear whether it also plays a role during indifferent gonad formation. We prevented mesonephros formation in the chick embryo by surgically blocking migration of the nephric duct, which is an essential inducer of the mesonephros. The opposite side of the embryo, where the duct was not blocked, served as a control. Molecular analysis using the mesonephric marker Pax2 confirmed complete absence of the mesonephros on the blocked side. Despite complete inhibition of mesonephros formation, timing of the appearance of the early indifferent gonad markers Lhx9 and Sf1 was not affected, although their spatial expression patterns were somewhat altered, and the germinal epithelium was

markedly thinner on the blocked side. Migration of PGC's into the gonadal epithelium also occurred relatively normally. By HH Stage 25-26, a morphologically identifiable gonad was visible on the blocked side, although it was somewhat smaller than the gonad on the control side. We conclude that the mesonephros is not required for the early stages of indifferent gonad formation, including activation of the molecular markers Lhx9 and Sf1, and for homing of PGC's to the developing gonad, although it may influence the gonadal growth rate.

Program Abstract #466
Early gonadal development and sex differentiation in a freshwater shrimp
Grace Okuthe
Walter Sisulu University, ZA
Crustaceans inhabit a wide range of aquatic environments, exhibit diverse life strategies, and show an array of sex determination mechanisms. Their significance in ecology, evolutionary biology, and toxicology has prompted us to choose *Caridina nilotica* as a model species of crustaceans to study development. The present work was carried out to describe post-embryonic gonad development in *C. nilotica*. Specimen were obtained from hatchery tanks at different stages of development and prepared for histological examination. Based on histological observations, primordial germ cells were observed at 5 days post hatch (dph) (total length,TL: 3.38±0.2 mm). Testicular differentiation started at 15 dph (TL: 5.15±0.4 mm), and was identified by the increase in the number of proliferating spermatogonial cells. At 37 dph (TL: 14.14 ±0.14 mm), initial ovarian differentiation was identified by the presence of degenerating testicular tissues, which was accompanied by the presence of gonial cell prolifereation. Mature females with no apparent testicular tissues in the ovary were identified from 56 dph onwards. These findings indicate that female germ cells are derived from sex changed males in *C. nilotica*, suggesting that these species can be classified as protandrous hermaphrodites. This study was supported by the Water Reseach Commission (WRC), RSA) grant to Dr. Muller WJ.

Program Abstract #467
HOP-1 presenilin is essential in the *C. elegans* adult gonad
Ruth Solomon, Donna Leet, Brian Brady, Valerie Hale, Caroline Goutte
Amherst College, USA
Notch signaling mediates many essential events over the course of *C. elegans* development. One of the critical steps in Notch receptor activation is the cleavage of its transmembrane domain by the gamma secretase protease. The catalytic subunit of the gamma secretase complex is the presenilin protein. In *C. elegans* there are two orthologs of presenilin: *sel-12* and *hop-1*. For most Notch signaling events, *sel-12* and *hop-1* appear to play redundant roles, hence single mutants in *sel-12* or *hop-1* are viable and fertile, and can be propagated as homozygous strains, while double mutants exhibit drastic Notch phenotypes (maternal-effect embryonic lethality and sterility due to lack of germ line proliferation). This redundancy supports two ideas: the *sel-12* and *hop-1* genes must have overlapping gene expression patterns and the encoded proteins must be functionally similar at the biochemical level. We have investigated a unique phenotype of *hop-1* null mutants, first suggested by Westlund *et al*. in 1999, namely a reduced brood size. We demonstrate that *hop-1* null hermaphrodites have self-brood sizes that typically range from 43% to 59% that of the wild type self brood size. To explore this defect further, we analyze mated brood sizes and brood sizes of females versus males that lack *hop-1* activity. By measuring embryo production over the course of adult life, we demonstrate that *hop-1* deficiency has its largest impact in older adults. This result is further supported by our finding that the gonads of adult *hop-1* null hermaphrodites cannot maintain germ cell proliferation throughout adulthood. We show that decreased germ cell proliferation does not affect sperm count in *hop-1* null hermaphrodites, but most likely reduces oocyte and nurse cell production in the adult. Taken together, our analysis shows that adult hermaphrodites rely on *hop-1* for full fecundity, and support the hypothesis that *hop-1* may be the only presenilin available for Notch signaling in the adult gonad.

Program Abstract #468
Cellular and molecular mechanisms behind deposition of ectolecithal egg capsules in planarian flatworms
Labib Rouhana, Jessica Steiner, Junichi Tasaki
Department of Biological Sciences, Wright State University, USA
The survival of oviparous species relies largely on the accumulation of nutrients to support embryonic development of fertilized ova post-deposition. For most organisms yolk accumulation during oocyte growth serves this purpose. In contrast, embryonic development in neoophoran species of flatworms relies on contributions from a specialized yolk cell-producing organ, the vitellaria. Yolk cells packaged during ectolecithal egg capsule production support both maturation of the egg capsules and embryonic development. Technical advancements for genetic analysis in the planarian flatworm

Schmidtea mediterranea have allowed us to dissect some of the cellular and molecular processes involved in formation of ectolecithal egg capsules. Analysis of germline defects caused by RNA-interference targeting *Smed-boule*, a member of the DAZ family of germline RNA-binding proteins, revealed that egg capsule deposition occurs independently of fertilization, ovulation, mating, or the presence of gametes. Additionally, analysis of a set of genes with preferential expression in vitellaria revealed five factors required for events that take place during egg capsule deposition. Amongst these, *SynaptotagminXV* is required for egg capsule shell formation, whereas homologs of C-Type Lectin, Tyrosinase, and Surfactant B are required for egg capsule "tanning" (a process of shell maturation that occurs post-deposition). These findings shed light on the evolution of ectolecithy and may prove useful in efforts to hinder the pathology and dissemination of parasitic flatworms.

Program Abstract #469

The molecular signature of polarity during the oocyte to embryo transition

Christine Reid[1], Taejoon Kwon[2], Edward Marcotte[3], Julie Baker[1]

[1]Stanford University, USA; [2]Ulsan National Institute of Science and Technology, South Korea; [3]University of Texas at Austin, USA

The vertebrate body plan is dependent on maternally localized determinants deposited into the egg. While many determinants have been identified, it remains unclear how many are localized and in what bias they are deposited within the egg. The whole oocyte, egg and embryo 30 minutes post fertilization were fixed and dissected into animal and vegetal halves and RNA-Sequencing was performed. We found 564 transcripts localized in the oocyte, with biases in both animal and vegetal halves. Surprisingly, nearly half of the transcripts localized in the oocyte are no longer localized in the mature egg, with the vast majority retaining vegetal localization. As overall transcript levels are consistent between these two cell types, the loss of transcript localization cannot be explained by selective degradation, but instead suggests that these transcripts are losing localization upon egg maturation. The loss of these localized transcripts also suggests that protein localization may drive cell fate determination during early embryogenesis. Tandem mass spectromtery performed on animal and vegetal halves of the egg revealed over 1700 proteins, 240 of which show an animal bias, and 235 of which show a vegetal bias. Very few proteins have localized transcripts, suggesting that protein translation may be locally controlled within the egg. Several of these biased proteins are associated with RNA-binding, suggesting that they play a role in localizing transcript during the oocyte maturation. Finally, by 30 minutes post fertilization, very few transcripts are localized in either half of the embryo, likely due to cortical rotation. Current work is focused on determining when transcripts are re-localized in the one cell embryo and what cytoskeletal elements are involved in protein and transript movement and localization. Taken together, our work provides a clear picture of maternally deposited determinants and suggests that these are driving the early patterning of the vertebrate embryo.

Program Abstract #470

Loss of Snail family genes disrupts stem cell function in the *Drosophila* germarium.

Victoria K. Jenkins, Majed Abbas, Horacio Frydman, Kim McCall

Boston University, USA

The Snail family of transcription factors are essential across Bilateria for facilitating such processes as the epithelial-to-mesenchymal transition, asymmetrical division, and maintenance of stem cell character. In the fruit fly *Drosophila melanogaster*, the three Snail family genes *snail*, *escargot*, and *worniu* have been shown to be essential to maintaining stem cells including neuroblasts, intestinal stem cells, and cyst cells in the testis. While much has been done to establish how *escargot* maintains somatic cyst cells in the testis, little is known about whether Snail genes play an analogous role in the ovary. In a screen for cell death effectors, we found that ectopic expression of *escargot* in female germline cells causes failures of multiple cell death events, both apoptotic and non-apoptotic. This suggested that Snail genes may also have a role in the ovary. Tracing the lineage of *escargot-Gal4* expressing cells showed expression in the germline niche and in the somatic cells that envelop the germline in each egg chamber, suggesting that it plays a role in the regulation or maintenance of ovarian stem cells. By generating mitotic clone cells that lack all three Snail family genes, we found that Snail genes are required to maintain function of somatic stem cells in the ovary. While we were able to recover control and *escargot* loss-of-function clones one week after clone induction, we found very few non-transient clones lacking all three genes. Ongoing work includes rescuing the triple deficiency with individual Snail genes, as well as with other genes that may act downstream of the Snail genes. By doing so, we can examine why stem cells lacking the Snail genes fail to produce new daughter cells - whether that be due to cell death, premature differentiation and loss, or failure to divide.
This work was funded by NIH grant R01 GM060574.

Program Abstract #471

Passing the baton: Sequential translational regulation of a germline RNA by temporally restricted translational repressors

Pooja Flora, Ryan Palumbo, Andrew Oligney, Prashanth Rangan
University at Albany, SUNY, USA

During oogenesis, maternal deposition of mRNAs to the developing egg is critical to establish the future generation's developmental program. These maternally deposited RNAs are under strict translational regulation, mediated by a myriad of translational repressors, to ensure proper spatio-temporal expression. However, the expression of translational repressors are themselves temporally regulated during development. We asked how maternal RNAs are continually repressed, using *Drosophila* germline as a model system. *polar granule component* (*pgc*), a maternal RNA, is translationally repressed throughout oogenesis, except for transient expression in the stem cell daughter. To determine cis and trans-acting factors that control *pgc* translation, we carried out a phylogenetic analysis of its 3'UTR and identified conserved binding sites for translational repressors Pumilio (Pum) and Bruno (Bru). We found that Pum, together with its binding partners Nanos and Twin, repress *pgc* translation only in the germline stem cells. Nanos levels drop in the stem cell daughter allowing for *pgc* expression. Intriguingly, we found that even though Nanos levels rebound during the intermediate stages of oogenesis, Pum complexes with Brat to repress Pgc translation. Pum levels dramatically drop and Bru levels increase past the intermediate stages. Consequently, we find Bru, not Pum, is required for repression of Pgc translation in late stages of oogenesis. Surprisingly, we discover that both Pum and Bru utilize the same cis-elements in the 3'UTR to suppress its translation. Collectively, our studies establish that *pgc* mRNA has evolved to be regulated by temporally restricted repressors. These repressors bind to the same cis-element in order to ensure constant translational repression throughout development. We are currently identifying a network of germline mRNAs that could be similarly regulated during *Drosophila* development.

Program Abstract #472

Age-specific changes in oogenesis and response to sex peptide in female *Drosophila melanogaster*

Margaret Bloch Qazi[1], Brian Hastings[1], Claudia Fricke[2]
[1]Gustavus Adolphus College, USA; [2]Westfaelische Wilhelms-University of Munster, Germany

Reproductive senescence is characterized as a decline in fecundity and fertility with increasing age. A need to better understand the nature and outcomes of female reproductive senescence on gametogenesis and offspring development is driven by an increasing awareness that females in natural populations continue to reproduce as they age and that research on oogenesis is largely focused on young animals. The pomace fly, *Drosophila melanogaster*, is a model system for exploring the various effects of increasing parental age on developmental processes. In females, reproductive senescence is attributed to decreased germline stem cell activity and oocyte provisioning. In this study, we examined the effects of female age on oogenesis and responsiveness to male-derived mating stimuli. We examined changes in oogenesis by mating young and old females to wild-type males, then counting and staging female egg chambers 24 h and 96 h post-mating. A decrease in ovariole activity with increasing age is consistent with a loss in the ability to modulate or maintain oogenesis. Changes in female response to the male seminal fluid protein "sex peptide" were explored by comparing egg chamber development between control and sex peptide receptor (SPR) null females. A difference in the rate of oogenesis with increasing female age between normal and SPR null females reflects a change in females' abilities to respond to male-derived stimuli. These experiments highlight the value of understanding aging as a dynamic condition that can affect various aspects of development and reproductive function.

Program Abstract #473

Germline regulation of Sex lethal in *Drosophila melanogaster*

Raghav Goyal, Kelly Baxter, Pradeep Kumar Bhaskar, Mark Van Doren
Johns Hopkins University, USA

In *Drosophila*, sex-determination is under the control of the "switch" gene *Sex lethal* (*Sxl*). While in some species the sex of the soma is sufficient to determine the sex of the germline via inductive signaling, sex-determination in the *Drosophila* germline also occurs cell-autonomously via intrinsic signaling dictated by its sex chromosome constitution. Interestingly, when *Sxl* is expressed in XY germ cells, they are able to produce eggs upon transplantation into a female somatic gonad, demonstrating that even in the germline, *Sxl* is the "switch" and is sufficient to activate female identity [1]. In the germline and soma, the presence of two X chromosomes leads to *Sxl* expression. However, its control is different in the germline at both *cis*- and *trans*-regulatory levels, and we are studying how this is regulated. The DNA elements responsible for the activity of *Sxl*'s sex-specific promoter (*SxlPe*) in the germline remain unidentified. To this end we are cloning different

DNA fragments covering the entire region upstream of *SxlPe* into a GFP reporter vector to test for sex-specific expression in the germline. Further, we are investigating the *trans*-acting factors that control *Sxl* expression in the germline. The X chromosome "counting genes" important for activating *Sxl* in the soma are not known to be required in a dose-dependent manner in the germline. However, we have found that knocking down *sisterless A* specifically in the female germline results in an ovarian tumor phenotype and reduced *Sxl* expression. Finally, we are searching for additional *trans*-regulators of *Sxl* through an RNAi screen of genes expressed sex-specifically in the undifferentiated germline. Our studies will further our understanding of how 'sex' in the germline is coordinated with the 'sex' of the soma, a failure in which leads to defects in gametogenesis in both flies and humans, this work is important for our understanding reproductive biology. [1] Hashiyama, K., et al. Science 333, (2011).

Program Abstract #474
Specifying germ cells via restrictive signals
Tara Fresques
Brown University, USA
Eggs and sperm are the only cells in adult sexually reproducing animals that contribute to the next generation. Therefore, one of the most important decisions in the development of an animal is: which cells will be fated to become an egg or sperm cell, aka the germ cell lineage. In some animals (ie fruit flies, round worms, and frogs) the embryonic cells that inherit germ cell factors from the egg are fated to become the germ lineage. However, in many other animals (ie mice, axolotl, crickets) secreted cellular signals instruct which cells will become the germ lineage. In mice (where this mechanism is most intensively studied) BMP and Wnt are required for germ cell specification, yet the signaling network that uniquely turns on a germ cell fate is still poorly understood. Sea stars may be a useful model to study signals that contribute to germ cell specification. Different from a sea urchin that specifies its germ line at the 5th cell division, the sea star does not appear to segregate its germ cells until well after gastrulation. We found that the mRNAs of two germ cell factors (Nanos and Vasa) localize broadly during early development, subsequently become restricted to smaller domains, and ultimately accumulate in a small pouch of approximately 100 cells in the larva stage. We tested if Nodal contributes to the restriction of the germ cell fate in two ways 1) by incubating embryos with pharmacological inhibitors and 2) by injecting an antisense translation blocking morpholino into eggs prior to fertilization. Our results show that Nodal is required to restrict germ cell factors during two stages of early development, which may be a conserved feature of the signaling networks that contribute to the germ cell fate in many other animals. Future comparisons of the sea urchin and sea star may reveal mechanisms that contribute to evolutionary transitions in germ cell specification.

Program Abstract #475
Primordial germ cell specification in *C. elegans* is regulated by xnd-1
Rana Mainpal[1], Luiz Sanchez-Pulido[2], Sijung Yun[3], Logan Russell[1], Tetsu Fukushige[3], Chris Ponting[2], Judith Yanowitz[1]
[1]Magee-Womens Research Institute, USA; [2]MRC Human Genetics Unit, UK; [3]NIDDK, USA
Despite the central importance of germ cells for the transmission of genetic material between generations, our understanding of the molecular programs that control primordial germ cell (PGC) specification and differentiation are limited. We recently reported that *xnd-1* (X nondisjunction factor-1), known for its role in regulating meiotic crossover formation, is an early determinant of germ cell fates. Maternal XND-1 proteins localize to the P4 germline progenitor, and are required to ensure that it divides into the PGCs, Z2 and Z3. *xnd-1* mutant embryos display a unique “one PGC” phenotype due to G2 arrest of P4. Zygotic XND-1 comes at on at the ~250 cell stage making it the earliest PGC marker in the worm. This protein then dictates the ultimate size of the germline: *xnd-1* mutants therefore have smaller germlines, reduced brood sized, and a subset are sterile due to complete lack of no germ cells. *xnd-1* acts redundantly with the Nanos paralogs, *nos-2* and *nos-1* to elaborate a germ line and the sterility in *xnd-1* and *nos-1/2* double and triple mutants is preceded by an increase in the activating histone marker, H3K4me2 suggesting that *xnd-1* functions to maintain transcriptional quiescence in the PGC. XND-1 proteins are associated with chromatin, although underrepresented on the X chromosomes, suggesting it may directly regulate gene transcription. Consistent with this interpretation, bioinformatic analysis of XND-1 identifies a helix-turn-helix motif that is conserved in family of nematode proteins, including 2 additional *C. elegans* paralogs. To identify XND-1 target genes, we have performed both ChIP-Seq (collaboration with ModEncode) and RNA-Seq analyses. XND-1 partially overlaps with MES-4, an H3K36 methyl transferase. *xnd-1;mes-4* double mutants show enhanced sterility suggesting XND-1 may function with MES-4 to regulate a subset of target genes required for germ line specification.

Program Abstract #476

Primordial germ cell survival depends on contact with the somatic gonad in the *C. elegans* embryo

Daniel McIntyre, Jeremy Nance

NYU Medical Center, USA

One of the most conserved aspects of stem cell biology is the close association of stem cells with niche cells – supporting cells that regulate stem cell activity and protect these cells from external influences. To understand how a niche functions, we have studied the germline stem cell niche in the nematode worm, *C. elegans*. In this simple genetic system, two germline stem cells and two somatic gonadal precursor cells (niche cells) interact predictably during embryogenesis to form the gonadal primordium. Niche formation is critical for the survival of the germline stem cells, and ultimately controls these cells' switch between quiescent and activated states. We have taken two approaches to investigate how this process occurs. First, using laser ablations we are examining the fate of germ cells that do not make contact with somatic gonadal precursor cells. Germ cell survival depends on early contact with the niche cells and we are working to uncover the molecular mechanisms underlying this phenotype. Second, previous research from our lab has implicated adhesion molecules including E-cadherin in gonadal primordium formation. We are taking advantage of the recent advances in genome editing technology to conditionally disrupt E-cadherin function in the niche and assay the role it plays in stem cell survival. The results will show if these molecules have signaling as well as adhesive roles in the germline stem cell niche. Niche cell function bears directly on many aspects of human health, including regenerative medicine and cancer biology, and the high degree of conservation previously observed between worms and mammals indicates our results will be applicable to problems in human stem cell biology.

Program Abstract #477

ELLI-1, a novel germline protein, modulates RNAi activity and P-granule accumulation in *C. elegans*

Karolina Andralojc, Anne Campbell, Ashley Kelly, Marcus Terrey, Paige Tanner, Ian Gans, Michael Senter-Zapata, Eraj Khokhar, Dustin Updike

Mount Desert Island Biological Laboratory, USA

Germ cells contain non-membrane bound cytoplasmic organelles that help maintain germline integrity. In *C. elegans* they are called P granules; without them, germ cells undergo partial masculinization or aberrant differentiation. Many key P-granule components play roles in both exogenous and endogenous small RNA pathways. CSR-1 represents a small-RNA binding P-granule protein that antagonizes the accumulation of sperm-specific transcripts in developing oocytes. Loss of CSR-1 and its cofactors cause a very specific, enlarged P-granule phenotype. To better understand the function of CSR-1 in P granules, PGL-1::GFP expressing worms were mutated and screened for enlarged P granules. Ten mutants were isolated, including multiple alleles of *csr-1* and its cofactors *ego-1*, *ekl-1*, and *drh-3*. Two alleles are in a novel gene now called *elli-1* (**en**l**li**ne granules). ELLI-1 becomes expressed in primordial germ cells during mid-embryogenesis and continues to be expressed in the adult germline. ELLI-1 forms cytoplasmic aggregates that do not co-localize with P granules, but instead accumulate in the syncytial cytoplasm of the adult germline. Genes encoding P-granule components, including those in the *csr-1* pathway, are up-regulated in *elli-1* mutants, as are several genes that promote RNAi. Our results show that *elli-1* enhances hypomorphic *drh-3* and *glp-1* alleles, suggesting that ELLI-1 functions with the CSR-1 pathway to modulate RNAi and P-granule accumulation.

Program Abstract #478

Understanding Mechanisms of Tissue-Specific Gene Regulation Using a Novel, Germline-Specific Factor in *Caenorhabditis elegans*

Catherine McManus, Valerie Reinke

Yale University, USA

As the only cells passed from generation to generation, the germ line of an organism is critical to the survival of the entire species. An early event in embryonic development in many species is the specification of the germ line as a distinct cell lineage from the soma. Germ cells undergo specialized processes such as meiosis and gametogenesis, meaning that somatic differentiation must be repressed in germline precursors in order to protect their unique fate. Conversely, the germline program must be silenced in somatic lineages so that somatic development can correctly proceed. In the nematode *C. elegans*, germ cells are subject to precise regulation as they progress from proliferation to differentiation to fertilization within each gonad arm. Deciphering the mechanisms that protect germ cells from somatic differentiation and that regulate their progression to a totipotent zygote is critical to the global understanding of cell fate specification. The Reinke laboratory discovered an uncharacterized, germline-specific zinc finger protein called F49E8.2 with several intriguing features. First, the factor is expressed early in embryonic development in the nucleus of the germline precursor

cell exclusively. Furthermore, F49E8.2 expression within the adult gonad depends on gamete fate, with distinct expression patterns for the spermatogenesis program versus the oogenesis program. Lastly, F49E8.2 mutants exhibit increased germ cell apoptosis that depends upon the synapsis checkpoint, suggesting that F49E8.2 may be a key meiosis regulator. With early, highly regulated, and tissue-specific expression, and with a potential role in a critical cellular process, F49E8.2 is an excellent candidate to illuminate key mechanisms of how germ cell fates are regulated and kept distinct from the soma. With this work, we will gain insight into the mechanisms of germ cell fate specification and regulation.
This work is in part supported by National Institute of Health (T32 GM007499).

Program Abstract #479
Identifying a novel role for ETR-1 in *Caenorhabditis elegans* reproduction and hermaphrodite physiological germ cell apoptosis
Anna Allen[1], Ruby Boateng[1], Kristina Ramirez[1], David Hall[3], Andy Golden[2]
[1]Howard University, USA; [2]National Institutes of Health, USA; [3]Albert Einstein College of Medicine, USA
ETR-1, a highly conserved ELAV-Type RNA-binding protein, is canonically known for its involvement in *C. elegans* muscle development. We have identified novel roles for ETR-1 in hermaphrodite and male gametogenesis, and in hermaphrodite "physiological" germ cell apoptosis. Previously we demonstrated that ETR-1 is expressed in the hermaphrodite somatic gonad and germline, and that ETR-1 depletion results in reduced hermaphrodite fecundity. Here we will show that ETR-1 is also localized throughout the male germline, and to specific locales in both spermatids and spermatozoa. Additionally, *etr-1(RNAi)* in RNAi hypersensitive *rrf-3* males results in reduced fecundity compared to control depleted animals. Intriguingly, we observe delayed spermatid activation in *in vitro* sperm activation assays when comparing sperm isolated from ETR-1-depleted and control-depleted males. Within *etr-1(RNAi)* hermaphrodite animals, the reduced fecundity appears to be due to an increase in the number of germ cells undergoing apoptosis as evident by increased CED-1::GFP positive pre-apoptotic cells and apoptotic acridine orange dyed germ cells. Transmission Electron Microscopy (TEM) reveals significant defects in the structure of the somatic gonadal sheath cells and a failure to properly engulf dying germ cells in *etr-1(RNAi)* animals. Investigating the two established engulfment pathways in *C. elegans*, we will demonstrate that co-depletion of CED-1 and ETR-1 suppresses the increase in the number of apoptotic bodies observed in *etr-1(RNAi)* animals, while initial experiments co-depleting CED-2 and ETR-1 appear to enhance the apoptotic phenotype. Combined this data identifies a novel role for ETR-1 in both hermaphrodite and male gametogenesis, and in the process of engulfment of apoptotic germ cells. Our studies are greatly increasing our understanding of important regulators involved in the reproductive success of an animal.
Funding from DoD, HU ADVANCE-IT, and Howard University.

Program Abstract #480
Germ cell localization during testis cord formation in the red-eared slider turtle
Ceri Weber, Blanche Capel
Duke University, USA
During vertebrate sex determination, a bipotential gonad receives signals that initiate either male- or female-specific development. In the red-eared slider turtle *Trachemys scripta elegans*, egg incubation temperature serves as a sex-determining switch. Germ cells inhabit the surface of the gonad upon the initiation of sex determination in the turtle. During male sex differentiation, germ cells relocate into the sex cords, which will become the future seminiferous tubules. In the female gonad, germ cells remain in the cortex. Sexually dimorphic localization of germ cells is not well understood, especially in terms of how these events are related to or regulated by temperature. Previous work by this lab suggested that testis cords form through involution of the cortical coelomic epithelium and envelop germ cells during their formation. However, we discovered that cords exist long before germ cells are found inside. Our recent work suggests that testis germ cells ingress at sites adjacent to SOX9 positive cells to form connections with preexisting testis cords. Germ cell relocalization in the turtle gonad may be regulated by temperature or by other signals as the gonad differentiates such as sex-specific steroids. These findings may indicate how testis cords incorporate germ cells during testis differentiation in other vertebrate species. This research is supported by NIH 5T32-GM007754 and NSF 1256675

Program Abstract #481
Discovery of the first protein-coding gene on an eliminated chromosome in songbirds.
Megan Nelson, Dr. John R Bracht
American University, USA
Conventionally, it is believed that DNA is the same in every cell of a multicellular organism. When chromosomes are

deleted or added, the consequences can be detrimental resulting in severe disorders or death. However, some species are able to dramatically rearrange their genomes, even eliminating chromosomes without any clear side-effects[7]. This effect is not limited to unicellular or microbial life: Zebra finches (*Taeniopygia guttata),* are well-studied songbirds that possess an additional chromosome restricted to the germline cells[2-5]. This germline restricted chromosome (GRC) is thought to be expressed only in the female ovary, silenced in the male testis, and eliminated in somatic tissue of both sexes[2-5]. Interestingly, no protein-coding genes from the GRC have ever been reported, even though it is the largest chromosome in the karyotype. We therefore took a genomic approach to discover genes from this chromosome. Hypothesizing that GRC genes are expressed in the ovaries, these genes would be detectable by deep-sequencing the germline transcriptome. Therefore, we isolated total RNA and DNA from juvenile finch ovaries and testis. After sequencing, the raw data was assembled *de novo* to produce 167,929 ovarian and 289,111 testicular transcripts using the Trinity software package[7]. We then filtered computationally against the somatic reference gemone[6] and a brain transcriptome dataset[1]. We performed validation by qPCR against genomic DNA, confirming 1 GRC gene (a SNAP protein) as present in the germ-line tissues (of both males and one female) and not detected within the somatic tissues. While there are still many unknowns, our discovery of this first GRC gene provides a glimpse into understanding the role and function of the GRC.

Program Abstract #482

Chromosome inheritance in *C. elegans* meiotic mutants

Mara Schvarzstein[1], Katherine A. Gomez Rivera[1], Gunar Fabig[2], Thomas Muller-Reichert[2], Anne Villeneuve[3]
[1]City University of New York, Brooklyn College, USA; [2]Dresden University of Technology, Germany; [3]Stanford University, USA

Errors in chromosome partitioning in meiosis result in aneuploid gametes that form embryos that are unviable or developmentally abnormal. In meiosis, each maternal homologous chromosome become connected to its paternal counterpart by crossover recombination. These connetions are key to enable the ordered partitioning of the genome in the two meiotic divisions. In the 1st division, connected homologous chromosomes are partitioned while co-oriented sister chromatids remain together until the 2nd division when the sister chromatids bi-orient and segregate. Analysis of spermatocytes in *C. elegans* meiotic mutants allowed us to inquire how aneuploidy arises in spermatocytes of sister chromatids that either bi-orient or co-orient in the 1st division. This analysis revealed that chromatids not only mis-segregate during the meiotic divisions, but can also impair the organization of the spindles themselves. Meiotic mutant with co-oriented chromatids in meiosis I (*e.g. spo-11*) congress at metaphase I, but do not undergo anaphase I and often form tetrapolar spindles. These mutants progress into the 2nd division, despite the lack of anaphase, forming a tetrapolar spindle rather than two separate bipolar spindles as normally happens in the second division of wild type spermatocytes. In the 2nd division chromosomes congress, bi-orient, segregate, and form spermatids with unequal amounts of chromatin. In contrast, sister chromatids in mutants lacking REC-8 cohesin, which are bi-oriented, segregate sister chromatids at anaphase I. This suggests that the ability of chromosomes to bi-orient may promote anaphase. Interestingly, γ-irradiation of *spo-11* mutants at doses providing an average of one double strand break and thus a single crossover per nucleus, suppresses the tetrapolar spindles. This implies that a single connected pair of homologous chromosomes that bi-orients at metaphase I is sufficient to promote anaphase I, and normal bipolar spindle formation in meiosis II.

Program Abstract #483

Dmrt1 is necessary for male sexual development in zebrafish

Katilyn Webster[1], Ursula Schach[3], Angel Ordaz[2], Jocelyn Steinfeld[1], Bruce Draper[2], Kellee Siegfried[1]
[1]University of Massachusetts Boston, USA; [2]University of California Davis, USA; [3]Max Planck Institute for Developmental Biology, Germany

The *dmrt1* (*doublesex and mab-3 related transcription factor 1*) gene is a key regulator of sex determination (SD) and/or gonadal sex differentiation across metazoans. This is unusual given that SD genes are typically not well conserved. The mechanisms by which zebrafish sex is determined have remained elusive due to lack of sex chromosomes and the complex polygenic nature of SD in lab strains. To investigate the role of *dmrt1* in zebrafish SD and gonad development, we isolated mutations disrupting this gene. We found that the majority of *dmrt1* mutant fish developed as fertile females. A small percentage of mutant animals became males, but were sterile with dysmorphic and dysgenic testes. Therefore, zebrafish *dmrt1* functions in male SD and testis development. *Dmrt1* mutant males display defects at the onset of gonadal sex-differentiation, with aberrant testis development and retention of ovarian cell-types resulting in juvenile intersex phenotypes. We hypothesize that these juvenile intersex individuals eventually become fertile females, leading to the female sex-bias seen in adult mutants. We found that *dmrt1* is necessary for normal transcriptional regulation of the *amh* (*anti-Müllerian hormone*) and *foxl2* (*forkhead box L2*) genes in testes, which are thought to be important for male or

female sexual development, respectively. Therefore, zebrafish Dmrt1 may specify male-specific cell types through the repression of female associated genes and activation of male associated genes as observed in other animals. We conclude that *dmrt1* is dispensable for ovary development but necessary for testis development in zebrafish, and that *dmrt1* promotes male development through transcriptional regulation of genes involved in gonadal development. Our future work is focused on identifying direct targets of Dmrt1 transcriptional regulation. The strong sex-ratio bias caused by *dmrt1* loss of function points to potential mechanisms through which sex-chromosomes may evolve.

Program Abstract #484

Ultrastructural features of spermatogenesis in Cuban lesser racer (*Caraiba andreae andreae*: Dipsadidae)

Ana Sanz Ochotorena[1], Yamilka Rodríguez Gómez[1], Javier Torres López[1], Reyna Lara Martínez[2], María de Lourdes Segura Valdéz[2], Luis Felipe Jiménez García[2]

[1]Faculty of Biology. University of Havana, Cuba; [2]Faculty of Sciences. National Autonomous University of Mexico, Mexico

Caraiba is a monotypic genus endemic to Cuba. *Caraiba andreae andreae* is a diurnally active, fast-moving, ground-dwelling, pan-Cuban species. There is a lack of knowledge on the biology of this species and the ultrastructure sexual cells of and spermatogenesis have not been published. Therefore, the present study was conducted to elucidate the spermatogenesis process in this species as well as the sperm ultrastructure. Five males were collected in Western Cuba during the breeding period. The individuals were killed ethically following established protocols. The gonads were extracted and fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.6. Afterwards, gonad tissue sample was processed and analyzed by Transmission Electron Microscopy. The testes were in spermatogenesis and sex cells, from spermatogonia to spermatozoa, are arranged in radial disposition as is characteristic in reptiles. Results showed the sperm of *C. a. andreae* has very elongated head, conic at the end because of the acrosome shape. The nucleus shows very compacted chromatin. At the tail, the axonema runs its entire length. It presents the typical pattern of doublets (9+2), and associated with these structures, similar to dynein arms. In the mid-piece nine mitochondria are seen, nine dense fibers and intermitochondrial bodies, this could be considered derivatives of intramitochondrial dense bodies present in other reptiles. In spermiogenesis process up to 19 mitochondria were observed. The evolutive significance of sperm variations remains poorly understood as the enigmatic specific aspects of sperm morphology favoring an enhancement in energy allocation to sperm motility by increasing tail length plus its associated mitochondria in the mid-piece, which suggest the presence of great risks of sperm competition in these snakes. The present findings are discussed in relation to other relevant studies.

Program Abstract #485

Using long term live-imaging and cell tracking in blastoderm stage crickets to understand how a well-ordered germ band is assembled

Seth Donoughe, Jordan Hoffmann, Chris Rycroft, Cassandra Extavour

Harvard University, USA

In the majority of insect lineages, development begins as a syncytial embryo. First, the nuclei divide and move through the viscous fluid of the shared cytoplasm, becoming a single layer around the yolk. Then, the nuclei undergo coordinated flows at the periphery of the egg, physically segregating distinct lineages of tissues. Such nuclear movements are poorly understood, even in the well studied *Drosophila melanogaster*. Moreover, those aspects of post-blastoderm dynamics that have been elucidated in *Drosophila* are unlikely to be representative of all insects. We are therefore examining these developmental events in an insect that branches basally with respect to the most well-studied model species. We use light sheet microscopy to live-image transgenic embryos of the cricket *Gryllus bimaculatus* with high temporal resolution. We automatically detect and track nuclei, and then quantitatively characterize early divisions and movements of thousands of nuclei in 3D space for up to 12 hours at a time. This has enabled us to uncover early differentiation of cellular behaviors in the absence of gene expression information. We also describe how the geometric organization of nuclei changes over time, prefiguring the spatial arrangement of newly formed embryonic cells. Taken together, this work sheds light on how early nuclear movements contribute to the formation of the insect embryo.

Program Abstract #486

Divide OR Conquer? Cell cycle arrest is required for cell invasive behavior.

David Matus

Stony Brook University, USA

Cell invasion occurs during normal development, immune surveillance and is dysregulated in cancer metastasis. Our

laboratory examines anchor cell (AC) invasion into the vulval epithelium during *C. elegans* larval development. Recently, we have identified a dichotomy between proliferation and invasive behavior. Specifically, our data links G1 cell cycle arrest to acquisition of an invasive phenotype. A single transcription factor, the conserved nuclear hormone receptor *nhr-67* (TLX), is required in the AC to prevent the AC from entering the cell cycle. NHR-67 maintains the AC in G1 cell cycle arrest, in part through upregulation of the cyclin-dependent kinase inhibitor *cki-1* (p21/p27). Loss of *nhr-67* results in non-invasive mitotic ACs that fail to express matrix metalloproteinases (MMPs) and actin regulators or form invadopodia, F-actin rich membrane-localized protrusions that are required for invasion. Strikingly, AC invasion can be rescued through induction of G1 arrest, preventing cell division and promoting differentiation. Downstream of G1 arrest, the AC requires the activity of a histone deacetylase, HDA-1, a key regulator of cell differentiation, to regulate the expression of pro-invasive genes and localize invadopodia. Through RNA interference (RNAi) screening, we have identified upstream transcriptional regulators of NHR-67 activity (*egl-43* and *mep-1*), new cell cycle regulatory components (*skr-2* (SKP1), *cdc-14* (CDC14A), *cul-1* (Cullin1) and epigenetic modifiers (*let-418* (Mi-2/CHD3) and SWI/SNF-components) that function in an NHR-67 pro-invasive gene regulatory network to maintain G1 cell cycle arrest and differentiate the invasive phenotype. Together our results suggest that the acquisition of the invasive phenotype is a post-mitotic differentiated state, which may help explain the paradoxical reports that the invasive fronts of many metastatic cancers are non-proliferative.

Program Abstract #487

The Heart Tube Forms and Elongates through Dynamic Cell Rearrangement Coordinated with Foregut Extension

Yukio Saijoh[1], Gary Schoenwolf[1], Hinako Kidokoro[2]

[1]University of Utah, USA; [2]National Cerebral and Cardiovascular Center, Japan

The vertebrate heart is formed by fusion of paired, splanchnic mesodermal primordia that flank the midline of the embryo. The primitive heart tube is initially short and rapidly extends craniocaudally, progressively adding myocardial progenitor cells to the nascent heart tube. Adequate elongation of the heart tube is essential for establishing functional hearts, as defects in elongation result in failure of proper looping and consequently cardiac malformations. However, the mechanism that drives rapid elongation of the heart tube remains to be elucidated. To tackle this, we performed extensive live tracking of heart precursor cells throughout heart tube formation using chick embryos. The data showed that both the first and second heart fields undergo convergent extension (CE) while forming the heart tube, to rapidly elongate it craniocaudally. The short mediolateral extent of the paired heart fields dramatically and directionally extended, with changing its orientation, to form the entire craniocaudal length of the elongated heart tube whereas narrowing the perpendicular plane. We further found that heart precursor cells undergo actomyosin-dependent cell-cell intercalation. Inactivating myosin markedly impaired CE of the heart primordia, resulting in severe inhibition of heart elongation. These results strongly suggest that heart tube elongation is driven in large part by myosin-dependent directional cell rearrangement. In addition, our data suggest that CE also promotes deployment of the second heart field into the heart tube through directional tissue movement. Taken together, CE plays key roles in lengthening of the heart tube by driving a directional tissue extension, as well as the progressive incorporation of progenitor cells. Finally, we show that the heart primordia and the associated endoderm coordinately form and elongate the heart tube/foregut through similar processes, and that oriented heart extension may be driven by endodermal movements/forces.

Program Abstract #488

Impaired development of left anterior heart field by retinoic acid causes transposition of the great arteries in chick embryonic heart

Mayu Narematsu, Tatsuya Kamimura, Toshiyuki Yamagishi, Mitsuru Fukui, Yuji Nakajima

Osaka City University Graduate School of Medicine, Japan

During early heart development, progenitors that originate from the left and right anterior heart field (AHF) in the anterior pharyngeal arches 1/2 and secondary heart field (SHF) in the visceral mesoderm of posterior pharyngeal apparatus migrate to form distinct conotruncal regions. Therefore, spatiotemporally restricted developmental alterations in AHF/SHF may cause distinct conotruncal heart defects. Transposition of the great arteries (TGA), in which the aorta is transposed ventrally to the pulmonary trunk and originates from the right ventricle, is one of the most common conotruncal heart defects diagnosed at birth in human. The aim of this study is to clarify responsible region in AHF/SHF and cellular and morphological mechanisms causing TGA. We placed a retinoic acid-soaked bead on the left or right, or on both sides of AHF/SHF of stage 12 to 14 chick embryos and inspected heart morphology at stage 34. When the left and right AHF were treated with retinoic-acid soaked beads at stage 12, TGA, dextroposed aorta, and persistent truncus arteriosus were diagnosed. TGA was found at high incidence in embryos to which a retinoic acid-soaked bead had been

placed on the left AHF at stage 12. AHF cells exposed to retinoic acid showed a disruption of cellular polarity and failed to migrate to the proximal outflow tract (conus region). Local administration of retinoic acid to the left AHF did not affect the migration of cardiac neural crest cells to the aortico-pulmonary septum. The expression of FGF8 and Isl1 was downregulated in retinoic acid-exposed AHF. In cultured AHF with retinoic acid, myocardial differentiation and expansion were suppressed. These results suggest that the impaired development of the left AHF at early looped heart stage causes defective subpulmonic conus, which subsequently may lead rotational arrest or inversion of the conotruncus resulting in the TGA morphology.

Program Abstract #489
Cell-ECM interactions in the development of the aortic arch arteries
Sophie Astrof
Thomas Jefferson University, USA
Development of the aortic arch arteries (AAAs) can be roughly divided into two major stages: formation of the three symmetrical pairs of the pharyngeal arch arteries (PAAs) and remodeling of the PAAs into the AAAs. While PAA remodeling is more widely studied, physiological mechanisms of PAA formation are much less clear. Specifically, the origin of PAA endothelium and the cellular mechanisms regulating PAA formation in the mouse are unknown. We used temporal lineage mapping and quantitative 3D imaging to determine how PAAs form and the origin of the PAA endothelium. Our work indicates that PAAs form by vasculogenesis and that the PAA endothelium originates from the second heart field (SHF). Initially, SHF-derived cells assemble into a uniform $VEGFR2^{+}PECAM1^{negative}$ plexus, which then acquires Pecam1 positivity. Subsequently, $VEGFR2^{+}PECAM1^{positive}$ plexus endothelium becomes rearranged into the PAA by the coalescence of plexus endothelial cells in the middle of the arch. We found that PAAs grow in diameter by proliferation and by the acquisition of endothelial cells from the plexus. Our studies suggest that PAA formation is regulated at a number of distinct steps, including: differentiation, proliferation and survival of $VEGFR2^{+}$ PAA progenitors in the SHF; exit of these progenitors from the SHF and migration into the pharyngeal mesenchyme; formation of endothelial plexus and the coalescence of the pharyngeal endothelium to form the PAA. Thus, our work establishes a platform for elucidating physiological mechanisms regulating PAA formation and for analyses of mutants interfering with this process. We used this platform to investigate the role of the extracellular matrix glycoprotein fibronectin (Fn1) in PAA formation. We found that the expression of *Fn1* in the Isl1 lineage was required for PAA formation, and regulated both the accrual of endothelial cells in the pharyngeal arches at the early stages and the assembly of the endothelium into the PAA. Funding: NHLBI, AHA

Program Abstract #490
Effects of polycyclic aromatic hydrocarbons (PAHs) on pharyngeal system development
Gina Cho
Smith College, USA
Pharyngeal arches have been a prominent topic of study for many years, as they give rise to craniofacial and cardiac structures. Failure for the arches to correctly form leads to various teratological effects, such as Treacher Collins–Franceschetti syndrome and facial clefting. As arches give rise to many cartilaginous structures, previous studies focused mainly on the role neural crest cells, the precursors to cartilage and ligaments, in arch formation. But only recently have we come to fully understand the process of arch formation, most notably how pouch formation drives arch morphogenesis. The pharyngeal endoderm receives various cellular regulatory signals and via actin web-assisted migration creates pharyngeal pouches, which are required for proper arch formation and differentiation. Through extensive past research on the effects of crude oil components on zebrafish mutagenesis, this project found that the most prominent water-soluble component, naphthalene, created reproducible phenotypes: a loss in the most posterior pouch of the pharyngeal system and a lack of differentiation between the last two arches. The pharyngeal structures of naphthalene-treated embryos were also highly disorganized, and showed diffuse pouches that lacked proper slit-like morphology. The hour of treatment and the treatment solution's depth caused variability in the severity of the teratogenic effects. These variables were found correlated to the small window of pre-arch development treatment and naphthalene's high volatility. Staining for F-actin filaments revealed that naphthalene causes highly disorganized actin webs and disrupts pouch morphogenesis. These results are consistent with the proposed model for pharyngeal system development, and shows naphthalene disrupts the process at the level of actin-aided endoderm migration. Future directions include narrowing down what specific cellular signals naphthalene disrupts to hinder proper cell differentiation and proliferation.

Program Abstract #491
Kir 2.1 is required for craniofacial development
Megan Josey, Steven Rose, Adam Almeida, Giri Dahal, Sarala Pradhan, Emily Bates
University of Colorado, AMC, USA
Mutations that disrupt an inwardly rectifying K+ channel, Kir2.1, cause dominantly inherited Andersen-Tawil Syndrome (ATS). Symptoms of ATS include cardiac arrhythmia, periodic paralysis, cognitive deficits, and morphological abnormalities in craniofacial and limb development. Although the known role of Kir2.1 in muscle and neurons may explain the phenotypes in muscle and brain, it is less clear how loss of this ion channel could affect morphogenesis. We show that deletion of Kir2.1 in mouse causes cleft palate, reduction of the size of the jaw, loss of craniofacial bone, and digit defects. Deletion of Kir2.1 drastically reduces proliferation of the cells in the palate shelf, but does not affect cell survival. Conditional deletion of Kir2.1 reveals that Kir2.1 is required in the cranial neural crest cells that make up the mesenchyme of the palate shelves, and not in the overlying ectoderm for palate closure. Further studies will determine the mechanism by which Kir2.1 impacts canonical developmental signaling pathways.

Program Abstract #492
Expression of a set of cranial neural crest regulatory genes in developing mouse teeth
Emily Woodruff, Alyssa Mangino, Jonathan Bloch, Martin Cohn
University of Florida, USA
During mammalian embryonic development, cranial neural crest cells are specified in the dorsal neural tube by a set of regulatory genes that distinguish these cells from adjacent non-migratory cells of the neural tube and the non-neural ectoderm. Cells derived from the cranial neural crest contribute to the mesenchymal tissue in the branchial arches that form the face, including the dental mesenchyme in developing teeth. Tooth development has been studied extensively in mice (*Mus musculus*) and the expression patterns of many genes necessary for proper tooth development are well documented. However, similarities in the genetic regulation of cranial neural crest development at early stages (specification and/or early migration) and during tooth development have not been explored in depth, despite the fact that dental mesenchyme is derived predominantly from neural crest. In order to test the hypothesis that a gene regulatory network that is initiated in the early cranial neural crest is later re-activated during dental development we investigated the spatial expression patterns of a set cranial neural crest regulatory genes in developing mouse teeth. Spatial expression patterns of these genes were examined at three key stages of dental development: bud (E12.5-13.5), cap (E14.5), and bell (E16.5-17.5) stages. We find that a subset of these genes are expressed in both the cranial neural crest cells and the dental mesenchyme, but others are restricted to the dental epithelium. Additional *in situ* assays to detect expression of other genes associated with the cranial neural crest will demonstrate whether the expression of this entire set of regulatory genes is held in common between cranial neural crest cells and their derivatives in the dental mesenchyme, or alternatively, if only part of this gene network is re-activated during tooth development.
This work was funded by an NSF DDIG to EDW.

Program Abstract #493
Shh and EGF systems cooperatively regulate branching morphogenesis of fetal mouse submandibular glands
Masanori Kashimata, Noriko Koyama, Toru Hayashi, Kenji Mizukoshi
Asahi Univ Sch of Dentistry, JP
The hedgehog family includes Sonic hedgehog (Shh), Desert hedgehog, and Indian hedgehog, which are well known as a morphogens that play many important roles during development of numerous organs such as the tongue, pancreas, kidney, cartilage, teeth and salivary glands. In *Shh* null mice, abnormal development of the salivary gland is seen after embryonic day 14 (E14). Shh also induced lobule formation and lumen formation in acini-like structures in cultured E14 SMG. In this study, we investigated the relationship between Shh and epidermal growth factor (EGF)/ErbB signaling in developing fetal mouse subamndibular gland (SMG). Administration of Shh to cultured E13 SMG stimulated branching morphogenesis and induced synthesis of mRNAs for EGF ligands and receptors of the ErbB family. Shh also stimulated activation of ErbB signaling system such as ERK1/2. AG1478, a specific inhibitor of ErbB receptors, completely suppressed BrM and activation of EGF/ErbB/ERK1/2 cascade in E13 SMGs cultured with Shh. The expressions of mRNA for *Egf* in mesenchyme and mRNA for *Erbb1, Erbb2* and *Erbb3* in epithelium of E13 SMG were specifically induced by administration of Shh. These results show that Shh stimulates branching morphogenesis of fetal mouse SMG, at least in part, through activation of the EGF/ErbB/ERK1/2 signaling system.

Program Abstract #494

Calcium and non-muscle myosin II mediate cell shape changes required for brain morphogenesis.

Jennifer Gutzman, Srishti Sahu, Mike Visetsouk, Ryan Garde, Leah Hennes, Constance Kwas
University of Wisconsin-Milwaukee, USA

One of the earliest, and most highly conserved vertebrate brain structures that forms during development is the tissue fold at the midbrain-hindbrain boundary (MHB). Specific cell shape changes occur at the point of deepest constriction of the MHB, the MHB constriction (MHBC), and are critical for proper formation of this structure. We are using zebrafish to study the molecular mechanisms that regulate the initial cell shape changes that form the MHBC: cell shortening and cell narrowing. These cell shape changes are mediated by mechanical forces generated within individual cells that are integrated to effect whole tissue shape. The generation of force within a cell often depends on motor proteins, particularly non-muscle myosins. The contractile state of the neuroepithelium is tightly regulated by non-muscle myosin II (NMII) activity; therefore, we tested the role of NMIIA (*myh9a* and *myh9b*) and NMIIB (*myh10*) in regulating cell shape changes that occur during initial MHB morphogenesis. These studies revealed that NMIIA and NMIIB have a differential role in regulating MHB formation. NMIIA is required for regulation of cell length, while NMIIB is required for regulation of cell width. However, the upstream signaling pathways that initiate differential regulation of cell shape during MHB morphogenesis are not known. Our current studies reveal that calcium signaling is critical for mediating MHBC cell shape. In complementary rescue experiments that couple modulation of intracellular calcium levels with manipulation of NMII function, we demonstrate that calcium signals to NMII to regulate cell length at the MHBC. We show specifically that calcium mediates phosphorylation of myosin light chain in the MHB tissue and that *calmodulin 1a* and MLCK are required regulators of MHBC cell length. Together these data indicate that calcium signals via NMII to mediate cell shape changes required for MHB morphogenesis.

Program Abstract #495

The zebrafish motility mutant frozen reveals a role for the unconventional myosin MYOXVIIIB in skeletal muscle morphogenesis

Philip Ingham[1,2,3], Ritika Gurung[1], Samantha Lee[1], Sarah Baxendale[3], Yosuke Ono[2], Stephen Moore[3]
[1]Institute of Molecular and Cell Biology, Singapore; [2]Lee Kong Chian School of Medicine, Singapore; [3]University of Sheffield, United Kingdom

Myosins are commonly classified into two types - conventional and unconventional - based on the sequences of their motor domains and are further subdivided into six families (I, V, VI, VII, IX and XVIII) according to their functional domains. The unconventional myosin MYOXVIIIB is encoded by a gene identified through its deletion in a human lung cancer cell line as well as its expression in human adult skeletal muscle. Consistent with the latter characteristic, two recent studies have associated mutations of *MYO18B* with Nemaline myopathy in human. In mouse, mutation of *Myo18B* results in early developmental arrest associated with cardiomyopathy precluding analysis of its effects on skeletal muscle development. Amongst a group of immotile mutants isolated in the 1996 Tübingen screen for zebrafish embryonic lethal mutations, *frozen* (*fro*) mutant embryos are characterized by a loss of birefringency in their skeletal muscle, indicative of disrupted sarcomeric organization. We have mapped the *fro* locus to the previously un-annotated zebrafish *myo18b* gene, the predicted protein product of which shares over 50% identity with human MYOXVIIIB. Expression of *myo18b* initiates in the developing myotome prior to myofibril assembly in the zebrafish embryo and is restricted to cells of the fast-twitch muscle lineage. We show that sarcomeric assembly is blocked specifically in nascent fast-twitch myofibres at an early stage in *fro* mutants, leading to the disorganised accumulation of actin, myosin and alpha-actinin and a complete loss of myofibrillar organisation. *fro* embryos also show defects in cardiomyocyte differentiation as previously observed in the mouse *myo18B* mutant, indicating a conservation of MYOXVIIIB function across species.

Program Abstract #496

Blood flow and vascular remodeling: analyses of individual endothelial cell behaviors by in vivo live-imaging and mathematical approach

Yuta Takase[1], Kenichi Nakazato[2], Atsushi Mochizuki[2], Yoshiko Takahashi[1]
[1]Department of Zoology, Graduate School of Science, Kyoto University, JP; [2]Theoretical Biology Laboratory, RIKEN, JP

Vascular structures undergo dynamic remodeling, which is prominently influenced by the blood flow (hemodynamics). To understand how the vascular remodeling is regulated in living embryos at the cellular and molecular levels, we use the yolk sac vasculature in chicken embryos as an experimental model, where vascular remodeling proceeds in a two-dimensional plane. We previously reported that the yolk sac vascular remodeling indeed depends on the blood flow revealed by a local experimental blockage of the flow. To understand how endothelial cells react to the flow, we have

performed *in vivo* time-lapse imaging analyses combined with local gene-transfer into remodeling blood vessels. In a fast-flow region cells move against the flow, whereas cells in a slow-flow region do not move, and become round and eventually detached from the vessel. These peculiar behaviors of endothelial cells are flow-dependent because when the flow is locally blocked, the cells not only stop the movement against the flow, but also become round and eventually detached from the blood vessel. We also present molecular regulation of such cellular behaviors, where mechanosensitive ion channels and RhoA play important roles. Furthermore, we assume that the flow-dependent changes in cell behaviors would mediate the macroscopic rearrangements of blood vessels during vascular remodeling. We are currently addressing this issue by mathematical simulation.

Program Abstract #497

Toddler affects Cxcr4a-dependent endoderm migration by regulating mesoderm patterning

Megan Norris[1], James Gagnon[1], Andrea Pauli[2], Alex Schier[1]

[1]Harvard University, USA; [2]Research Institute of Molecular Pathology, Austria

Internalization and patterning of endoderm and mesoderm (mesendoderm) during early gastrulation is highly conserved but the pathways that regulate these processes remain poorly understood. Toddler/Apela is a secreted peptide that signals via the Apelin receptor (AplnR) and loss of Toddler signaling leads to fewer mesendodermal cells, which internalize and migrate more slowly during gastrulation. The role of Toddler, namely the tissue in which it signals and its direct cell biological function, remain an open question. In this study, we asked whether the primary defect in *toddler* mutants is the reduced specification of endoderm or the reduced migration of mesendoderm. To test the first model, we increased the amount of endoderm in *toddler* mutants by knocking out Lefty2, a known repressor of mesendoderm. Though endodermal cell number was indeed increased in *toddler/lefty2* double mutant embryos, *toddler*-like patterning defects remained, suggesting lack of endodermal cells is not sufficient to explain the *toddler* mutant phenotype. To test the cell migration model, we knocked out the endodermal migration regulator Cxcr4a. In *toddler/cxcr4a* double mutants patterning of endodermal cells resembled wildtype supporting the hypothesis that the endoderm defect seen in *toddler* mutants is due to aberrant cell migration. However, neither loss of Lefty2 nor Cxcr4a rescued the patterning defects in *toddler* mutant mesoderm, which we show to be the predominant location of AplnR expression. Taken together, these results support the hypothesis that Toddler signaling regulates mesodermal cell internalization and migration and that Toddler's effect on endodermal migration is indirect via Cxcr4a-dependent endodermal tethering to mesoderm.

Funding: NICHD

Program Abstract #498

Rho GTPase function during gastrulation in the cnidarian, *Nematostella vectensis*

Craig Magie[1], Spencer Hess[1], Colby Ledoux[1], Setareh Khalili[2], Alexa McWhinnie[1], Jack Wheeler[1]

[1]Quinnipiac University, USA; [2]California State University, Fresno, USA

The starlet sea anemone *Nematostella vectensis* has become an important model organism in recent years. Because cnidarians are the sister group to the bilaterians, bilaterally-symmetric animals including all our standard model organisms, study of *Nematostella* development promises to provide unique insight into the evolution of developmental mechanisms. In particular, we are interested in the evolution of cell-biological mechanisms underlying the cell movements and shape changes that occur during gastrulation. Gastrulation in *Nematostella* occurs via endodermal invagination, though the molecular details underlying this process remain to be elucidated. One group of genes that is a likely candidate for regulating cell behaviors during gastrulation is the Rho family of small GTPases, important regulators of various cellular processes including actin cytoskeletal rearrangement, transcriptional activation, and cell adhesion. Phylogenetic analyses have identified *Nematostella* orthologs of Rho, Rac, and Cdc42, and in situ hybridization analysis has revealed that all are expressed ubiquitously during the gastrula stage. By the larval stage these genes appear to be more highly expressed in the endoderm. We are currently utilizing a variety of approaches to perturb function of Rho GTPases and their downstream effectors. Treatment of embryos with a pharmacological inhibitor of Rho-kinase (ROCK), a downstream target of Rho, affects cell morphology and the ability of treated embryos to complete gastrulation. Additionally, microinjection of morpholinos targeting Rac or mRNA constructs encoding dominant-negative Rac have shown similar defects in cell morphology, and pharmacological inhibition of Rac results in a dose-dependent failure to complete cleavage divisions, possibly indicating a role in cell division. Our data suggest that the molecular mechanisms underlying Rho GTPase function in *Nematostella* may be distinct from those in bilaterian taxa.

Program Abstract #499

Changing yolk cell microtubules dynamics during zebrafish gastrulation

Ashley Bruce, Zhonghui Fei, Koeun Bae

Univ of Toronto, CA

The first morphogenetic movement during zebrafish development is epiboly. Epiboly describes the vegetal movement of the blastoderm and the underlying yolk syncytial layer to cover the yolk cell. A longitudinal microtubule array, associated with the yolk syncytial layer nuclei, extends from the yolk syncytial layer towards the vegetal pole and shortening of this array is proposed to provide a vegetally directed pulling force during epiboly. Despite evidence implicating yolk microtubules in epiboly, their exact function remains unclear. To investigate yolk cell microtubule function, we examined the movements of the microtubule plus end associated protein EB3 fused to GFP (EB3-GFP), which binds to actively polymerizing microtubules. The current model is that the microtubule array is established before epiboly and shortens during epiboly, predicting little polymerization during early epiboly. In contrast, EB3-GFP tracking revealed widespread polymerization in the yolk syncytial layer and extensive vegetally directed microtubule growth in the yolk from high stage to 60% epiboly. Strikingly, after 60% epiboly, EB3-GFP was not detected in the yolk, suggesting that the yolk microtubules are stabilized at 60% epiboly. To investigate this possibility, antibody staining for markers of dynamic and stabilized microtubules as well as FRAP analysis of GFP-labeled microtubules was done. Our data are consistent with the hypothesis that yolk cell microtubules are more dynamic at early epiboly stages than at late epiboly stages, suggesting that they perform distinct functions at these stages. Previous work proposed that the yolk cell microtubules pull the yolk syncytial layer nuclei towards the vegetal pole starting at 60% epiboly and we are currently investigating the possibility that the yolk cell microtubules must be stabilized in order to perform this function. Funding from NSERC.

Program Abstract #500

p120-Catenin couples cadherin switching to Epithelial to Mesenchymal Transition (EMT) during mouse gastrulation.

Rocío Hernández-Martínez[1,2]

[1]*Sloan-Kettering Institute, USA;* [2]*Memorial Sloan-Kettering Cancer Center, USA*

p120-catenin regulates cell-cell adhesion through interaction with cytoplasmic tails of cadherins. Experiments show that *p120-catenin* promotes cadherin stability on the cell surface, apparently by inhibiting its endocytosis. It was known that deletion of *p120-catenin* causes embryonic lethality in mice, but the basis of that lethality was unclear. We generated mouse embryos lacking *p120-catenin* in all cells, or specifically in the epiblast. We observed that about half of mutant embryos had a duplication of the posterior body axis, shown by the ectopic expression of primitive streak markers *Wnt3* and *T*. The remaining mutants developed a cellular bulge at the streak due to defects in mesoderm migration. In addition to these morphogenetic defects, mesoderm production is greatly impaired, leading to a paraxial mesoderm deficit and a lack of a complete set of somites. Accompanying the defects in axis specification, *p120-catenin* mutant embryos exhibit apoptosis at the streak. During normal gastrulation Wnt signaling stimulates an EMT and E-cadherin is down-regulated at the streak. In *p120-catenin* mutant embryos, cells at the primitive streak down-regulate E-cadherin, but some of those cells remain in the epiblast and aberrantly express N-cadherin, indicating that p120-catenin couples cadherin switching to EMT. Moreover, there is an early expansion of the Wnt-signaling domain at the streak in *p120-catenin* mutant embryos, accompanied by strong nuclear localization of β-catenin, suggesting that junctional β-catenin amplifies Wnt signaling at the site of EMT in the absence of *p120-catenin*.

This research was supported by the Pew Latin American Fellows Program in the Biomedical Sciences, USA., and CONACYT-México.

Program Abstract #501

The Role of the small GTPase rab25a during zebrafish epiboly.

Patrick Willoughby, Molly Allen

University of Toronto, CA

Epiboly, the thinning and spreading of a multilayered sheet of cells is the first morphogenetic movement during zebrafish gastrulation. The three layers of the early gastrula, the epithelial enveloping layer (EVL), deep cells and yolk-syncytial layer all undergo epiboly to close the blastopore at the vegetal pole of the embryo. Despite being required for development, the molecular mechanisms controlling epiboly have yet to be fully elucidated. rab25a is a small GTPase associated with the apical recycling endosome in epithelial cells and it was identified by our lab to be up-regulated during epiboly through RNA-Sequencing. To investigate whether rab25a is critical for the normal progression of epiboly, a translation blocking morpholino was used to knockdown rab25a in the early embryo. rab25a morphant embryos exhibited

an epiboly delay in all three layers when compared to wild-type embryos. To our surprise, despite rab25a being expressed in an EVL specific manner, the underlying deep cells displayed the largest epiboly delay. To verify that the delay was the result of rab25a functioning explicitly in the EVL, we restored rab25a to the EVL in rab25a morpholino injected embryos using a transgenic approach. This EVL–specific rescue was able to partially rescue the epiboly delay in all three layers of the embryo. This leads us to believe that rab25a could be functioning in the EVL to indirectly regulate deep cell motility during epiboly. To provide evidence for this model and to further characterize rab25a during epiboly, a mutant was generated using the CRISPR/Cas9 gene-editing system. Preliminary data suggest that the rab25a mutant phenotype matches the morphant phenotype by exhibiting a similar epiboly delay which occurs primarily in the deep cells. Funding from National Science and Engineering Research Council of Canada (NSERC).

Program Abstract #502

Aquaporin3b plays a role in tissue architecture and convergent extension during *Xenopus* gastrulation

Jennifer Forecki, Kaitlyn See, Christa Merzdorf
Montana State University, Bozeman, MT, USA

Aquaporins and aquaglyceroporins are members of a family of membrane channel proteins that allow the movement of water and other small molecules like glycerol across plasma membranes. Previous work has identified essential roles of aquaporins in the renal system and during wound closure. Our results indicate that aquaporins play more complex roles within tissues in addition to allowing water movement. We have identified an expression pattern for *aqp3b* in the sensorial layer of the *Xenopus laevis* animal cap and dorsal margin, which indicated a possible role in gastrulation. Using an *aqp3b* morpholino to disrupt expression of *aqp3b* in the dorsal margin, we found that Aqp3b participates in the maintenance of tissue architecture and in convergent extension (CE). Specifically, the loss of *aqp3b* expression resulted in the loss of fibril fibronectin, and subsequently these cells failed to respect tissue borders during mesoderm involution. Interestingly, there was no loss in the fibronectin matrix around individual cells, indicating a role for aquaporins in only the mechanism of fibril assembly, not in fibronectin synthesis and secretion. Further, we have identified a role for aqp3b in noncanonical Wnt signaling during convergent extension. Aqp3b is expressed in the dorsal margin of *Xenopus* embryos, and dorsal margin explants (Keller explants) undergo CE. When aqp3b is inhibited in Keller explants, both convergence and extension were impaired. Co-injected dvlDeltaDIX rescued these CE defects, demonstrating that Aqp3b acts through noncanonical Wnt signaling. Further rescue experiments showed that Aqp3b exerts its influence specifically through the Wnt/Ca2+ pathway, rather than through Wnt/PCP or Wnt/Ror2 noncanonical Wnt signaling. We are in the process of determining by which mechanism Aqp3b is able to interact specifically with and/or regulate Wnt/Ca2+ signaling and whether there is a relationship to fibril fibronectin formation. Funded by NSF.

Program Abstract #503

Exploring the mechanobiology of Brachet's cleft at the level of individual cell pairs

Serge Parent, Ashley Bruce, Rudolf Winklbauer
University of Toronto, CA

Tissue separation and boundary formation are critical for animal development and tissue maintenance. One example of this is Brachet's cleft in *Xenopus laevis* and zebrafish gastrulae, which is composed of ectoderm on one side and mesoderm on the other. It has long been known that when tissues are dissociated, mixed, and reaggregated they separate out from one another in a process known as cell sorting. Despite being a topic of interest for many years, the physical and cellular basis for the separation of tissues is unclear. The well-known differential adhesion hypothesis posits that cell sorting occurs due to differential energies of adhesion, with less adhesive cells forming concentric rings around more adhesive ones. This, however, does not result in the formation of a clear-cut boundary and instead results in the general sorting of cells. Interestingly, as we have reported in a recent publication, this separation behaviour that underlies Brachet's cleft can even be observed between individual cells during cell reaggregations forming an interaction we termed "cleft adhesions". The implications of this are that we can observe the establishment of boundaries and separation behaviour in addition to suggesting that separation behaviour is an intrinsic property of these cells. Furthermore, these cleft adhesions were observed to be very dynamic, with the ectoderm and mesoderm moving laterally relative to one another. To investigate the physical basis of cleft adhesion formation and therefore tissue separation I developed an image analysis program using Python to quantify the adhesiveness of cells based on contact angle as well as contact surface. Preliminary results appear consistent with the prediction that cleft adhesions are less adhesive than are homotypic adhesions. On-going experiments continue to characterize the kinetics and cellular basis of cleft adhesion formation. This work is supported by grants from NSERC and CIHR.

Program Abstract #504

Genetic and functional characterization of planarian head and tail patterning

Dayan Li[1,2], Peter Reddien[1]

[1]MIT, USA; [2]Harvard Medical School, USA

For proper regeneration to occur, newly generated cells must adopt the correct identities and positions to replace exactly what is missing. This process, referred to as patterning, is likely orchestrated by precisely timed and coordinated instructive cues. How cell-cell signaling consistently and faithfully organizes new tissue into a replica of the old remains incompletely understood. Given their remarkable regenerative ability and simple body plan, planarians serve as ideal organisms to study patterning. Here we examine patterning along the anterior-posterior (AP) and medial-lateral (ML) axes of the planarian *Schmidtea mediterranea.* Our study is informed by clusters of cells at the tip of the head and tail, respectively termed anterior and posterior poles, that are defined by the expression of Wnt signaling genes (e.g. *notum* in the anterior pole and *wnt1* in the posterior pole) and genes encoding transcription factors (e.g. *foxD* in the anterior pole and *pitx* in the posterior pole). To understand the likely roles of the poles as signaling centers that direct head and tail patterning, we collected anterior and posterior pole transcriptomes via bulk and single-cell RNA sequencing. This allowed us to uncover new genes, some pole-specific and others more broadly expressed in the head and tail, that have enriched pole expression. Among these include a transcription factor gene important in AP patterning, and a receptor tyrosine kinase-like gene involved in ML patterning. RNAi of these genes results in ectopic head structures and neural cells characteristic of aberrant patterning. In addition to further highlighting the importance of regionalized expression of genes in maintaining proper body axes, detailed histological characterization of these AP and ML phenotypes has provided new insights into the molecular logic of patterning. This study was funded by the HHMI, the Paul and Daisy Soros Fellowship for New Americans, and the NIH MSTP grant

Program Abstract #505

Physiological Networks Stochastically Regulate Anterior-Posterior Polarity in Regenerating Planaria: Permanent Reprogramming of Regenerative Anatomy

Fallon Durant[1], Junji Morokuma[1], Christopher Fields[2], Katherine Williams[1], Michael Levin[1]

[1]Tufts University, USA; [2]Sonoma, CA, USA

It is commonly assumed that an animal's regenerative pattern is uniquely specified by its genome. We present an assay in regenerating planaria showing that their normal anterior-posterior structure can be stably re-written by a brief perturbation of physiological signaling. Using the transient gap junction blocker, octanol, we created a stochastic change of the target morphology of planaria. This temporary inhibition of gap junction communication results in a proportion of treated planaria with axial duplication and another proportion of planaria with normal morphology that we originally attributed to incomplete penetrance. However, upon a second amputation of these apparently normal planaria in water, we observed a stochastic reemergence of axial duplication, suggesting the current morphology of these planaria was not indicative of the target morphology (the shape to which the planaria would regenerate upon damage and cause regeneration to cease). Remarkably, the same stochastic distribution of double head outcomes continues to arise in subsequent amputations in water, with no further perturbation. To investigate what could be permanently storing the aberrant target morphology in a planarian that is genetically and, morphologically, phenotypically wild-type, we tested several candidate molecular mechanisms that could control axial polarity and control body-wide morphological outcomes. We implicated membrane voltage patterns as primary drivers of target morphology storage, highlighting the importance of physiological networks for information storage in regeneration. These data have significant implications for understanding biological variability, control of patterning outcomes, and the interplay of genetics and physiology in specifying large-scale anatomy.

Program Abstract #506

Quantitative analysis of Left-Right Organizer Morphogenesis

Agnik Dasgupta, Andrew E. Jacob, Jeffrey D. Amack

SUNY Upstate Medical University, United States of America

Left-right (LR) patterning of the vertebrate embryo is critical for proper development of the asymmetric cardiovascular and gastrointestinal systems. In several vertebrates, LR asymmetry is generated by a transient organ containing motile cilia that we refer to as the left-right organizer (LRO). Mechanisms that guide LRO development remain poorly understood. Using zebrafish as a model, we have identified distinct steps of LRO formation. The zebrafish LRO, called Kupffer's vesicle (KV), is formed from a group of precursors known as dorsal forerunner cells (DFCs). During gastrulation, DFCs proliferate and migrate toward the vegetal pole of the embryo to form a tight cluster. Within this cluster, DFCs form multiple foci enriched in apical membrane and junction proteins at cell-cell interfaces. DFCs then

undergo rearrangements that bring these foci into close proximity with one another to form a rosette-like structure that gives rise to the KV lumen where cilia elongate. The cellular behaviors of DFCs during these distinct stages of LRO development remain unclear. To address this, we developed a mosaic-labeling and time-lapse imaging approach to track individual DFC/KV cells. We have quantified multiple aspects of cell behavior and morphology during LRO formation. To test the significance of these behaviors we used reverse genetic approaches to interfere with Junction plakoglobin (Jup), a multifunctional armadillo-related molecule associated with cadherin complexes that is enriched in the DFC/KV lineage. Jup is predominantly expressed at DFC/KV cell-cell junctions and perturbing Jup function disrupted KV formation. Quantitative cell morphometrics revealed that Jup is involved in regulating morphology of KV cells during development. Taken together, these results quantify novel cellular dynamics during LRO morphogenesis and provide new candidate pathways/mechanisms that regulate LRO development and LR patterning of the embryo.

Program Abstract #507

Establishment of the Ventral Embryonic Midline is a Dynamic Process that Requires Bilaterally Symmetric BMP and Hedgehog Signaling

Alaa Arraf, Ronit Yelin, Inbar Reshef, Thomas Schultheiss
Technion Israel Institute of Technology, IL

The vertebrate body plan is bilaterally organized around a central axis located in the body midline. During embryogenesis, the first midline structure to emerge is the notochord, which is located in the dorsal midline and which secretes multiple factors, including the BMP antagonists Noggin, Chordin, and Follistatin, as well as Sonic hedgehog (Shh). Subsequently other midline structure form in more ventral regions, including the descending aorta, primitive linear heart tube, dorsal and ventral mesenteries that connect the gut tube and its derivatives to the body wall, sternum, umbilicus, urinary bladder, uterus, and external genitalia. The mechanisms that ensure the alignment of these ventral midline structures with the dorsal midline are not well understood. The current study uses the dorsal mesentery (DM) as a model for investigating the positioning of ventral midline structures. We document formation of the DM by way of epithelial-to-mesenchymal transition (EMT) and medial ingression of the coelomic epithelium (CE) of the lateral plate mesoderm, in which newly generated mesenchyme cells from the two sides of the CE migrate medially between the aorta and the endoderm until they meet in the ventral midline, where they generate the DM and other ventral body structures. Inhibition of BMP signaling in the CE on one side of the embryo caused a deflection of the DM towards the treated side, attributable at least in part to reduction in the rate of EMT of the CE on that side. Because BMP antagonists are expressed in the dorsal midline, this suggests a mechanism in which factors secreted from the dorsal midline regulate the rate of medial ingression of CE-derived cells, thereby ensuring alignment of the dorsal and ventral embryonic midlines.
Funding: Israel Science Foundation, Niedersachsen-Israel Research Fund, Rappaport Institute

Program Abstract #508

A novel large-scale protein localization screen in multiciliated cell identified new regulators of ciliogenesis

Fan Tu, Jakub Sedzinski, Edward M Marcotte, John B Wallingford
The University of Texas at Austin, USA

Multiciliated cells (MCCs) are essential for normal functioning in vertebrate tissues such as the airway, brain, and reproductive tracts. Previously we showed that the conserved transcription factor *RFX2* was required for ciliogenesis and cilia function, and we identified 911 direct target genes of *RFX2*. As expected, this dataset contains many genes (~100) known to be involved in cilia-related processes, such as IFT components, axonemal dyneins, and centriolar proteins. However, the functions of the vast majority of *RFX2* targets remain unknown. To address this issue, we carried out a large-scale screen for targets with distinct subcellular localization in MCCs. Taking the advantage of human ORFeome collection and the Gateway cloning system, we systematically made over 300 fluorescently tagged clones driven by an MCC specific promoter. The screen identified dozens of novel proteins localized to basal bodies, axonemes, the cytoskeleton, and other cellular structures. Guided by this localization data, functional tests identified novel players in basal body docking and axoneme assembly.

Program Abstract #509

Studies on the effects of bisphenol A and bisphenol S, individually and in combination, on the development of *Xenopus laevis* (African clawed frog) larvae

Lauren Chukrallah, Lisa E. Thottumari, Nicholas F. Antonucci, Frances S. Raleigh, Ph.D., Laura H. Twersky, Ph.D.
Saint Peter's University, USA

Bisphenol A (BPA) is an industrial chemical used in the production of polycarbonate plastics and epoxy resins, which are

used in food and drink packaging, including metal cans. We are comparing the effects of bisphenol A, a known environmental endocrine disruptor (EED), and bisphenol S (BPS), one of its replacements, individually and in combination, on larval development of *Xenopus laevis* (African clawed frog). BPA is known to stimulate cellular responses by binding to estrogen receptors as well as being a thyroid hormone antagonist. BPA and BPS have been found in urine samples; BPS's effects are not yet understood. Larval development of *X. laevis* has been used as a model system to determine effects of EEDs due to dependence on hormone regulation, transparency of the tadpoles, and relatively rapid rate of development. Eight groups of *X. laevis* tailbuds, starting at Nieuwkoop and Faber stages 26-27 were compared: four groups were incubated in solutions with two concentrations each of BPA and BPS (5µg/mL and 10µg/mL), three groups with 3µg/mL BPA:6µg/mL BPS, 6µg/mL BPA:3µg/mL BPS, and 5µg/mL BPA:5µg/mL BPS, and a control. Survivorship and rate of development were measured, and morphological changes were observed. Low doses resulted in higher incidence of malformations; tadpoles exposed to 5µg/mL BPA:5µg/mL BPS, 3µg/mL BPA:6µg/mL BPS, and 5µg/mL BPA exhibited head malformations, and the BPS groups exhibited a marked decrease in rate of development. The 10µg/mL BPS group was at stage 52 in development while the controls were at stage 63, and none of the 5µg/mL BPS group were alive. The only surviving member at 6µg/mL BPA:3µg/mL BPS from trial 1 halted development at stage 58 with no visible forelimb formation. Simultaneously, controls continued to develop normally to stage 66. Highest rates of mortality were observed in 10µg/mL BPA, 5µg/mL BPA, and the 5µg/mL BPA:5µg/mL BPS groups. Results suggest BPS might have harmful effects on human health.

Program Abstract #510

Cdx4 transcription factor controls the 'determination front' during somitogenesis, linking segmentation and patterning processes

Saptaparni Bandyopadhyay[1], Vanessa Fleites[2], Mejdi Najjar[3], Isaac Skromne[1]
[1]University of Miami, USA; [2]University of Pennsylvania, USA; [3]University of Central Florida, USA

The vertebrate paraxial mesoderm is segmented into somites, each bestowed with its own unique identity. While somite number and identity can vary significantly between species, little if no variation is seen within species. How is somite number and identity matched? We addressed this question in zebrafish deficient for Cdx4, a transcriptional regulator of *hox* identity genes, whose inactivation results in shortening of the primary axis. Here we show that axial shortening in Cdx4-deficient embryos is not due to the loss of segments, but instead is due to a reduction in segment size. We ruled out that segment size reduction was due to defects in cell proliferation or apoptosis, and instead show to be caused by defects in segmentation. In particular, we demonstrate that Cdx4 does not affect the rate of somitogenesis (somite clock), but instead, the size of the region in which the tissue is competent to segment (determination front). Epistatic analysis between Cdx4 and determination front components FGF, Wnt and Retinoic Acid further supports this model. Thus, by regulating the determination front that controls somite size and the expression of *hox* identity genes, Cdx4 coordinates paraxial mesoderm segmentation and patterning.

Program Abstract #511

Somite scaling - wave vs gradient -

Kana Ishimatsu, Tom Hiscock, Zach Collins, Sean Megason
Harvard Medical School, USA

Somite formation shows temporal periodicity, which is controlled by cellular oscillators. Although a lot is known about the molecular basis of the somite clock, how the temporal periodicity is converted into the spatial periodicity is largely unknown. In this study, by focusing on somite scaling, we found the most feasible model for somite spacing. In order to study scaling in zebrafish, we developed a size reduction technique that allows us to make smaller embryos without any genetic modification. Combining this technique with high-resolution live imaging, we quantified the size of somites and precursor tissue of somites (PSM) over long periods of time. As a result, we found somite size scales with PSM size both among individuals and over time. We next searched for a model that accounts for the somite scaling. We tested the following three models: 1. The clock and wavefront model is the model which is the most widely accepted in the somite field, in which the repetitive somite formation is explained by the interaction between an oscillator and moving positional information in the PSM. Importantly, the speed of the positional information movement is believed to be controlled by tail elongation. 2. The wavelength model explains somite size determination by the spatial pattern of the oscillator (i. e. the phase gradient). The cellular oscillator shows wave-like expression pattern in PSM and this model assumes that the wavelength of this wave pattern determines the size of the somites. 3. Clock and scaling morphogen model is the refined version of clock and wavefront model, where the driving force of the positional information is not only the tail elongation, but also the scaling dynamics of a gradient in PSM. By quantitatively measuring multiple parameters in vivo, we found

that the clock and scaling morphogen model is the only model that can account for somite scaling and thus propose this model as the most feasible model for somite size control.

Program Abstract #512
A 3-D Model for Skeletal Patterning in Sea Urchin Embryos
Cynthia Bradham, Michael Piacentino, Daniel Zuch
Boston University, USA
Skeletal patterning in sea urchins is a simple model for morphogenesis, in which the ectoderm directs the migration of the skeleton-producing mesoderm cells, the PMCs. We have discovered a new set of skeletal patterning genes that are expressed in the ectoderm via an RNA-seq-based screen, including SLC26a2, BMP5-8, and Univin/Alk4/5/7. Individually, these signals mediate ventral, left, and anterior patterning, respectively. Together, these data support a new model for skeletal patterning along the DV, LR, and AP axes of the embryo, and thus provide significant new insights into the mechanisms underlying morphogenesis of the sea urchin skeleton.

Program Abstract #513
Wnt signaling regulates progenitor cell identity and collective cell migration during organogenesis
Hillary McGraw[1], Austin Forbes[1], Yuanyuan Xie[2], Richard Dorsky[2], Alex Nechiporuk[1]
[1]1Department of Cell, Developmental and Cancer Biology. Oregon Health & Science University, Portland, OR 97239, USA; [2]2Department of Neurobiology & Anatomy, University of Utah, Salt Lake City, UT 84112, USA
Collective cell migration, which is movement of cells as a cohesive group, is a critical process during embryonic organ formation, wound healing and is inappropriately coopted during the invasion of certain cancers. Although many of the cellular hallmarks of collective cell migration have been defined, the genetic pathways that regulate these processes are not well understood. Development of the zebrafish lateral line has proven to be an elegant model for studying collective cell migration, as it is amenable to live imaging and genetic manipulation. The posterior lateral line (pLL) forms from the posterior lateral line primordium (pLLP), a cohort of ~100 cells which collectively migrate along the trunk of the developing zebrafish embryo. The pLLP is comprised of proliferative progenitor cells and organized epithelial cells that will form the hair cell-containing mechanosensory organs of the pLL. Wnt signaling is active in the leading progenitor zone of the pLLP and regulates cellular proliferation, survival and maintenance. Here we examine the downstream targets of Wnt signaling and their role in mediating pLLP progenitor cell behavior. We used RNA-sequencing to identify genes that are altered in zebrafish *lef1* mutant pLLPs as compared to wild-type controls. One of the genes we selected for further analysis is the tumor suppressor Fat1b, which is strongly expressed in the wild-type pLLP and is downregulated following loss of Wnt signaling. CRISPR-Cas9-mediated mutation of Fat1b function results in failed pLLP migration in a manner that is similar to previously described canonical Wnt signaling mutants. These data suggest that our approach will allow us to refine our understanding of how canonical Wnt signaling pathway regulates various cellular behaviors.

Program Abstract #514
Plus-end tracking proteins of the TACC family regulate microtubule dynamics during embryonic development
Erin Rutherford, Leslie Carandang, Patrick Ebbert, Alexandra Mills, Jackson Bowers, Laura Anne Lowery
Boston College, USA
Early embryonic morphogenesis relies on the successful orchestration of several dynamic processes dependent on cell motility. The early nervous system in particular undergoes numerous developmental changes involving the coordinated movement and guidance of cells, notably including early neurite outgrowth and neural crest cell migration. In addition to the well-established roles of the actin cytoskeleton, important roles for microtubules (MTs) in these motile processes have recently received more attention. Plus-end tracking proteins (+TIPs), a conserved family of proteins which localize to the growing ends of polymerizing MTs, are known to regulate microtubule dynamics and functionality in a variety of ways and thus are significant players in the dynamic process of embryogenesis. We have shown that transforming acidic coiled coil (TACC) proteins 1 and 3 are +TIPs which localize to the distal-most plus ends of MTs, in front of EB1, where they influence MT dynamics in multiple *Xenopus* embryonic cell types. Here, we demonstrate the plus-end localization of TACC2, the final and least-studied TACC protein. Additionally, we assess the mRNA expression patterns of the three TACCs along a developmental time course, as a first step in determining which processes each TACC protein may help facilitate during early development. Moreover, differential overexpression phenotypes obtained from quantification of MT dynamics in cultured *Xenopus* cells provides evidence of both functional redundancy and functional divergence within the TACC family. Finally, we demonstrate that TACC3 manipulation impacts pharyngeal arch morphology, a finding which supports others' claims that TACC3 plays a role in cell motility, and suggests that this may be true in the context of neural

crest migration. Collectively, our data support an emerging role for the TACC family in cytoskeletal regulatory processes that are crucial for proper embryogenesis. (L.A.L. funding: NIH R00 MH095768.)

Program Abstract #515

Basement membrane type IV collagen is crucial for lung alveolarization and septation.

Maria Loscertales[1], Fotini Nicolaou[1], Marion Jeanne[2], Mauro Longoni[1], Douglas Gould[2], Yunwei Sun[1], Nandor Nagy[3], Patricia Donahoe[1]

[1]Massachusetts General Hospital/ Harvard Medical School, USA; [2]University of California San Francisco, USA; [3]Semmelweis University, Hungary

The last stages of lung development are essential to build the blood-gas exchange units, the alveoli. Despite intensive lung research, the morphogenetic events that direct alveologenesis are poorly understood. Type IV collagen is the main component of the basement membrane that gives strength to the blood-gas barrier. Homozygous mutations of *Col4a1* and *Col4a2* are lethal after mid-embryogenesis (E10.5-E11.5) because of impaired basement membrane stability. Heterozygous mutants have reduced viability and developed severe cerebral, ocular, renal, and vascular abnormalities. Surviving *Col4a1*$^{+/\Delta ex41}$ pups are often cyanotic undergoing respiratory distress, dying soon after birth. Little research has been done to understand the role of the basement membrane in alveolar development. In the present work, we used the *Col4a1*$^{+/\Delta ex41}$ and conditional *R26-Cre*^{ER}*; Col4a1*$^{+/Flex41}$, and *Tie2-Cre; Col4a1*$^{+/Flex41}$mice to explore the role of type IV collagen in alveologenesis. We found that the *Col4a1*$^{+/\Delta ex41}$ and postnatal conditional *R26-Cre*^{ER}*; Col4a1*$^{+/Flex41}$ mutant lungs displayed an imbalance between epithelial progenitors and differentiated cells, which were apparent as early as saccular formation. We also showed that *Col4a1*$^{+/\Delta ex41}$ and *R26-Cre*^{ER}*; Col4a1*$^{+/Flex41}$ mutants exhibited disrupted myofibroblast proliferation, differentiation, and migration. Specific *Col4a* mutation driven by the *Tie2* promoter, *Tie2-Cre; Col4a1*$^{+/}$Flex41, was sufficient to cause defects in vascular development, and blood-gas barrier formation also observed in *Col4a1*$^{+/\Delta ex41}$ and *R26-Cre*^{ER}*; Col4a1*$^{+/Flex41}$. Lastly, we showed in studies *in vitro* that COL4A1 protein can induce myofibroblast proliferation and migration in monolayer culture and increased the formation of mesenchymal-epithelial septal-like structures in co-culture. We conclude that type IV collagen is crucial for alveologenesis and we show a novel role in alveolar myofibroblast development, thus directing septal development.

Program Abstract #516

Matrix metalloproteinase 13 proteolytic activity in development and macrophage migration in zebrafish

Christopher Small

University of New Brunswick, CA

Matrix metalloproteinases (MMPs) are a family of secreted enzymes present in the genomes of all vertebrates. Classically, they were thought to be responsible solely for degrading and remodeling the extracellular matrix (ECM) however they are now implicated in the catabolism of many non-matrix substrates, both extracellularly and intracellularly, as well in non-proteolytic functions. The human and zebrafish genomes encode about two dozen MMPs, however the zebrafish genome includes several paralogous MMPs due to the teleost genome duplication, and lacks orthologues of several MMPs found in mammalian genomes. Matrix metalloproteinase 13a and -b are the only collagenases in the zebrafish genome. Mmp13a is known to be important for the development of bone and cartilage as well as for macrophages migrating through the ECM to a site of injury, and Mmp13a morphant embryos develop with severe spinal curvature, craniofacial defects, and abnormal fin rays. There is currently no functional data for Mmp13b. Furthermore, because MMPs are secreted as inactive zymogens, transcriptional analysis can be misleading. We have used immunofluorescence to characterize the distribution of Mmp13 proteins at various stages of development, and combine this with analysis of the effects of pharmacological inhibition of Mmp13 activity and established agonists of *mmp13a* gene expression (glucocorticoids and H_2O_2). We hypothesize that Mmp13-mediated migration of macrophages is triggered by production of glucocorticoids and H_20_2 at the sites of injury and/or infection. Using Tg(*mpeg1:eGFP*) embryos, which express GFP in macrophages, we will investigate this possibility.

Program Abstract #517

Irx3 and Irx5 regulate sister chromatid segregation and limb bud shape

Hirotaka Tao[1], Jean-Philippe Lambert[2], Danyi Li[1,3], Kimberly Lau[1], Vijitha Puviindran[1], Xiaoyun Zhang[1], Noah Hahn[1,3], Xiao Xiao Chen[3], Kendra Sturgeon[1], Gregory Anderson[1], R. Henkelman[1], Yasuhiko Kawakami[4], Anne-Claude Gingras[2,3], Chi-chung Hui[1,3], Sevan Hopyan[1,3]

[1]The Hospital for Sick Children, CA; [2]Mount Sinai Hospital, CA; [3]University of Toronto, CA; [4]University of Minnesota, USA

The spatial pattern of specialised tissues is critical for organ function.It has long been recognised that the emerging shape of organ primordia profoundly influences pattern formation, although factors that link these processes remain unclear. Anterior skeletal pattern of the vertebrate limb is regulated by the TALE class homeodomain proteins Iroquois 3 and 5 (Irx3/5), in part through direct promotion of *Gli3* transcription. Interestingly, *Irx3/5* double mutant mice also exhibit deficient anterior mesoderm and a misshapen early limb bud. Here we show that Irx3/5 regulate morphogenetic mechanisms that shape the limb bud primordium. Using BioID, we identified multiple partners of Irx3 and Irx5 that regulate sister chromatid segregation, including Cux1 and the Cohesin subunit Smc1. Super-resolution microscopy and proximity ligation assay confirmed that Irx3 partially colocalises and binds with Cux1 and Smc1 *in vivo*. In anterior limb bud mesenchyme, Irx3/5 maintain Cux1 and Smc1 proteins in a nontranscriptional fashion and are required to segregate sister chromatids during cell division. Nascent daughter cells fail to separate and intercalate among their neighbours in the absence of *Irx3/5*. This unexpected function of Irx3/5 shapes the morphogen field in advance of overt pattern formation.

Program Abstract #518

Interdigit BMP signaling is essential for programmed cell death and digit outgrowth during mouse limb development

Maria Kaltcheva[1], Matthew Anderson[1], Brian Harfe[2], Mark Lewandoski[1]

[1]NIH, National Cancer Institute - Frederick, USA; [2]Department of Molecular Genetics and Microbiology, University of Florida College of Medicne, USA

During mouse development, the distal limb bud differentiates into digital rays connected by interdigital (ID) tissue, which is removed by programmed cell death (PCD). The absence of PCD results in webbed limbs. Our previous work showed that BMP signaling regulates ID PCD *indirectly* by modulating nearby *Fgf* expression, which encodes a cell survival activity to the ID. We also recently showed through an ID-specific inactivation of *Bmpr1a* that BMPs are *direct* triggers of ID PCD through a non-SMAD dependent mechanism. Both the direct and indirect BMP roles in ID PCD converge on the production of reactive oxygen species. While examining redundancy between *Bmpr1a* and *Bmpr1b* in ID PCD we serendipitously discovered a potential instructive role of the ID tissue in digit formation. Mendelian *Bmpr1b* null digits are short due to abnormal development of their phalanges. This defect is rescued when we inactivate *Bmpr1a* specifically within the ID. To test whether only the retention of ID tissue is sufficient to rescue the *Bmpr1b* phenotype we examined the *Bmpr1b* null defect in two separate webbed mutants; in one we inactivated the genes encoding BAX and BAK necessary for apoptosis and in a second we increased FGF activity. Neither of these manipulations rescued the BMPR1B phenotype suggesting that webbing is not sufficient to restore digit development. It has been previously shown that genetically increasing BMP signaling in the digits of *Bmpr1b* mutants partially restores normal digit formation. We hypothesized that in our rescued digits the absence of ID-specific BMPR1A allows unbound ligand to diffuse from the ID to the developing digit and restore normal BMP levels. In support of this, we inactivated *Bmp7* within the ID of the rescued animals and restored the *Bmpr1b* null phenotype. Thus we provide genetic evidence for the role of ID BMP signaling in digit formation. Currently, we are further examining the role of the ID as an essential signaling center in digit formation.

Program Abstract #519

FGF mediated rescue of fin bud initiation in a zebrafish model of Holt-Oram Syndrome

Haley Stinnett[1], Qiyan Mao[2], Robert K. Ho[1]

[1]University of Chicago, USA; [2]Developmental Biology Institute of Marseille, France

Holt-Oram Syndrome is a congenital birth defect linked to Tbx5 haploinsufficiency, wherein patients exhibit heart and limb defects. The human limb abnormalities range from a loss of anterior digits to the loss of the entire forelimb. In the zebrafish, Tbx5a is expressed in the lateral plate mesoderm (LPM) prior to pectoral fin bud formation. Loss of Tbx5a function results in the complete absence of the pectoral fin due to mismigration of fin precursor cells. Previously, Fischer, Draper and Neumann (2003) demonstrated that Fgf24 is activated downstream of Tbx5a and that in Fgf24 mutants and morphants, precursor cells also fail to migrate properly to form the fin bud. In Mao, Stinnett and Ho (2015), we showed that the mismigration phenotype of Fgf24 morphants can be alleviated by supplying an ectopic source of FGF at the proper time and location. In order to determine if Tbx5a deficient cells can also respond to an ectopic FGF source, we implanted FGF-coated microbeads in the LPM of Tbx5a morphant embryos. Using live imaging and cell tracking analysis, we found that fin progenitors remain closer to the FGF-coated bead than a control bead, indicating the Tbx5a-deficient cells retain an ability to respond to a local source of FGF protein. This work is funded by a National Institutes of Health Developmental Biology Training Program grant [HKS] a National Science Foundation Graduate Research Fellowship [HKS] and a National Institutes of Health grant [RKH].

Program Abstract #520

Characterization of folic acid metabolism genes in the roundworm *Caenorhabditis elegans*

Nancy Brant, Jessica Sullivan-Brown
West Chester University, USA

Folic acid supplementation has been shown to decrease the risk of a congenital condition known as neural tube defects (NTDs). NTDs include spina bifida and anencephaly and symptoms can include leg weakness, paralysis, and in some cases death. The goal of this project is to help better understand how folic acid contributes to development using the roundworm *Caenorhabditis elegans* as a model organism. We will be analyzing folic acid metabolism mutants in *C. elegans* and determining embryonic and larval phenotypes. Currently, we are studying mutations in the *mtrr-1* gene which is homologous to the vertebrate methionine synthase reductase gene. Our results suggest that *mtrr-1* is not required for embryogenesis but rather has important roles in later larval development. *mtrr-1* mutants are not able to develop past the first larval stage of development, implying that folic acid metabolism may be important in growth and development in *C. elegans.* Through this research, we hope to gain further understandings into the role folic acid plays in the cellular and molecular events regulating early stages of development.

Program Abstract #521

Determining the Expression of Folate Metabolism Genes during Neural Tube Closure in the frog *Xenopus laevis*

Patricia Bianchino, Jessica Sullivan-Brown
West Chester University, USA

Neural tube defects (NTDs) are a common human birth defect. NTDs are structural malformations of the central nervous system that result from disruption of neural tube closure during embryonic development. To date, the only known NTD prevention strategy is maternal folic acid supplementation. However, the mechanisms through which folate influences neural tube closure remain unclear. To better understand the roles of folate in the neural tube, we are using the frog *Xenopus laevis* as a model system. We will perform RT-PCR and RNA *in situ* hybridization experiments with three folic acid metabolism genes (*mthfr*, *mthfd1,* and *slc19a1*) during different stages of development. Preliminary RT-PCR results suggest that *mthfr*, *mthfd1,* and *slc19a1* are maternally supplied, as well as expressed at gastrulation and neurulation stages. Although we do not yet have data from our RNA *in situ* hybridization experiments, we hypothesize that the expression of folic acid metabolism genes will be enriched in the neural plate and the surrounding neural folds. By characterizing the RNA expression profiles of folic acid metabolism genes during development, we hope gain further insights into the roles folic acid may play in neural tube closure.

Program Abstract #522

Investigating the role of calcium in neural tube closure through an ENU-induced mutation in the calcium pump SPCA1

Joel Brown, Maria Garcia-Garcia
Cornell University, United States

Experimental evidence from whole embryo culture studies suggests that calcium levels are important during neural tube closure. For instance, removal of calcium from embryos grown in culture causes a collapse of the neural folds and a subsequent failure to neurulate. The same effect is also observed in embryos cultured with drugs that inhibit calcium uptake. Despite this evidence, knowledge about the specific roles of calcium in neural tube closure is still lacking. A recent ENU mutagenesis screen in our laboratory identified *1D*, a mouse mutant that exhibits hindbrain exencephaly and disrupts the Secretory Pathway Calcium ATPase isoform 1 (*Spca1*). We found that dorsolateral bending of the neural folds fails to occur in *Spca1*^{1D} mutants despite normal dorsal-ventral pattering of the neural tube. The SPCA1^{1D} protein is properly localized to the trans-Golgi network, but MEFs from *Spca1*^{1D} embryos showed alterations in cytoplasmic and intra-Golgi calcium levels, indicating that the *1D* mutation disrupts SPCA1 activity and calcium homeostasis. Interestingly, we have found that cofilin 1, an actin severing protein that regulates actin dynamics, is preferentially phosphorylated (inactive) in SPCA1^{1D} mutants and fails to co-localize with actin in the neuroepithelium. Together, our studies on SPCA1 provide evidence supporting that calcium is required during neurulation and that it promotes neural tube closure by regulating actin dynamics and apical contraction in the neural tube.

Program Abstract #523

Morphogenesis of the semicircular canal ducts in the zebrafish ear

Sarah Baxendale, Esther Maier, Elvira Diamantopoulou, Sarah Burbridge, Nicholas J. van Hateren, Celia J. Holdsworth, Tanya T. Whitfield

University of Sheffield, UK
Disorders of the vestibular system, including dizziness and vertigo, are common conditions that can be debilitating for the patient and difficult to treat in the clinic. Vestibular anomalies are associated with some syndromic disorders, such as CHARGE and Down Syndrome, and can result in developmental delay in infants, while degeneration of the vestibular system is thought to be a cause of falls and fractures in the elderly. We are using the zebrafish as a model system to understand development and function of the semicircular canal ducts of the inner ear. Morphogenesis of the canal system begins when projections of tissue, driven by extracellular matrix production, move into the lumen of the otic vesicle. Here, they fuse to form pillars of epithelium that span the vesicle lumen and become the hub of the developing canal ducts. In the *gpr126* mutant, the epithelial projections fail to fuse, resulting in a swollen ear that lacks all three semicircular canal ducts. Expression of extracellular matrix genes, including *versican*, remains at abnormally high levels in the *gpr126* mutant ear. The *gpr126* gene codes for an adhesion class G protein-coupled receptor that is expressed in the epithelial projections. In a second mutant, *cloudy*, the lateral canal duct develops normally, but the anterior and posterior ducts are truncated. We are using light-sheet microscopy to image semicircular canal development in the embryo in these two mutant lines. We are also using an in situ hybridisation-based assay to screen for compounds that can rescue Gpr126 signalling and restore normal levels of *versican* expression in the developing *gpr126* mutant ear. Homozygous *gpr126* and *cloudy* mutants are adult viable. We are using the light-sheet microscope to characterise inner ear anatomy in adult fish, and are correlating our anatomical data with vestibular behavioural deficits, quantified using an automated movement tracking system.
Funding: BBSRC

Program Abstract #524
Role of the Apela Signaling Pathway in Mouse Development
Laina Freyer[1], Chih-Wei Hsu[2], Sonja Nowotschin[1], Andrea Pauli[3], Pamela Hoodless[4], Alexander F Schier[5], Mary E Dickinson[2], Anna-Katerina Hadjantonakis[1]
[1]Memorial Sloan Kettering Cancer Center, USA; [2]Baylor College of Medicine, USA; [3]Research Institute of Molecular Pathology, Austria; [4]BC Cancer Agency Research Centre, Canada; [5]Harvard University, USA
A novel signaling molecule called APELA (also known as Ende, Toddler, and Elabela) was identified in zebrafish as a factor that promotes cell movement during gastrulation and is required for cardiovascular development. APELA signals via the G protein-coupled receptor APJ whose canonical activating ligand is APELIN. *Apj* null mice have developmental heart defects resulting in reduced viability. However, *Apelin* null mice develop normally, indicating that an additional molecule, likely APELA, is critical for activating APJ during embryonic development. In mice, *Apela* and *Apj* expression patterns suggest that secretion of APELA from the endoderm may be required to activate APJ signaling in mesodermal derivatives. The goal of this study is to investigate the functional conservation of the Apela signaling pathway in mice. $Apela^{H2B\text{-}GFP}$ knock-in mice were generated using CRISPR/Cas9 and homozygotes are not recovered at birth in expected Mendelian ratios. At late gastrulation stages (E7.0-7.5), *Apela* null mutants have defects in extraembryonic and embryonic mesoderm. By E9.5, *Apela* null mutants have a range of embryonic lethal and non-lethal phenotypes including pericardial edema, heart malformations, and defects in remodeling of embryonic and extraembryonic vasculature. Our results suggest that APELA-APJ signaling may regulate endothelial cell behavior during vasculogenesis. We hypothesize that expression of *Apela* near the node then later in the ventral neuroectoderm overlying the midline may enhance mesendoderm formation and angioblast specification and/or cell migration during stages that precede the onset of cardiovascular defects. Furthermore, preliminary phenotypic analysis of *Apela;Apelin* double mutant embryos suggests that APELAis the critical ligand for activating APJ signaling during mouse development. This work was supported by a Ruth L. Kirschstein fellowship from the National Institutes of Health (1F32GM115089, awarded to LF), and grant 1R01DK084391 (AKH).

Program Abstract #525
Apoptotic morphogenesis of Urogenital System
You Chi Tang, Katie Stewart, Maxime Bouchard
Goodman Cancer Research Centre, Department of Biochemistry, McGill University, CA
Apoptosis is a crucial and tightly regulated process during embryonic development for tissue morphogenesis. Previously, or lab has described an exquisite system of apoptosis involved in early urogenital system (UGS) maturation, where the elimination of common nephric duct (CND) results in ureter-bladder connection. In this system, progressive CND elimination is shown to strictly depend on regulated apoptosis, reaching to 50% of cells dying at the CND-bladder connection region, to bring the ureter in contact with the bladder. If this apoptotic rate is altered, diseases such as ureter obstruction and reflux will be resulted. And importantly, the high rate of apoptotic cell death must be compensated by an

equally high rate of resolution of the "gaps" created by cell removal. Recent observations from confocal analysis of CND cells showed interesting phenotypes that the apoptotic bodies (usually small, condensed) lay within other duct epithelial cells containing a normal columnar nucleus, surrounded by the same cell membrane (E-cad). Due to the high apoptotic rate in the CND, there are usually more than half of the duct epithelial cells showing this phenotype. Evidence in the literature has demonstrated that the exposure of phosphatidylserine (PtdSer) on apoptotic cells signal phagocytes for cell clearance. However, the mechanisms of apoptotic cell engulfment by epithelial cells are largely unknown. We then hypothesize **apoptotic-cell phagocytosis by epithelial cells act as the main driver for tissue morphogenesis in the CND, through PtdSer signaling.** A series of experiments will be performed to visualize cell behaviors with respect to changes in apoptosis levels, and to detect changes in engulfment when blocking PtdSer to further elucidate molecular mechanisms for apoptotic cell clearance. Since inappropriate cell clearance is linked to various diseases, our findings will additionally provide better insight for tissue homeostasis.
Funding: Kidney Foundation Canada

Program Abstract #526
Formation of the vaginal opening: sexual differentiation of the urethra and the role of androgen
Christine Larkins, Ana Enriquez, Martin Cohn
University of Florida, USA
Development of the vaginal opening in the vulva is a process that is often disrupted in disorders of sexual differentiation, but it is not clear how the vaginal opening normally forms. The vagina develops from the Mullerian ducts, which are attached to the developing urethra at a structure called the sinus ridge. The sinus ridge, while initially identical in males and females, undergoes sexual differentiation, shifting its relative position along the urethra in females with the attached Mullerian ducts until it reaches the perineum to allow development of the vaginal opening. Conversely in males, the sinus ridge is maintained just distal to the bladder neck and becomes the site of ejaculatory duct entry. Thus, development of the vaginal opening is dependent on sexual differentiation of the sinus ridge, but how this sexual differentiation occurs is not clear. To determine the role that androgen plays in sexual differentiation of the sinus ridge, we first defined the time-point when sexual differentiation of the sinus ridge begins in mice. We then defined the time window when androgen exposure prevents the shift in sinus ridge position and formation of the vaginal opening. Finally, we conditionally deleted the androgen receptor in the urogenital sinus epithelium or mesenchyme and performed mosaic analysis to determine where androgen acts to prevent movement of the sinus ridge. These results highlight the impact that sexual differentiation of the urethra has on formation of the vaginal opening and defines when and where androgen signaling can inhibit this process.

Program Abstract #527
Glandular Morphogenesis in the Mouse Uterus
Zer Vue[1,2], Ying Wang[1], C. Allison Stewart[1], Gabriel Gonzalez[1], Jichao Chen[1], Richard Behringer[1,2]
[1]MD Anderson, USA; [2]Baylor College of Medicine, USA
Endometrial glands secrete substances that are essential for uterine receptivity to the embryo, implantation, conceptus survival, development and growth. In uterine gland knockout animal models, females are infertile due to defects in implantation and early pregnancy loss, suggesting their role for fertility. Adenogenesis is the formation of glands within the stroma of the uterus, which forms after birth but before sexual maturity. In the mouse at P5, gland formation initiates from the luminal epithelium. By P12, the glandular epithelium invades the adjacent stroma. In domestic animals, glands are highly coiled and branched. However, the morphology of mouse glands is poorly understood because it is based on two-dimensional histology. With the use of optical projection tomography and lightsheet microscopy, labeling the uterine epithelial cells with an antibody, we have generated 3D models of uterine glands, providing meaningful insights into their structure and distribution. Cumulatively, we conclude that glands are "buds" at P8 and become elongated tubes by P11. By P21, these elongated tubes will be curved and sometimes branched. To establish the cellular mechanisms of elongation, we treated mice with EdU and data suggest that glands form through generalized proliferation. We used a conditional fluorescent reporter (*Wnt7a-Cre; R26R-mTmG*) to examine the cell shapes of glands and showed that they have diverse shapes. Our current work focuses on identifying factors involved in adenogenesis through an unbiased transcriptome approach (RNA-seq).

Program Abstract #528
Rab10-mediated secretion synergizes with tissue movement to build a polarized basement membrane architecture for organ morphogenesis
Adam Isabella[1], Sally Horne-Badovinac[1,2]

[1]*Committee on Development, Regeneration, and Stem Cell Biology, The University of Chicago, USA;* [2]*Department of Molecular Genetics and Cell Biology, The University of Chicago, USA*
During development, basement membranes (BMs) must be continually remodeled to accommodate changes in tissue size and shape. BM remodeling can also play an instructive role in directing organ morphogenesis. Very little is known, however, about the molecular and cellular mechanisms that control BM dynamics in developing tissues. We are using the *Drosophila* egg chamber as a highly tractable system to study BM remodeling during organ morphogenesis *in vivo*. The initially spherical egg chamber elongates dramatically as it grows. This morphogenesis depends on a surrounding BM that is secreted and remodeled by underlying follicular epithelial cells. Egg chamber elongation correlates with the generation of a polarized network of fibrils in the BM. Formation of these fibrils requires rotation of the egg chamber within the BM in the direction of polarization. BM fibrils are proposed to drive elongation by constraining growth along the polarization axis. Here, we use live imaging and genetic manipulations to determine how these fibrils form. We find that BM fibrils are assembled from newly synthesized proteins in the pericellular spaces between the egg chamber's epithelial cells, and undergo oriented insertion into the BM by directed epithelial migration. We identify a Rab10-based secretion pathway that promotes pericellular BM protein accumulation and fibril formation. Finally, by manipulating this pathway, we show that BM fibrillar structure influences egg chamber morphogenesis. This work shows how coordinated cellular behaviors can precisely regulate BM structure during development, and how matrix architecture can play an important role in shaping tissues.

Program Abstract #529

Cellular Remodeling of *C. elegans* Germ Cells Through Developmentally Regulated Cell Cannibalism

Yusuff Abdu

NYU School of Medicine, USA

Cells remodel to carry out specialized functions and to dispose of unneeded components. We have discovered a novel mechanism of morphogenesis where large portions of a cell are removed by neighboring cells. During *C. elegans* embryogenesis, the primordial germ cells (PGCs) extend large protrusions (lobes) that are shortly after embedded in adjacent endodermal cells; the PGC lobes disappear before the embryo hatches. Using live fluorescent imaging, we observed that lobes are cannibalized and subsequently digested by the endoderm, along with cellular content such as mitochondria. Lobe cannibalism required the cell corpse engulfment gene *ced-10/rac,* which we showed functioned in the endoderm by promoting actin recruitment to the neck of lobes. In *ced-10/rac* mutants, lobes persisted within endodermal cells while maintaining a thin membrane connection to PGC cell bodies. Null mutations in other cell corpse engulfment genes, such as like *ced-1/CD91* and *ced-5/DOCK180,* did not affect lobe cannibalism, suggesting that *ced-10/rac* acts in a novel pathway to promote lobe cannibalism. In a large-scale chemical mutagenesis screen to find other regulators of lobe cannibalism, we uncovered a hypomorphic mutation in the sorting nexin *lst-4* that leads to a defect in the removal of PGC lobes, but unlike *ced-10/rac*, does not cause defects in cell corpse engulfment. RNAi knockdown of *lst-4* phenocopies the newly uncovered mutation and leads to failure of actin and dynamin localization to lobe necks within the endoderm. Our study will facilitate better understanding of other similar cellular remodeling events, such as glia-driven neurite pruning, as well as provide a foundation for determining the biological significance of PGC-endoderm interactions. Funding from HHMI International Student Research Fellowship.

Program Abstract #530

Mitogen-Activated Protein Kinase (MAPK) Pathway Regulates Branching and Nephron Differentiation

Anneliis Ihermann-Hella[1], Frank Costantini[2], Mart Saarma[1], Satu Kuure[1]

[1]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland;* [2]*Department of Genetics and Development, Columbia University Medical Center, New York, United States of America*

Renal development is a multistage process where branching of the ureteric bud (UB) and nephrogenesis are tightly linked. Inductive interactions between the epithelium and mesencyhme evoke extensive intracellular signaling, driving morphogenesis although the roles of specific pathways have remained unclear. MAPK pathway acts downstream of receptor tyrosine kinases that mediate signaling from different growth factors, of which GDNF plays a major role in UB morphogenesis, while FGFs are important regulators of both UB and metanephric mesencymal (MM) processes. MAPK pathway is known to regulate an extensive amount of targets and while UB branching and proliferation has been linked to MAPK, more elaborate role during kidney development is probable. Notably, congenital renal defects are among the most common birth disorders, ranging from dysplasia to cancer and aplasia. Nevertheless, the genetic bases of different defects are currently poorly understood. Therefore, we aim to define the roles of MAPK pathway during UB branching morphogenesis, nephrogenesis and maintenance of nephron progenitor population. We have characterized the UB

epithelium specific MAPK pathway deficiency *in vivo* where branching, proliferation and adhesion defects were found, leading to kidney hypodysplasia. Furthermore, our observations of MAPK activity in the MM and during nephrogenesis has led us to investigate the role of MAPK pathway in the MM where its functions have not been previously described. MAPK pathway regulates early nephron differentiation, MM cellular properties and proliferation, thus resulting in hypoplastic kidneys with reduced nephron number and impaired morphology. Our fundamental goal is to understand the vital process of renal morphogenesis and differentiation that will ultimately determine the functional capacity of the kidney and the viability of the individual. Funding: Doctoral Programme in Biomedicine, Jane&Aatos Erkko Foundation, Sigrid Juselius Foundation, Munuaissäätiö

Program Abstract #531

Role of the Vasculature in Villus Development: Notch Signaling Modifies Vascular Pattern and Villus Growth

Katherine Walton, Deepa Chandrasekhar, Deborah Gumucio
University of Michigan Medical School, USA

In the adult intestine, finger-like projections called villi increase the absorptive surface area by 10-fold. Villi are composed of an endodermally derived epithelial layer and an underlying mesodermally derived core of supporting tissues including a tightly coupled vascular network. De novo villus formation occurs primarily during fetal development when villi begin to emerge from a flat tube of epithelium at embryonic day 15 in the mouse. We previously found that villus emergence requires the formation of tight aggregations of Hedgehog responsive mesenchymal cells (clusters) that grow with the emerging villi to become part of each villus core. These clusters are intimately associated with the villus core vasculature and both clusters and villi fail to form when vasculature is inhibited. To investigate the role of vasculature in villus development, we sought to alter vascular pattern and examine the affect on villus development. Since Notch signaling is a critical regulator of vascular development controlling tip (angiogenic sprouts) versus stalk cell fate, we used endothelial specific genetic loss or gain of Notch signaling to alter vascular pattern. Cre mediated endothelial specific loss of Notch signaling in two different genetic models (loss of $Rbpj^{f/f}$ or expression of $Rosa^{DNMAML}$, Dominant Negative Mastermind-like) resulted in increased vascular sprouting and very large, highly vascularized villi with multiple clusters and branches. Increased endothelial Notch signaling by Cre mediated expression of the Notch Intracellular Domain (NICD) decreased vascular sprouting and reduced cluster formation and villus emergence. Thus, alterations in endothelial sprouting and vascular pattern in turn modulate mesenchymal cluster pattern and villus growth. Targeted applications to induce endothelial cell sprouting may provide means to induce villus formation in the treatment of diseases where villus structure or number is compromised.

Program Abstract #532

Role of c-Myc Gene Family during adult intestinal stem cell development

Morihiro Okada, Thomas Miller, Yun-Bo Shi
National Institutes of Health, USA

For proper organ development, the balance between cell proliferation and differentiation is an important factor. If the balance tips in favor of proliferation, stem cells can over-accumulate, leading to cancer. If the balance tips toward differentiation, stem cells can disappear, leading to the deterioration of organs. Thus, it is important to understand how adult stem cells are formed and maintained in a well-balanced manner during vertebrate development. The thyroid hormone (T3)-dependent frog metamorphosis resembles mammalian postembryonic development and offers a unique opportunity to study how adult stem cells are formed and how the balance between proliferation and differentiation are maintained as it is easy to manipulate the externally developing frog embryos. We carried out microarray analysis of intestinal gene expression during metamorphosis and identified a number of novel candidate adult stem cell related genes. Among them is the Mad gene, an antagonist of the c-Myc. Previous studies have shown that Mad inhibit transcription leading to cell differentiation while c-Myc induces proliferation. In this study, we revealed epithelium specific induction of Mad in the intestine during metamorphosis. We further provided evidence that Mad expression is induced by T3, just prior to c-Myc expression and adult stem cell formation. High levels of Mad expression is localized to apoptotic cells while c-Myc expression is present in adult stem cells. In addition, to investigate the role of Mad during adult stem cell formation, we adapted the recently developed TALEN technology to knockout the endogenous Mad. Interestingly, we found that knocking out the endogenous Mad reduced the adult stem cell population. Our findings suggest that Mad/c-Myc balance is likely critical for cell fate determination during adult stem cell formation. These observations provided the first example for the involvement of Mad/c-Myc pathways in adult stem cell formation in vertebrate.

Program Abstract #533

Regulation of histone H3 lysine 27 methylation and RNA polymerase II by Piwi proteins in the *Drosophila* ovary.

Jamy Peng[1], Anton Valouev[2], Haifan Lin[3]

[1]St Jude Children's Research Hospital, USA; [2]University of Southern California, USA; [3]Yale University, USA

The *Drosophila* ovary is an excellent model to interrogate molecular regulation of tissue stem cells and the interplay between somatic and germline stem cells. We performed a genetic screen for suppressors of *piwi* mutant phenotypes in order to elucidate how Piwi protein regulates the somatic niche and germline stem cells. Our effort isolated multiple *piwi* suppressors: *corto, c-fos,* and Polycomb Group genes. *Corto* is a chromatin factor genetically and physically interacting with Polycomb and Trithorax Group proteins. c-Fos is a classic proto-oncogene with wide influence over development and disease. The Polycomb Group proteins are chromatin modifiers of histone proteins for creating repressive chromatin structure, which in turn inhibits transcription by RNA polymerase II. Biochemical assays of Piwi and Polycomb Group complexes PRC1 and PRC2 in ovarian cell extract and as recombinant proteins showed that Piwi directly associate with PRC2 complex subunits Su(z)12 and Esc. Moreover, Piwi negatively regulates the binding of the PRC2 complex to chromatin as well as PRC2-mediated methylation of lysine 27 on histone H3 (H3K27m3). Analysis of multiple replicates of chromatin immunoprecipitation-deep sequencing revealed increased H3K27m3 levels and decreased RNA polymerase II levels at chromatin of *piwi* mutant ovarian cells when compared to wild type. However, Piwi proteins bind very few genomic sites, suggesting that Piwi proteins influence PRC2, PRC1, and RNA polymerase II in the nucleoplasm away from the chromatin. The gene regulatory effect of Piwi proteins is validated by RNA-seq showing the expression of genes known to affect the somatic niche and/or germline stem cell functions is affected by the Piwi-PRC2 interaction. Our findings suggest that negative modulation of the Polycomb Group protein activities is necessary to maintain the appropriate gene expression profiles for stem cells and oogenesis. This work was funded by the ALSAC and an NIH grant (R00HD071011).

Program Abstract #534

Silencing of repetitious elements in the *Drosophila melanogaster* genome through heterochromatin formation

Sarah C R Elgin[1], Michael Lee[1], Tingting Gu[2], Pui Pik Law[3], Richard Festenstein[3], Elena Gracheva[1]

[1]Washington University in St Louis, USA; [2]Nanjing Agricutural University, China; [3]Imperial College London, UK

Within eukaryotic genomes, repetitious sequences are silenced by heterochromatin formation. The *Drosophila melanogaster* DNA transposon remnant *1360* is an effective target, promoting silencing of an adjacent *hsp70*-driven *white* gene, resulting in a Position Effect Variegation (PEV) phenotype. However, PEV is only observed when the reporter construct *P{1360, hsp70-white}* is present within or close to a heterochromatic domain (Haynes et al 2006 Curr Biol 16: 2222-7). At the 1198 "landing pad" site at the base of chromosome arm 2L, a copy of either *1360* or *Invader4* (a retrotransposon remnant) can switch a euchromatic region to a heterochromatic one, with increased HP1a and H3K9me2; the variegating *hsp70-white* shows a ~2-fold decrease in expression (Sentmanat & Elgin, PNAS 109:14104-9). Recently we observed that 256 copies of a 36 bp *lacO* fragment induces even stronger PEV, an ~8-fold decrease in expression of the *hsp70-white* reporter. Surprisingly, *lacO* mediated silencing shows an inverse temperature effect, with loss of silencing at lower temperatures (G. Reuter; EG). A fragment with 310 copies of the GAA triplet repeat (the driver in Friedreich's Ataxia) in the 1198 site similarly results in eight-fold silencing. Introducing HP1a mutations results in a loss of silencing in all of these cases, confirming that heterochromatin assembly is required. Histone H3K9 methylation appears to be less important for the *lacO* repeats than for other cases. We find that *lacO*-induced silencing, the PEV observed in the w^{m4} line, and the PEV phenotype of a reporter in the pericentric heterochromatin (118E-10), are all sensitive to nicotinamide, an HDAC inhibitor, supporting a general role for histone deacetylation in heterochromatin formation in *Drosophila*. The heterochromatin-based mechanism for silencing the tandem *lacO* repeats apparently differs in some important aspects from the heterochromatin formation used to silence TEs and GAA_{310}. Supported by NIH GM068388 & NSF MCB-1243724.

Program Abstract #535

Heterochromatin protein 1ß regulates neural and neural crest development via repression of the pluripotency-associated gene Oct25

Chih-liang Tien, Chenbei Chang

University of Alabama at Birmingham, USA

Formation of the vertebrate nervous system starts at neurulation, whereby neural and neural crest cells are induced in the dorsal ectoderm and give rise to central and periphery nervous systems respectively during later embryogenesis. Multiple growth factor signals and transcription factor networks have been implicated in this process, but how epigenetic

mechanisms regulate neural and neural crest development is less understood. In this study, we examined the function of heterochromatin protein 1 beta (HP1β), a histone H3 lysine 9 trimethylation binding protein involved in modulation of chromatin structure, in early development of the nervous system in *Xenopus*. We found that all three HP1 paralogs, HP1α, β, and γ, were expressed in the neural and neural crest regions during neurulation. Antisense morpholino (MO)-mediated knockdown of HP1β and HP1γ, but not HP1α, reduced the expression of the late neural marker NRP1 and the neural crest markers Sox9 and Snail2, but did not affect the early neural marker or the neural plate border marker. As the embryos were most sensitive to the level of HP1β-MO, we focused our studies on this protein. We showed that HP1β, but not HP1α or HP1γ, rescued the defects in HP1β morphant, implying that HP1 paralogs had distinct activities. As early genes induced in the dorsal ectoderm were not affected by HP1β-MO, but late neural and neural crest markers were reduced, we hypothesized that HP1β might regulate switch between precursor and more differentiated cell types. In agreement with this idea, we observed expansion of Oct25, a *Xenopus* Oct4 homolog associated with pluripotency, in HP1β morphant. Overexpression of Oct25 phenocopied HP1β-MO in regulation of NRP1, Sox9 and Snail2; and knockdown of Oct25 partially rescued the expression of these markers in HP1β morphant. Taken together, our data indicate that HP1β promotes neural and neural crest differentiation at least partially via repression of the pluripotency-associated gene Oct25.

Program Abstract #536

Interfering with Serine 31 phosphorylation on histone variant H3.3 impairs gastrula transition in *Xenopus laevis*

David Sitbon[1,2], Geneviève Almouzni[1,2]

[1]Institut Curie, PSL Research University, CNRS, UMR3664, Equipe Labellisée Ligue contre le Cancer, F-75005 Paris, FR; [2]Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 3664, F-75005 Paris, FR

Proper packaging of eukaryotic DNA into chromatin is required for functional genome organization. This involves the use of a fundamental motif, the nucleosome, which is constituted of DNA wrapped around histones. The role of distinct histone variants during the development of an organism has been a longstanding issue. Interfering with their function gives rise to different outcomes in different organisms (*cf.* for review Filipescu *et al.*, 2014). The case of H3 variants is particularly striking considering that, in mammals, the replicative forms H3.1 and H3.2 and the replacement form H3.3 share more than 96% of sequence identity. However, their respective functional roles are still not accurately characterized. Previous work in our laboratory revealed that down-regulation of H3.3 expression leads to major developmental defects during *Xenopus laevis* gastrulation, which can only be rescued by the very same variant H3.3 (*cf.* Szenker *et al.*, 2012). To understand the basis of this specificity, we explored how each single amino acid in H3.3 residues that differ from H3 could be critical. For this, we took advantage of a complementation assay using exogenous H3.3 constructs mutated at each distinct residue to try to rescue the gastrulation phenotype due to depletion of the endogenous H3.3. Among the 4 distinct amino acids, serine 31 in H3.3 proved critical to allow the embryo to undergo gastrulation. Our data points to a particular importance of phosphorylation on this residue. We will discuss our most recent data and their implications regarding unanticipated roles for H3.3 in cell function that culminate at particular developmental transitions.

David Sitbon is a recipient of a Ph.D. fellowship from the Ministère de l' Éducation Nationale, de l'Enseignement Supérieur et de la Recherche with PSL University. This work was supported by la Ligue Nationale contre le Cancer (Equipe labellisée Ligue).

Program Abstract #537

Epigenetic Regulation of Embryonic Lymphatic Integrity

Matthew Menendez, Joanna Podsiadlowska, Vijay Muthukumar, Courtney Griffin

Oklahoma Medical Research Foundation, USA

Embryonic lymphatics arise from blood vessels and play important roles in maintaining tissue fluid homeostasis. While several factors required for lymphatic formation have been identified, little is known about what regulates lymphatic stability and integrity during development. We recently generated murine embryos with deletion of the chromatin-remodeling enzyme BRG1 in developing lymphatic endothelial cells (LECs) that have aberrant blood-filled lymphatics and lethal lymphatic rupture. These phenotypes correlate with upregulation of the programmed necrosis (necroptosis) executor gene *Ripk3* in developing LECs. Genetic deletion of *Ripk3* rescues *Brg1* mutants from embryonic lethality, indicating that LEC necroptosis compromises blood/lymphatic vascular separation and causes lethal lymphatic rupture. Since BRG1 regulates transcription of its target genes, we initially hypothesized that BRG1 directly represses *Ripk3* transcription in LECs. However, we found no evidence that BRG1 binds *Ripk3* regulatory elements and instead discovered that *Ripk3* transcripts are aberrantly stabilized in BRG1-depleted LECs. Because we see similar *Ripk3* transcript stabilization in LECs after depletion of the miRNA processing enzyme DICER1, we currently hypothesize that BRG1 regulates a *Ripk3*-destabilizing miRNA in LECs to protect them from aberrant necroptosis. This research is

significant because it is the first to describe causes and effects of necroptosis in the lymphatic vasculature and it highlights the detrimental impact of this cell death modality on embryonic lymphatic integrity.

Program Abstract #538
Novel parent of origin imprinting events during gastrulation.
Chelsea Marcho, Jesse Mager
University of Massachusetts, USA
Appropriate epigenetic regulation of gene expression during lineage allocation and tissue differentiation is essential for successful development. Genomic imprinting, defined as parent-of-origin mono-allelic expression is established due to epigenetic differences arriving in the zygote from sperm and egg haploid genomes, and maintained by allele specific chromatin alterations. In the mouse, there are approximately 150 known imprinted genes, many of which are coordinately regulated in imprinted clusters. We have previously shown that the imprinted genes *Igf2r* and *Airn* undergo temporal and tissue specific epigenetic modifications during gastrulation. In order to expand these findings of novel epigenetic regulatory events during gastrulation, we have performed allele-specific RNA sequencing and whole genome bisulfite methylation on gastrulation tissues (E6.5 epiblast, E6.5 visceral endoderm, E7.5 embryo, E7.5 visceral endoderm). We have identified numerous novel imprinted loci that are not associated with defined imprinting control regions, suggesting epigenetic regulatory mechanisms distinct from gametic DNA methylation. Our ongoing analysis is aimed at identifying the epigenetic regulatory features of these previously unidentified imprinting events.

Program Abstract #539
Effect of incubation conditions on DNA methylation in turtles with environmental dependent sex determination.
Itzel Sifuentes, Boris Tezak, Jeanette Wyneken, Sarah Milton
Florida Atlantic University, USA
Experimental studies with turtles known to have temperature-dependent sex determination (TSD), a form of environmental sex determination suggest that moisture conditions during incubation may influence development and sex determination. Wetter substrates produce more males, whereas drier substrates produce more females. This modifier is consistent with findings in the field that sex ratios obtained from Loggerhead turtle (*Caretta caretta*) nesting beaches show a poor relationship with temperatures recorded in situ. However, when the relative moisture level (particularly rainfall) is added to nest temperatures, sex ratio trends become more predictable. Still, the mechanisms by which environmental factors (temperature or moisture) affect sex determination remain unknown. It has been suggested that embryonic sex differences are initially determined by different patterns of nuclear DNA methylation. Evidence supporting this hypothesis was found in the American alligator (*Alligator mississippiensis*), where males showed elevated aromatase promoter methylation compared to females, while the opposite occurred in the *Sox9* promoter, displaying an inverse relationship between methylation and expression levels. Together, these results led us to hypothesize that moisture influences sex ratios in turtles via an epigenetic mechanism regulating the expression of sex determination/differentiation genes. Here we tested the effects of moisture on sex determination by exploring DNA methylation in the gonadal aromatase and *Sox9* promoters of the freshwater turtle *Trachemys scripta*. Such a mechanism would affect gene expression, and hence sex ratios, in species with environmental sex determination.

Program Abstract #540
Polycomb-mediated chromatin remodeling during mammalian sex-determination
Sara Garcia Moreno, Isabella Salamone, Danielle Maatouk
Northwestern University, USA
Prior to sex determination XY and XX gonads are indistinguishable, and the somatic cells that compose them are bipotential with the ability to differentiate into either Sertoli cells (XY) or pregranulosa cells (XX). Disruption of the regulatory network underlying the establishment of either the XY or XX fate leads to disorders of sex development (DSDs). Interestingly, mutations of several chromatin-modifying proteins cause DSDs suggesting a critical role for chromatin remodeling in sex determination. Loss of the Polycomb-group (PcG) subunit CBX2 leads to male-to-female sex reversal.CBX2 maintains gene repression by binding the repressive histone modification H3K27me3. We hypothesize that PcG directs bipotential gonadal cells toward the XY pathway by maintaining sexually dimorphic patterns of H3K27me3 on SD genes. To investigate the role of PcG during sex determination, we performed ChIP-qPCR for H3K27me3 on purified Sertoli and pregranulosa progenitor cells at E10.5 (before sex determination) and E13.5 (after sex determination). In accordance with our hypothesis, ChIP-qPCR revealed H3K27me3 enrichment at XX-determining genes in Sertoli cells but not in pregranulosa cells at E13.5, suggesting an important role for PcG-repression of the XX pathway

during testis development. Furthermore, similar levels of both H3K27me3 and the active modification H3K4me3 on SD genes in XY and XX progenitor cells at E10.5 suggests that SD genes are bivalent and poised for sex determination at the bipotential stage. Our results are the first to uncover that genes required for XX development are PcG targets and to demonstrate dynamic histone modifications involved in the establishment and maintenance of mammalian sex-determination. Future ChIP-seq studies will uncover genome-wide PcG targets in XY and XX supporting cells and their potential role in sex determination.
Funding: Cellular and Molecular Basis of Disease T32 GM08061

Program Abstract #541
Epigenetic regulation of hematopoiesis by DNA methylation
Aniket Gore, Brant Weinstein
NICHD, NIH, USA
During embryonic development cell type-specific transcription factors promote cell identities, while epigenetic modifications are thought to contribute to maintaining these cell fates. Our understanding of how genetic and epigenetic modes of regulation work together to establish and maintain cellular identity is still limited, however. Here, we show that *DNA methyltransferase 3bb.1* (*dnmt3bb.1*) is essential for maintenance of hematopoietic stem and progenitor cell (HSPC) fate as part of an early *notch-runx1-cmyb* HSPC specification pathway in the zebrafish. HSPC-specific expression of Dnmt3bb.1 is regulated byexpressed in HSPC downstream from *notch1* and *runx1*, and loss of Dnmt3bb.1 activity leads to reduced *cmyb* locus methylation, reduced *cmyb* expression, and gradual reduction in HSPCs. Ectopic overexpression of *dnmt3bb.1* in non-hematopoietic cells is sufficient to methylate the *cmyb* locus, promote *cmyb* expression, and promote hematopoietic development. Our results reveal an epigenetic mechanism supporting maintenance of hematopoietic cell fate via DNA methylation-mediated perdurance of a key transcription factor in HSPCs.

Program Abstract #542
Establishment and maintenance of heritable patterns of chromatin structure during early embryogenesis
Shelby Blythe[1,2], Eric Wieschaus[1,2]
[1]Princeton University, USA; [2]Howard Hughes Medical Institute, USA
Throughout the life cycle, the functional specializations of cells and tissues are directly reflected in the patterns of chromatin structure. During embryogenesis, genetic loci involved in cell fate specification and differentiation –committed decisions that restrict a cell's developmental potential– must be packaged in such a way that they can ultimately respond to precise spatial and temporal developmental cues. Furthermore, a mechanism must exist for faithfully replicating such chromatin states in daughter cells following mitosis. We have measured by ATAC-seq how the initial patterns of chromatin structure are established and maintained during early *Drosophila* embryogenesis with three-minute time resolution for the three cell cycles preceding the midblastula transition (MBT). During this period, the embryo undergoes large-scale zygotic genome activation and initiates the process of pattern formation. However, because such embryos are also simultaneously engaged in mitotic amplification, one longstanding question has been to what extent stable chromatin structure could be established in the context of an extremely rapid cell cycle. We find that extensive heritable chromatin structure can be established under these conditions. Over this period, thousands of genomic features acquire chromatin accessibility at specific times, in response to different biological timing mechanisms. Enhancers, for example, are accessible from very early in development, whereas the majority of promoters acquire accessibility at or around the MBT. The majority of these promoters gain accessibility in response to the biological timer that measures the nucleo-cytoplasmic ratio and controls the onset of the MBT. In addition, we observe that once established, these patterns of accessibility are maintained in metaphase chromatin, suggesting that the major mechanism for epigenetic inheritance of embryonic chromatin structure relies on acquisition of mitotic stability or chromatin "bookmarking".

Program Abstract #543
Brd2 paralogs in zebrafish may act antagonistically during development and play a role in the proper formation of the pronephric duct, and the circulatory and central nervous systems.
Gregory Branigan, Kelly Olsen, Angela DiBenedetto
Villanova Univ, USA
Brd2 is a member of the bromodomain-extraterminal domain (BET) family of transcriptional co-regulators and functions as a histone-directed scaffold in chromatin modification complexes. Brd2 facilitates expression of pro-proliferation genes and helps control apoptosis in mammalian adult tissues, while the *Drosophila* homolog is an upstream regulator of Hox genes in development. In zebrafish, brd2 has duplicated and diverged during evolution of telosts, resulting in brd2b and

brd2a paralogs with both overlapping and divergent expression patterns in developing embryos. We probed the developmental role of Brd2b through microinjections of antisense morpholino (MO) oligonucleotides and compared the morphant phenotype to that of the already characterized Brd2a paralog, which exhibits increased apoptosis and brain and central nervous system defects. Brd2b morphants show similar defects in brain but novel trunk defects. Whereas Brd2a knockdowns exhibit increased numbers of pax2a-positive cells in the peripheral blood island at 24 hpf, Brd2b knockdowns show reduced numbers of cells in this region, with heart defects and lack of circulation at later stages. Brd2b knockdowns also fail to form hollowed out pronephric ducts. Remarkably, co-knockdown of both Brd2b and Brd2b shows rescue of both brain and trunk defects, suggesting an antagonistic relationship between the paralogs. All morphant phenotypes are being verified using Crispr/Cas9 injections globally into 1 cell embryos and MO injections locally into the peripheral blood island of segmentation stage embryos, in addition to comparative phenotypic analysis of BET inhibitor-treated embryos, and of brd2b mutant lines obtained from ZIRC. Mitosis, apoptosis, and expression of blood stem cell markers are being assessed as additional phenotypic endpoints. Brd2a and 2b paralogs in zebrafish provide a unique opportunity to analyze both conserved and more recently derived functions of this important epigenetic regulator.

Program Abstract #544

Charge-Dependent Gene Repression by CBX2, a Polycomb Group Protein, is Required for Patterning the Mouse Axial Skeleton

Matthew Schwartz[1], Mei Sheng Lau[1,2], Clifford Tabin[1], Robert Kingston[1,2]

[1]Department of Genetics, Harvard Medical School, USA; [2]Department of Molecular Biology, Massachusetts General Hospital, USA

Pattern formation is the process by which cells determine their location within a tissue or organ in order to acquire the appropriate cell fate in space and time during embryonic development. Polycomb and Trithorax group proteins are epigenetic regulators which stably maintain gene silencing or activation respectively over many cellular generations in order to ensure that cells maintains their appropriate gene expression program. Mechanistic studies of Polycomb Repressive Complex 1 (PRC1) have mainly focused on its enzymatic function to ubiquitylate histones, but recent studies demonstrate that the ubiquitylation activity of PRC1 is dispensable for silencing. The ability of CBX2, a component of PRC1, to compact chromatin directly in a charge-dependant manner has been proposed as an alternative mechanism to explain the role of PRC1 in maintaining gene repression. To investigate the role of charge-dependent chromatin compaction by CBX2 in pattern formation during development, we generated three independent mouse lines in which six, 13, or 23 positive amino acids in the putative CBX2 compaction domain were mutated to alanine. CBX2 charge mutant mice exhibit posterior homeotic transformations across the mouse axial skeleton and the loss of repression results in early activation of *Hox* gene expression in the developing mouse anterior-posterior axis. Our results demonstrate that charge-dependent chromatin compaction by CBX2 is required in a dosage-dependent manner for the maintenance of gene silencing and for properly patterning the mouse axial skeleton.

Program Abstract #545

CreLite: a red light-inducible Cre tool for mouse and zebrafish

Shuo-Ting Yen[1,2], Jian MIn Deng[2], George T. Eisenhoffer[2], Richard R. Behringer[1,2]

[1]Baylor College of Medicine, USA; [2]UT MD Anderson Cancer Center, USA

Precise manipulation of gene expression with spatial and temporal control has been an important goal for developmental biology studies. Currently, the Cre-*lox*P binary system allows gene expression control using known ubiquitous or tissue-specific regulatory elements, which limits the possibilities for the control of gene expression at desired times and places. Recently, light-inducible proteins have become a powerful tool for genetic manipulations and confer precise control of various biological systems. A blue light-inducible Cre system has been engineered and applied in fly and a cell culture system. However, the low tissue penetrance and high photo-toxicity of blue light may hinder further applications for live, thick tissue or animals. Here, we present an engineered red light-inducible Cre system, CreLite, for potential use in both mouse cell culture and zebrafish embryos. The red light-inducible Cre system consists of two fusion proteins, PhyB-CreC and PIF6-CreN. The Cre activity is blocked by splitting Cre into an N-terminal half (CreN) and a C-terminal half. By fusing the two halves of Cre with the Arabidopsis thaliana light-inducible binding partners, PhyB and PIF6, the split Cre can become active by red light (660 nm) illumination. Transgenic mice, *CreLite*, carrying both synthetic genes have been generated. Mouse embryonic fibroblasts (MEFs) derived from *CreLite; R26R-YFP* mouse embryos successfully turned on the YFP reporter after exposure to red light. Furthermore, microinjection of *CreLite* mRNAs and its cofactor (phycocyanobilin; PCB) into Zebrabow embryos and red light illumination resulted in Cre activity and the generation of diverse color profiles in various embryonic tissues, including heart, skeletal muscle and epithelium. CreLite is a new tool

for precise temporal and spatial control of gene expression in cell culture, *ex vivo* organ culture, and animal models for developmental biology studies.
Funding source: NIH R21 OD019764

Program Abstract #546
Exploration of long non-coding RNAs in Hox clusters
Zainab Afzal[1,2], Bony De Kumar[1], Robb Krumlauf[1,2]
[1]Stowers Institute for Medical Research (SIMR), USA; [2]Kansas University Medical Center, Department of Anatomy and Cell Biology (KUMC/ACB), USA
Hox genes encode highly conserved transcription factors that play important roles during animal development by assigning anterior-posterior identity to developing cells and organs along the embryonic axis. Hox gene expression is in part established through mechanisms involving local enhancers and long-range/global regulation. Part of the transcriptional complexity of Hox clusters involves the presence of a large number of non-coding RNA, embedded within and adjacent to these clusters that are expressed in a manner that correlates with the timing of expression of adjacent Hox coding regions. LncRNAs are also associated with rapid epigenetic changes in chromatin states in the Hox clusters. These lncRNA transcripts may be acting as an additional regulatory input into roles of Hox clusters in development and organogenesis. We have found that the *Hobbit1* lncRNA shares a regulatory region with the adjacent Hox coding genes (Hoxb4 & Hoxb5). Deletion of a shared DE-RARE reduces *Hobbit1* expression and its response to RA treatment in ES cells and mouse embryos. *In situ* experiments show that the *Hobbit1* is expressed in the brachial arches and developing limb bud. Another genomic region (*Heater*) upstream of Hoxa cluster rapidly induces multiple lncRNAs upon treatment with retinoic acid (RA). The *Heater* transcripts are responsive to RA and may be involved in regulating the downstream Hoxa1 gene. We have mapped RA responsive enhancers that appear to regulate the Heater region (H-AR1) and have demonstrated that they contain several retinoic acid response elements (RAREs) bound by RAR and RXRs during RA induced differentiation of ES Cells. We are conducting regulatory analyses on these enhancers and find that in zebrafish reporter assays mouse HAR1 is able to direct expression in pharyngeal arches, ganglions, and axons adjacent to the lateral line. Together this work is helping to provide insight on the in vivo role of these Hox lncRNAs.
Research supported by funds from the SIMR.

Program Abstract #547
Specification of neural vs epidermal fates: Role and evidence of microRNAs and their target mRNAs
Vrutant Shah, Amy Sater
University of Houston, USA
During early embryonic development, ectoderm cells choose between neural or epidermal fates in response to paracrine signals. The fundamental gene regulatory networks (GRNs) that underlie these initial states of specification have been delineated; however, the roles of microRNAs (miRs) in establishing and maintaining these networks have not been examined. To investigate the role of miRs in ectodermal cell fate, we compared miRs and miR-targeted mRNAs in presumptive neural and epidermal ectoderm in *Xenopus laevis*. We identified over 400 miRs expressed in midgastrula ectoderm expressing either noggin or a constitutively active BMP4 receptor, reflecting anterior neural or epidermal ectoderm respectively. The miRs expressed in neural and epidermal ectoderm are broadly similar. Transcripts targeted by miRs were identified by co-immunoprecipitation of Argonaute (Ago) ribonucleoprotein complexes and sequencing of associated RNAs. The Ago-RNA pools from these tissues represent distinct subsets of genes, with significant overlap. Moreover, the profiles of Ago-RNAs differs substantially from the profiles of total RNAs in both tissues. Notably, genes associated with neuronal functions were represented in both neural and epidermal Ago-RNA pools. Two genes in the neural GRN, *foxD4L1* and *sox11*, are represented in Ago-RNAs from epidermal ectoderm, suggesting that miRs might be involved in the stabilization of epidermal fate. *FoxD4L1* is a critical transcription factor in the early immature neural GRN, where it up-regulates 11 early neural transcription factors. Overexpression of the *foxD4L1* 3'UTR leads to upregulation of genes in the early neural GRN. Analysis of the *foxD4L1* 3'UTR sequence revealed potential binding sites for several miRs within a "hot-spot" region; current studies assess the functional significance of translational control at this region. Overall, our results suggest that miRs contribute to cell fate specification and the restriction of developmental potency.

Program Abstract #548

Androgens alter the promoter activity of anti-Müllerian hormone (AMH) and Cyp26b1 genes in prepubertal Sertoli cells

Nadia Edelsztein[1], Helena Schteingart[1], Rodolfo Rey[1,2]

[1]Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) - CONICET - FEI, Hospital de Niños Ricardo Gutiérrez, Argentina; [2]Dpto. Biología Celular, Histología, Embriología y Genética, Facultad de Medicina, Universidad de Buenos Aires, Argentina

In the testis, pubertal Sertoli cell maturation is characterized by a decrease in AMH expression at the time when germ cells enter meiosis induced by retinoic acid (RA). In earlier stages of development, RA action is blocked by CYP26B1 expressed in peritubular and Sertoli cells. Since AMH inhibition and meiosis initiation are simultaneous, we sought to test if androgens could have an inhibitory effect on both *AMH* and *Cyp26b1* gene regulation. To test androgen effect on *AMH* promoter activity, a prepubertal Sertoli cell line (SMAT1) was transiently co-transfected with the androgen receptor (AR) and *AMH* promoter-luciferase constructs. Treatment with dihydrotestosterone (DHT, 10^{-7} M) resulted in a significant decrease in the activity of a (-3068 to -1)bp- and a (-423 to -1)bp-*AMH* promoter, but not of a (-3068 to -1916)bp promoter, suggesting that the proximal region of the *AMH* promoter is involved in the inhibition. Since this region lacks known androgen response elements, we tested if inhibition could be due to interaction of the AR with *AMH*-transactivating factors. DHT inhibition persisted when GATA4- and AP1-, but not SF1-, sites were mutated. Regarding androgen effect on the *Cyp26b1* promoter, in conflict with our hypothesis, a significant increase in promoter activity was seen for the constructs including ~9kb 5'-upstream of the first ATG or 3kb, 2.2kb, 1.3kb or 0.6 kb 5'-upstream of *Cyp26b1* promoter, showing a positive regulation of *Cyp26b1* by androgens. DHT treatment inhibits *AMH* but increases *Cyp26b1* promoter activity in presence of the AR in SMAT1 cells. AMH inhibition by androgens requires intact SF1 sequences in the proximal region of the *AMH* promoter.

Supported by grants of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), Argentina

Program Abstract #549

Analysis of cis-regulatory modules in *Drosophila* oogenesis: patterning, distributions, and perturbation tools

Nicole Pope, Robert A. Marmion, Maira Farhat, Nayab Kazmi, Nir Yakoby

Rutgers University- Camden, USA

A monolayer of epithelial cells surrounding the developing oocyte, the follicle cells, are extensively patterned before folding into the *Drosophila* eggshell. Follicle cell patterning has been extensively studied, but the spatiotemporal regulation of these genes remains mostly unknown. The FlyLight collection contains over 7,000 intergenic and intronic DNA fragments that can potentially drive the transcription factor GAL4. We cross listed the 84 genes known to be expressed during oogenesis with the 1200 genes in the FlyLight collection and found 22 common genes that are represented by 281 lines. Of these lines, 61 show expression patterns in the follicle cells when crossed to a UAS-GFP reporter. Of the 61 lines, 19 recapitulate the full or partial pattern of the endogenous gene pattern. Interestingly, while the average DNA fragment is ~3kb in length, the vast majority of fragments show one type of a spatiotemporal pattern. Mapping the distribution of all 61 lines, we found a significant enrichment of enhancers in the first intron in comparison to the 5' proximal or distal regions in the genes' model. All of the lines drive a GAL4 transcription factor, thus offering valuable tools to disrupt the tissue in a domain-specific manner. Our screen provides further evidence that complex gene-patterns are assembled combinatorially by different enhancers controlling the expression of genes in simple domains.

Program Abstract #550

Uncovering dynamic chromatin changes underlying mammalian sex determination

Isabella Salamone, Alexandra García-Moreno, Danielle Maatouk

Northwestern University, USA

Spatial organization within the nucleus is a critical regulator of chromatin function and gene regulation. This is seen during cellular differentiation, wherein genes have been observed moving towards or away from the nuclear periphery coupled with changes in their transcriptional profiles. Despite our growing understanding of functional nuclear domains and their impact on gene expression, the role of nuclear organization during the initiation and maintenance of differentiated cell fates *in vivo* remains unclear. An ideal model system to study this is mammalian sex determination, during which a population of bipotential progenitor cells directs the fetal gonad to become either testis or ovary. XX and XY gonads, initially indistinguishable, express components of the developmental pathways of both sexes, suggesting that progenitor cells are poised to initiate either fate. In XY gonads, expression of *Sry* triggers progenitor differentiation into

Sertoli cells; lacking *Sry*, XX progenitors differentiate into pregranulosa cells. Highlighting the true plasticity of this system, mutation of sex-determining (SD) genes such as *Sry* leads to male-to-female sex reversal at the SD stage. However, mutation of female SD genes does not disrupt initial progenitor cell differentiation and female-to-male sex reversal occurs postnatally. The ability of the adult gonad to transdifferentiate suggests that XX and XY gonadal cells retain a memory of their progenitor state. To investigate the role of chromatin organization in driving this memory, we are using 3D DNA-FISH to examine gene localization as well as local enhancer-promoter looping at key SD genes. Our initial results show that sex-specific genes repressed after sex determination do not localize to the nuclear periphery in either XX or XY supporting cells. This more interiorly positioning, perhaps poising genes for rapid reactivation, serves to retain the balance between differentiated cell fates and maintenance of plasticity.

Program Abstract #551

Fam172a is critically required for neural crest cell development and male sex determination in mice.

Catherine Bélanger, Félix-Antoine Bérubé-Simard, Élizabeth Leduc, Nicolas Pilon
Université du Québec à Montréal, Canada

Via an insertional mutagenesis screen for genes with key roles in neural crest cells (NCCs), we have obtained a mouse line – named *Toupee* – in which homozygotes exhibit multiple NCC defects. This screen was based on the random insertion of a *Tyrosinase* (*Tyr*) minigene in the FVB genetic background. Owing to its specific expression in melanocytes, the *Tyr* minigene rescues the albino phenotype of FVB mice and thus provides a visible – and generally uniform – pigmentation marker for transgenesis. Since melanocytes are derived from NCCs, this genetic tool also proved to be a potent indicator of abnormal NCC development via identification of non-uniform pigmentation patterns. The *Toupee* transgene insertion site is located in *Fam172a*, resulting in the almost complete loss-of-expression of this poorly characterized gene. In addition to multiple NCC-related malformations notably affecting the cranio-facial skeleton, cranial nerves and inner ears, homozygous *Toupee* mice (*Toupee*$^{Tg/Tg}$) also exhibit decreased fertility due to genital anomalies affecting both males (e.g. cryptorchidism and germ cell loss) and females (e.g. delayed puberty and smaller uterine horns). These genital anomalies together with our observation that the sex ratio of *Toupee*$^{Tg/Tg}$ mice is distorted towards females prompted us to evaluate the concordance between phenotypic and chromosomal sex. Very interestingly, PCR-based sexing revealed that approximately 25% of Toupee$^{Tg/Tg}$ males are phenotypically females. Finally, to gain insight into the molecular function of Fam172a, we performed MBP-mediated pull-down assays coupled to mass spectrometry using extracts of NCC-derived Neuro2a cells. Based on the interactants identified, Fam172a appears to regulate gene expression at multiple levels including chromatin remodeling, transcriptional elongation and RNA splicing. Altogether, our data thus indicate that Fam172a is a new regulator of gene expression that is critically required for NCCs and pre-Sertoli cells.

Program Abstract #552

Pdgf1a promotes Hif-1a activation in zebrafish neural crest cells

Jaime A. Espina[1,2], Cristian L. Marchant[1,2], Ariel E. Reyes[1,2]
[1]*Universidad Andrés Bello, Chile;* [2]*Interdiciplinary Center for Aquaculture Research (INCAR), Chile*

The hypoxia inducible factor (HIF-1) is the master regulator of the cellular response to low oxygen levels (hypoxia). This factor is constitutively expressed and traduced, and is degraded by proteasome under normal oxygen levels (normoxia). Under hypoxia, HIF-1α is stabilized, translocates to the nucleus, and regulates genes required for oxygen transport, glucose uptake, and angiogenesis. In neural crest cells (NCCs), Hif-1α regulates the epithelial-mesenchymal transition and chemotaxis. How this factor is stabilized in NCCs *in vivo* is poorly understood. It is known that a) NCCs exist under an epidermal cell layer and b) maximum oxygen exposure in NCC explants causes cells to behave normally, suggesting that Hif-1α is activated by an oxygen-independent mechanism. Multiple oxygen-independent factors regulating Hif-1α have been described *in vitro*. In this study, we searched for these factors *in vivo* in NCCs and assessed relationships with Hif-1α activation. For this, zebrafish NCCs (Sox10+) were isolated by FACS, and the presence of previously described factors was assessed by qPCR. One of the most overrepresented factors was *pdgf1a*, previously described as necessary for correct neural crest development. To test if *pdgf1a* regulates Hif-1α in zebrafish NCCs, morpholino injection fail-of-function experiments for *pdgf1a* were used to analyze genes activated by Hif-1α both generally (i.e. *glut1, glut3, phd3*) and specifically in NCCs (i.e. *twist and cxcr4*). Finally, to corroborate that gene changes were the result of *pdgf1a*- regulated Hif-1α, we co-injected the *pdgf1a* morpholino with *hif-1α* mRNA. *pdgf1a* fail-of-function reduced Hif-1α-activated genes, a reduction restored when *pdgf1a* morpholinos were co-injected with Hif-1α mRNA. Altogether, these data indicate that Pdgf1a regulates Hif-1α in vivo in NCCs.

Program Abstract #553

Signature of homolog pairing in haplotype-resolved chromosome organization

Jelena Erceg[1], Anton Goloborodko[2], Bryan R. Lajoie[3], Geoffrey Fudenberg[2], Nezar Abdennur[2], Maxim Imakaev[2], Ruth B. McCole[1], Son C. Nguyen[1], Eric F. Joyce[1], Tharanga Niroshini Senaratne[1], Mohammed A. Hannan[1], Guy Nir[1], Job Dekker[3], Leonid A. Mirny[2,4], Ting (C.-ting) Wu[1]

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA; [2]Department of Physics, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA; [3]Howard Hughes Medical Institute and Program in Systems Biology, Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605-0103, USA; [4]Institute for Medical Engineering and Science, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA

Nuclear organization involves many chromosomal interactions in three-dimensional space, including interactions that bring the homologous regions of maternal and paternal chromosomes together. The consequences of such homolog interactions, called homology effects, include processes as potent as X-inactivation, parental imprinting, monoallelic expression, and transvection. Nevertheless, the mechanism by which homologs come together remains elusive. Here, we describe Hi-C studies conducted with *Drosophila melanogaster*, which pairs its homologs in all somatic cells throughout development, focusing on the earliest stages of embryogenesis in order to reveal how parental genomes behave when they encounter each other for the first time. In particular, we have generated Hi-C maps distinguishing *cis* and *trans* inter-homolog interactions in a haplotype-resolved manner and, using stringent filtering, have identified a Hi-C signature for inter-homolog interactions, *i.e.* homolog pairing. Our data also indicate prominent contacts between chromosomal arms, consistent with a Rabl configuration. Furthermore, our haplotype-resolved Hi-C maps show highly concordant TADs between maternal and paternal chromosomes, with TAD boundaries correlated with transcriptionally active regions.
This work was supported by NIH/NIGMS (RO1GM085169, 5DP1GM106412) and Quad Seed funding from Harvard Medical School to C.-t.W., an EMBO Long-Term Fellowship to J.E., a William Randolph Hearst Award to R.B.M., and NIH/NCI (Ruth L. Kirschstein NRSA) to E.F.J. Work in the Dekker and Mirny laboratories is supported by the NIH/NHGRI (R01 HG003143, U54 HG007010, U01 HG007910), the NCI (U54 CA193419), the NIH Common Fund (U54 DK107980, U01 DA 040588), the NIGMS (R01 GM 112720), and the NIAID (U01 R01 AI117839).

Program Abstract #554

Programmed DNA elimination in nematodes

Jianbin Wang, Yuanyuan Kang, Richard Davis

University of Colorado School of Medicine, USA

Maintenance of genome integrity is essential. However, programmed DNA elimination removes specific DNA sequences from the genome during early development. In the human and pig parasitic nematode *Ascaris*, we found that ~13% of the genome is eliminated during DNA elimination. The eliminated DNA consists of specific repetitive and unique sequences, including ~700 genes. The eliminated genes are primarily expressed in the germline, suggesting that DNA elimination in *Ascaris* is an irreversible mechanism for silencing a subset of germline-expressed genes in somatic tissues. We identified ~50 sites where chromosomes break and are healed by telomere addition. A closely related horse parasitic nematode *Parascaris* also undergoes DNA elimination. The majority of the DNA breaks and eliminated genes are conserved between *Ascaris* and *Parascaris*, suggesting a regulated and specific mechanism for DNA elimination in these nematodes. Intriguingly, we found no sequence motifs or other characteristics that might mark the conserved breakpoint regions for chromosomal breakage. We hypothesize that (1) histone modifications, (2) small RNAs, (3) chromosome structure and organization, and/or (4) DNA replication timing may be involved in the identification and generation of chromosomal breaks for DNA elimination. To facilitate a better understanding of DNA elimination, we are building chromosomal level genome assemblies using PacBio long reads and BioNano optical maps. A chromosomal level comparison between these nematodes will provide information on DNA elimination and the consequent re-organization of the chromosomes; it will also allow us to determine if DNA elimination serves to generate a specific set of somatic chromosomes (1n = 36) from drastically different numbers of germline chromosomes (1n = 1 vs. 24) in the two nematodes.

Program Abstract #555

Crosstalk Among Helix-Loop-Helix proteins: Lessons from the Da-Emc-Ato regulatory Network in Neuronal Differentiation

Ke Li, Nicholas E. Baker

Albert Einstein College of Medicine, USA

bHLH transcription factors play pivotal roles in a wide range of developmental events. E proteins and Inhibitor of

Differentiation (ID) proteins are broadly expressed dimerization partners of bHLH transcription factors, whose balance defines bHLH-dependent activities. Besides their established roles in development, E- and ID proteins are found disrupted in numerous cancers; emerging evidence also link them to neurodevelopmental disorders and other diseases. Previously we reported an unexpected feedback regulation between Daughterless (Da) and Extra macrochaetae (Emc), respectively the sole E- and ID protein in *Drosophila*. We hypothesize that the balance of E/Da proteins and ID/Emc proteins is a key aspect of cellular regulation, possibly acting throughout life beyond developmental fate specification. Although Da has been long studied as a transcriptional regulator, we found Da regulates *emc* both transcriptionally and post-transcriptionally. By performing gene dose experiments *in vivo*, we found Da is the dominant determinant of Emc protein levels. Interestingly, Emc is short-lived and becomes stable when dimerized with Da. We further discovered that Atonal (Ato), a proneural bHLH protein, interferes with Emc stabilization. Our results suggested that proneural proteins may destabilize Emc by competing to dimerize with Da, which could be a general mechanism for bHLH proteins interaction and regulation. Our data also indicated a novel mechanism other than the canonical transcriptional regulation in neuronal differentiation. Our long-term goal is to understand every aspect of bHLH proteins regulation and their relationship to diseases. This work is funded by NIH grant GM047892 and by an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology and Visual Sciences.

Program Abstract #556

Control of endoderm gene expression by oocyte-localized transcription factors in *Xenopus tropicalis*

Kitt Paraiso, Masani Coley, Ira L. Blitz, Margaret B. Fish, Ken W.Y. Cho

University of California, Irvine, USA

Metazoans rely on localization of specific mRNA transcripts in the oocyte for germ layer patterning. Many of these encode transcription factors that regulate germ layer specific expression in the embryo. We screened for transcription factor mRNAs localized in the vegetal region of *Xenopus tropicalis* and found that a very small percentage of known transcription factors are localized and only a handful of these are expressed at relatively high levels: Vegt, Sox7 and Otx1. We sought to identify the individual contributions of these three factors in inducing endodermal gene expression. Experiments utilizing ectopic expression in the future ectoderm reveal that the three factors induce different subsets of endodermal genes, suggesting that they are responsible for different sub-networks of the endodermal gene regulatory network. Additionally, identification of transcription factor binding sites using ChIP shows that these factors interact with the same cis-regulatory regions, indicating a possible requirement for combinatorial action of these factors to achieve proper expression of endodermal genes. Together, these experiments further elucidate the control of gene expression in the endoderm lineage during germ layer formation.

These studies were supported by NIH HD073179 and KDP is a recipient of NIH T32 HD60555.

Program Abstract #557

TAEL: A zebrafish-optimized optogenetic gene expression system with fine spatial and temporal control

Stephanie Woo[1], Anna Reade[1], Laura Motta-Mena[2], Kevin Gardner[2], Didier Stainier[3], Orion Weiner[1]

[1]Univ of California-San Francisco, USA; [2]City University of New York, USA; [3]Max Planck Institute for Heart and Lung Research, Germany

Here we describe an optogenetic gene expression system optimized for use in zebrafish. This system overcomes the limitations of current inducible expression systems by enabling robust spatial and temporal regulation of gene expression in living organisms. Existing optogenetic systems cause toxicity in zebrafish, therefore, we re-engineered the blue-light activated EL222 system, renamed TAEL, to have minimal toxicity with a large range of induction, fine spatial precision, and rapid kinetics. We validate several strategies to spatially restrict illumination and thus gene induction with the TAEL system. As a functional example, we show that TAEL is able to induce ectopic endodermal cells in the presumptive ectoderm via targeted *sox32* induction. We also demonstrate that TAEL can be used to resolve multiple roles of Nodal signaling at different stages of embryonic development. Finally, we show how inducible gene editing can be achieved by combining the TAEL and CRISPR/Cas9 systems. This toolkit should be a broadly useful resource for the fish community.

Program Abstract #558

The Gene Expression Database (GXD): integrated access to mouse developmental data.

Jacqueline Finger, Terry Hayamizu, Ingeborg McCright, Constance Smith, Jingxia Xu, Janan Eppig, James Kadin, Joel Richardson, Martin Ringwald

The Jackson Laboratory, USA

The Gene Expression Database (GXD) provides the developmental biology research community with an extensive,

integrated, and curated resource of mouse expression information. Curators collect expression data from the literature and through collaboration with large-scale data providers. These data are from RNA in situ hybridization, immunohistochemistry, reporter knock-in, northern, western and RT-PCR experiments and are integrated using standard genetic nomenclature, controlled vocabularies, and an extensive anatomical ontology. Currently, GXD contains 277,372 expression images and over 1.5 million annotated expression results for 14,098 genes covering all stages of mouse development, with data from wild-type mice and from 2,402 mouse mutants. GXD is part of the Mouse Genome Informatics (MGI) resource (www.informatics.jax.org). Thus, the expression data are integrated with genetic and phenotypic information. The Gene Expression Data Query Form allows many types of searches. One can search for expression data for specific genes or sets of genes. Gene sets can be based on gene nomenclature, on functional, phenotypic, or disease classification, or they can be user-defined lists of genes uploaded using the new batch search utility. One can search for expression in anatomical structures and developmental stages and/or for expression in wild-type mice or in specific mouse mutants. By combining parameters very specific queries can be built. Search results are represented at different levels of detail via tabbed data summaries for genes, assays, assay results, images, and tissue x gene and tissue x stage matrix views. Summaries can be sorted in different ways and interactively refined using data filters. Following links from summaries to assay details, users can see expression images together with their annotations. Visit the GXD Home Page at www.informatics.jax.org/expression.shtml to explore GXD's utilities. GXD is supported by NIH/NICHD grant HD062499.

Program Abstract #559
Rapid Diversification of Endogenous Retrotransposon-Driven Gene Networks in Mammalian Oogenesis and Early Development
Alexei Evsikov
University of South Florida, USA
Retrotransposons profoundly impact mammalian gene expression, notably in germ cells. Previously, we reported specific classes of LTR retrotransposons initiate regulated expression of multiple genes during the egg-to-embryo transition. Recent experimental work demonstrated co-option of retrotransposon promoters directly impacts mouse oocyte physiology. We tested the hypothesis that transposons, by acting as alternative promotors, establish functional modules within gene networks responsible for critical events during oogenesis and egg-to-embryo transition. We analyzed transposon-driven gene expression in the oocytes from four mammals: mice, yaks, cows and humans. We developed a pipeline for discovery and mapping gene transcripts containing alternative transposon-derived exons at the 5'-ends. We used this pipeline to analyze RNAseq data of total oocyte RNA in each species. Gene Ontology-based pathway enrichment tools were used to find network modules associated with these genes. Our analysis demonstrated alternative promoters from transposons drive a large proportion of expressed genes (5-10%) across all species. Remarkably, subsets of transposon-driven genes are substantially different among these species and overlaps are significantly smaller than expected. Network enrichment analysis using GO revealed distinct functional modules among transposon-driven genes in each species (e.g., RNAi pathway module is present in mice, but not bovids or humans). The profound dissimilarities among gene sets and modules indicate independent origins putatively shaped by natural selection in the case of humans and artificial selection pressures for cows, yaks and inbred laboratory mice. Our data further underscores mammalian oogenesis as unique system using novel, retrotransposon-driven, modules within functional gene networks.

Program Abstract #560
The Compensatory Embryonic Response to Perturbation of the Notch Signaling Pathway
Andrew Halleran, Caroline Golino, Brian Rabe, Ron Smith, Joshua Puzey, Greg Smith, Margaret Saha
College of William and Mary, USA
The Notch signaling pathway is conserved across all metazoans and plays a critical role in the establishment of neuronal patterning. Mis-expression of Notch at the two-cell stage in *Xenopus laevis* embryos initially alters the ratio of differentiated neuron and neural progenitor cells observed at neurula and early tailbud stages. However, as development progresses past tailbud stages the imbalance in differentiated neurons lessens and by swimming tadpole stages is no longer detectable by in situ hybridization or qRT-PCR. Using an RNA-Sequencing approach we have identified molecular pathways of interest implicated in the observed compensatory response to Notch signaling perturbation. Exploiting *Xenopus laevis*' pseudo-tetraploid genome as an ideal animal system to study polyploidy, further genomic and transcriptomic analysis has been performed to identify the extent of homeolog expression bias throughout the course of *Xenopus laevis* development. Funding Sources: Beckman Scholars Program, Arnold and Mabel Beckman Foundation

to AH; The Howard Hughes Medical Institute Science Education Program Grant to the College of William and Mary; NSF funding to MS (NSF 1257895).

Program Abstract #561

Using ATAC-seq to detect novel gene regulatory elements in development and its application for studying lens formation in *Xenopus tropicalis*

Sumanth Manohar[1], Takuya Nakayama[1], Ann Rose Bright[2], Marilyn Fisher[1], Gert Jan C. Veenstra[2], Robert M. Grainger[1]
[1]University of Virginia, USA; [2]Radboud Institute for Molecular Life Sciences, Netherlands

The mechanisms leading to cell determination remain largely unsolved, including genes and their regulatory elements (RE's) underlying this process. Lens development is an exceptional model to study these mechanisms. A classical approach to identify RE's relies on finding conserved regions among organisms but overlooks RE's that are not highly conserved. *In vivo* genome analyses such as ChIP-seq, DNase-seq, and recently ATAC-seq have provided us with tools to identify functional RE's. Using ATAC-seq we are tracking the epigenetic landscape identifying stable or dynamic regions of accessible chromatin during lens commitment. As expected, an ATAC-peak is localized on a conserved enhancer (CE1) previously identified for the key lens specification gene *foxe3* (Ogino *et al*., '08), indicating a link between ATAC-peak and enhancer activity. We noticed many ATAC-peaks on regions that are not broadly conserved among different organisms and theorized that such "non-conserved" peaks may correspond to unidentified enhancers. Many such "non-conserved" peaks are actually conserved with *X. laevis*, allowing us to use genome comparisons to identify putative transcription factor (TF) binding sites, providing a powerful tool to find potential enhancers. Among those, OCE1 (Open Chromatin Element1) is located between CE1 and the *foxe3* promoter and drives expression in the lens when fused to a heterologous promoter-GFP reporter gene. In silico analysis of OCE1 predicted several TF binding sites, including a putative otx/pitx site, which in CE1 has been shown to be crucial for lens formation. Currently we are identifying other enhancers based on above-mentioned criteria, performing functional studies by mutation analysis and using CRISPR to generate enhancer deletion mutants. This strategy could be a generally powerful tool to understand GRNs in development.
Funding: GJCV - EU PP FP7 REA 607142DC; RMG - Sharon Stewart Trust, NIH R01 EY017400, EY018000, EY022954

Program Abstract #562

Functional and evolutionary insights from a comprehensive *Ciona* notochord transcriptome

Wendy Reeves, Matthew Harder, Yuye Wu, Michael Veeman
Kansas State University, USA

Notochord fate specification in the simple chordate *Ciona* is relatively well understood, but the gene regulatory networks driving morphogenesis and differentiation are largely undefined. Previous studies have identified fewer than 100 notochord upregulated genes. To define a comprehensive notochord transcriptome, we performed RNAseq on flow-sorted GFP-expressing notochord cells from timepoints spanning key steps in notochord morphogenesis and identified >1300 enriched genes. To validate this dataset, we have tested 60 genes from two timepoints by in situ hybridization and confirmed that ~90% are detectably enriched in the notochord. We have also identified several genes showing regionalized expression within the notochord, supporting our previous work that this is not a spatially homogenous organ. We compared our gene set to previously identified mouse notochord genes and identified 26 evolutionarily conserved notochord genes that are strong candidates for future functional studies. To test whether gene expression in the notochord depends upon ongoing morphogenesis, we electroporated *Ciona* embryos with Bra>Prickle, which disrupts intercalation via the PCP pathway, and performed RNAseq on whole embryos. Strikingly, we detected no transcriptional differences between experimental and control embryos, suggesting that the notochord GRN does not involve a morphogenetic checkpoint for the successful completion of intercalation. We also revisited a famous *Ciona* experiment based on ectopic expression of the key notochord transcription factor Brachyury but assessed the transcriptional differences by RNAseq. We found that relatively few of the notochord-specific genes we have identified by RNAseq on sorted cells were among the 926 induced by Brachyury overexpression, suggesting that the notochord GRN is deep and complex.
Funding: Morphogenetic effector networks in the *Ciona* notochord (NIH 1R01HD085909), CMADP COBRE (NIH P20GM103638), Kansas INBRE (P20GM103418).

Program Abstract #563

High-throughput spatial cis-regulatory analysis by turning chaos into order

Catherine Guay, Sean McQuade, Jongmin Nam
Rutgers, USA

Cis-regulatory elements (CREs) are essential for cell-type specific gene expression patterns and contain abundant gene regulatory information in their DNA sequences. The biggest roadblock for the efficient utilization of gene regulatory information contained within CREs is the lack of a high-throughput method for *cis*-regulatory analysis. To address this critical challenge, we have developed a novel high-throughput and quantitative method for spatial *cis*-regulatory analysis using sea urchin embryos as a test bed. The new method takes advantage of i) stochastic and mosaic incorporation of reporter constructs in early embryos upon transgenesis and ii) a novel method for high-throughput, single embryo-resolution measurement of the copy numbers of expressed and incorporated reporter constructs in many mosaic embryos. Because the level of reporter expression in an embryo is determined by the combination of intrinsic activity of a given CRE and cells that harbor the construct at the time of measurement, we hypothesized that the profile of reporter expressions measured at single-embryo resolution in a sufficiently large number of mosaic embryos is determined solely by spatial activity of a given CRE. Our proof-of-principle experiment showed that the new method can rapidly classify ≥100 CRE::reporter constructs based on their spatial activities indepentent of imaging tools. The new method has the potential to increase the throughput of spatial *cis*-regulatory analysis at least three orders of magnitude compared to traditional imaging-based analyses. We anticipate that the new method can vastly accelerate discovery of cell-type specific CREs, identification of gene regulatory networks, and evolutionary comparison of CRE functions.

Program Abstract #564

Gene dosage of actin-capping protein CAPZB regulates craniofacial morphogenesis

Kusumika Mukherjee[1,3], Kana Ishii[1,3], Michael Talkowski[1,3], Richard Maas[2,3], Cynthia Morton[2,3]
[1]Massachusetts General Hospital, USA; [2]Brigham and Women's Hospital, USA; [3]Harvard Medical School, USA

Craniofacial malformations are among the common congenital anomalies, with a strong genetic basis. While significant advances have been made towards identifying the genetic loci involved in craniofacial development, the role of the cytoskeleton and its modifiers remain poorly understood. Utilizing a functional genomics gene discovery pipeline, an isolated disruption of *CAPZB* was identified in a patient with cleft palate (CP), micrognathia and hypotonia. *CAPZB* encodes an actin capping protein and regulates the growth of actin filaments. We exploited the zebrafish to model the phenotypes of the proband and carried out detailed analysis of developmental processes to determine the function of *capzb* in craniofacial morphogenesis. The spatiotemporal gene expression of *capzb* shows maternal inheritance and ubiquitous expression. Analysis of the musculo-skeletal structures of *capzb* mutants generated from an insertional mutagenesis screen show that the lower jaw elements are smaller and retrusive (micrognathia), the palate is only partially fused with a cleft in the anterior palate (CP) and the myofibrils are highly disorganized (hypotonia). Lineage tracing experiments suggest that defective migration of the cranial neural crest cells (CNCCs) cause the CP. Importantly, transient overexpression of *capzb* by mRNA injection in zebrafish also perturbs normal craniofacial development, suggesting that a correct dosage of *capzb* is critical to CNCC development and maintenance. Experiments are currently underway to further test the effects of gain of function of *capzb* in craniofacial morphogenesis. Taken together, this study identifies that the gene dosage of *CAPZB* is important in morphogenesis, excess or deficiency of which is pathologic for development. It also underscores how disruption of a basic cellular process like regulation of actin cytoskeletal dynamics by modulating the dosage of the actin-capping protein *CAPZB* can cause distinct morphologic derangements in an organism.

Program Abstract #565

Interplay of BMP and JAK/STAT in Developmentally Regulated Apoptosis

Alexandra Mascaro, Antoine Borensztejn, Kristi Wharton
Brown University, United States of America

Somatic follicle cells are critical for multiple aspects of oocyte development in the *Drosophila* oovary, from generating specialized structures of the eggshell to establishing signaling centers that initiate the future A/P and D/V axes of the embryo. A group of one somatic cell type, the stalk cell, form a linear structure that separates developing egg chambers such that each produces a properly patterned mature oocyte. Without an interfollicular stalk, egg chambers can merge, which disrupts their highly organized structure, and fail to produce a viable oocyte. We found that during normal oogenesis the stalk cells within a single interfollicular stalk are produced in excess and reduce in number as oogenesis proceeds. This reduction arises as a result of apoptosis, a process critical in many developmental events requiring defined numbers of cells as well as sculpting organs. Interestingly, while stalk cell death occurs normally, mechanisms are in place to prevent an excessive loss. We find that excessive apoptosis is prevented by JAK/STAT signaling, a process distinct from its proapototic role in defining exactly two polar cells at the anterior and posterior poles of each egg chamber. We find that BMP signaling is also required to inhibit abnormal loss of stalk cells through apoptosis. If either JAK/STAT signaling or BMP signaling is specifically reduced in the stalk cells, we observe a more significant loss of

stalk cells and the complete fusion of egg chambers may occur. The converse is true when JAK/STAT signaling or BMP signaling is increased. The reduced numbers of stalk cells observed when knocking down JAK/STAT signaling can be rescued through the overexpression of the BMP 5/6/7 ortholog Gbb. Similarly, the 'long' stalks produced by overactivation of JAK/STAT signaling can be suppressed by downregulation of *gbb*, supporting an intimate relationship between these two signaling pathways in defining the precise number of cells that make up interfollicular stalks.

Program Abstract #566

Minibrain and Wings apart control organ growth and tissue patterning through downregulation of transcriptional repressor Capicua

Liu Yang[1], Sayantanee Paul[1], Kenneth Trieu[1], Francesca Froldi[2], Kieran Harvey[2], Louise Y. Cheng[2], Gerardo Jimenez[3,4], Stanislav Y. Shvartsman[5], Alexey Veraksa[1]

[1]University of Massachusetts Boston, USA; [2]Peter MacCallum Cancer Centre, Australia; [3]Institut de Biologia Molecular de Barcelona-CSIC, Spain; [4]Institució Catalana de Recerca i Estudis Avançats (ICREA), Spain; [5]Princeton University, USA

The transcriptional repressor Capicua (Cic) controls tissue patterning and restricts organ growth, and has been recently implicated in human brain cancer. Cic has emerged as a primary sensor of signaling downstream of the receptor tyrosine kinase (RTK)/extracellular signal-regulated kinase (ERK) pathway, but how Cic activity is regulated in different cellular contexts remains poorly understood. We found that the *Drosophila* kinase Minibrain (Mnb, ortholog of mammalian DYRK1A), acting through an adaptor protein Wings apart (Wap), physically interacts with and phosphorylates the Cic protein. Mnb and Wap inhibit Cic function by limiting its transcriptional repressor activity. Downregulation of Cic by Mnb/Wap is necessary for promoting the growth of multiple organs, including the wings, eyes, and the brain, and for proper tissue patterning in the wing. Sites of Cic phosphorylation targeted by Mnb do not overlap with those targeted by ERK. We have thus uncovered a previously unknown mechanism of downregulation of Cic activity by Mnb and Wap. This mechanism operates in parallel to ERK-dependent control of Cic, indicating that Cic functions as an integrator of upstream kinase signals that are essential for tissue patterning and organ growth. Finally, since DYRK1A and CIC exhibit, respectively, pro-oncogenic versus tumor suppressor activities in human oligodendroglioma, our results raise the possibility that DYRK1A may also downregulate CIC in human cells.

Program Abstract #567

The role of IMPDH polymerization in *Drosophila* oogenesis

Jacqueline Simonet[1], Sajitha Anthony[2], Jeffrey Peterson[1]

[1]Fox Chase Cancer Center, USA; [2]Drexel University College of Medicine, USA

CTP synthase (CTPS) and inosine monophosphate dehydrogenase (IMPDH) are two rate-limited enzymes in nucleotide biosynthesis, which have been found to polymerize into filaments under conditions of nucleotide depletion or elevated demand for nucleotides in many different species and cell types. For example, these enzymes polymerize in nutrient-starved mammalian cells lines and CTPS polymerizes during normal *Drosophila* oogenesis, where germ cells undergo rapid cycles of endoreplication and rRNA synthesis. Our lab has demonstrated that these assemblies are present during *Drosophila* egg development and has pioneered the use of this model system to understand their function and regulation. Our hypothesis, based on accumulating biochemical and genetic data, is that filament assembly enhances their nucleotide biosynthesis activity and is necessary to maintain adequate nucleotide levels in growing and proliferating cells. In order to test how and why these filaments are assembled under normal biological conditions I am utilizing mutants of the single *Drosophila* IMPDH gene as well as functional rescue with transgenic expression of human IMPDH2 constructs to assess the role of IMPDH filament assembly in oogenesis. I will present progress toward generating transgenic *Drosophila* expressing point mutants of human IMPDH2 that either inhibit or promote filament assembly without abolishing enzyme activity. This will allow me to assess the role of assembly in a biologically important in vivo context. In parallel we are evaluating the effect of these mutants on assembly and catalytic activity in vitro. These experiments will provide insight into the biological function of IMPDH filaments in vivo in a cell type undergoing rapid cycles of genomic replication and RNA synthesis. Thus, it may provide insights the developmental regulation of an evolutionarily conserved pathway important for endoreplication, cell proliferation, and growth. Funded by: R01 GM083025 and T32 CA009035-41

Program Abstract #568

Rho and Ras signaling regulate *Caenorhabditis elegans* ovulation

Alyssa Cecchetelli, Coleman Clifford, Erin J Cram

Northeastern University, USA

Mechanotransduction, converting a mechanical signal into a biochemical response, is vital for proper development, tissue function and homeostasis. Defects in a cell's ability to sense and respond to external stimuli can lead to complications in development and diseases such as asthma. The *C. elegans* spermatheca, the site of fertilization within the worm, provides an ideal *in vivo* model to study this biological process. Stretch of the incoming oocyte is converted into waves of calcium potentiated by PLC-1 that culminates in acto-myosin contractility and expulsion of fertilized embryos into the uterus. What remains unknown however, is what activates PLC-1. PLC-1 is a multi-domain protein that can respond to both calcium and small GTPases, such as RHO-1/RhoA and LET-60/Ras. Overexpression of a RHO-1 gain of function (gf) allele and RHO-1 depletion via RNAi results in a highly constricted spermathecae with rapid transit and embryo trapping respectively. RHO-1 is required for normal calcium oscillations, however it may not be working through PLC-1 because deletion of the unique RHO-1 interaction site of PLC-1 does not result in a defective phenotype. In contrast, deletion of the CDC25 Ras guanine nucleotide exchange factor (GEF) domain of PLC-1 causes embryo trapping and broodsize defects comparable to the *plc-1*-null mutant. LET-60/Ras depletion via RNAi also leads to embryo trapping and abnormal ovulations. These results suggest a role for Ras signaling in the activation of PLC-1. Given the conservation of PLC-1, RHO-1, LET-60 and the pathway that regulates *C. elegans* ovulation, this work should provide insights into the biological mechanisms that regulate mechanotransduction *in vivo*. This work is currently funded by the NIH R01 grant #GM110268-01.

Program Abstract #569
Investigating the self-correction of craniofacial defects in pre-metamorphic *Xenopus laevis* tadpoles
Kaylinnette Pinet, Kelly McLaughlin
Tufts University, USA
In the wake of a recent spike in reported microcephaly cases, the frequency of craniofacial birth defects is now greater than 1 in every six hundred births worldwide. Currently there are little to no medical treatment options for children born with craniofacial defects such as microcephaly, cleft palate, and fetal alcohol syndrome. Thus, research that can lead to novel or improved treatment options for craniofacial abnormalities has never been more critical. Over the past decades, there have been numerous studies that focused on understanding the causes of craniofacial defects observed in vertebrate model organisms. Amongst these studies, only one has provided evidence of an animal being able to naturally remodel malformed craniofacial structures. This study (Vandenberg *et al.*, 2012) revealed that pre-metamorphic *X. laevis* tadpoles are capable of self-correcting craniofacial defects caused by a specific genetic perturbation applied during embryogenesis. Despite this initial observation, the underlying mechanisms that regulate this self-correction, as well as the limitations of this remodeling response, have not been characterized. Our lab has since established that pre-metamorphic *X. laevis* tadpoles are able to self-correct some, but not all, malformed craniofacial features resulting from a range of genetic, mechanical, or chemical perturbations. Our current research aims to elucidate the mechanisms that mediate the ability to remodel mispatterned craniofacial tissues. By fully understanding how pre-metamorphic *X. laevis* tadpoles detect, and subsequently correct, abnormal craniofacial morphology, we will provide valuable knowledge for establishing better or novel treatments for craniofacial abnormalities in humans.

Program Abstract #570
Dynamic multiplexing enables ligand discrimination in the Notch pathway
Nagarajan Nandagopal
California Institute of Technology, USA
A common feature of all developmental signaling pathways is their use of multiple receptors and ligands. These receptors and ligands often interact promiscuously with each other, and yet ligands cannot functionally replace each other in many contexts, suggesting that they induce different responses in signal-receiving cells. How can different ligands activate such different responses, especially through the same receptors? This behavior is particularly puzzling in the Notch pathway, because all ligands activate Notch through the same biochemical mechanism, providing no obvious means of discriminating among them. Here, we show that the Notch pathway uses *dynamic multiplexing* to discern ligand identity: the ligands Dll1 and Dll4 activate Notch1 with different dynamics, which produce distinct patterns of gene expression and cell fate decisions in signal-receiving cells. Using quantitative single-cell imaging, we found that Dll1 activates Notch1 in discrete, frequency-modulated pulses that selectively activate the Notch target gene Hes1. By contrast, Dll4 generates sustained, amplitude-modulated Notch1 activation that up-regulates the targets Hey1 and HeyL. The functional consequences of ligand discrimination could be observed in developing chick embryos, where expression of Dll1 in neural crest cells enhanced myogenic differentiation in somites, while expression of Dll4 inhibited this process. These results could help explain the use of Notch ligands like Dll1 and Dll4 in specific developmental contexts and, more generally,

provide a new framework to understand the use of distinct receptor-ligand combinations in development, physiology, and disease.

Program Abstract #571
Role of the ERK/MAPK pathway in hematopoiesis.
Jean Charron[1,2], Laurent Beret[1], Simon-Pierre Fortier-Beaulieu[1], Rifdat Aside[1], Émilie Pic[1]
[1]Centre de recherche sur le cancer de l'Université Laval, CA; [2]CHU de Québec, L'Hôtel-Dieu de Québec, CA
The MEK1 and MEK2 dual-specificity kinases responsible for ERK/MAP kinase activation are involved in diverse physiological outputs including cell fate determination, differentiation, proliferation, survival, migration, growth, and apoptosis. To investigate the role of the ERK/MAPK pathway in hematopoiesis, we performed the tissue-specific deletion of *Mek1* in hematopoietic cell lineages in a *Mek2* null background using the *Vav-iCre*. $Mek1^{f/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ embryos died in the first 24 hrs of life presenting a severe anemia. In contrast, mutant mice carrying one functional allele of *Mek1* ($Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$) or *Mek2* ($Mek1^{f/f}Mek2^{+/-}Tg^{+/Vav\text{-}iCre}$) were viable. However, both triple mutants presented splenomegaly and perturbed homeostasis of blood cell populations. 50% of the $Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ females developed a severe anemia between 5 and 14 months of life. Already at 4 months of age, $Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ females showed reduced hematocrit and hemoglobin levels. The number of bone marrow and spleen hematopoietic progenitors appeared normal as well as the erythropoiesis responses to splenic stress and erythropoietin. The necropsy of $Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ females revealed a severe glomerulonephritis with fibrosis. The glomerulonephritis was associated with deposits of antibodies. $Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ mutants also developed autoantibodies against nuclear proteins and dsDNA, a hallmark of autoimmune diseases such as the systemic lupus erythematosus (SLE). A normal proportion of the immune cells was maintained in $Mek1^{+/f}Mek2^{-/-}Tg^{+/Vav\text{-}iCre}$ mutant spleens, but the absolute numbers of B cells, $CD4^+$ and $CD8^+$ T cells, and myeloid cells were increased. Thus, deregulation of the ERK/MAPK pathway in hematopoietic cell lineages perturbs myeloid cell functions and affects B and T cell differentiation causing phenotype reminiscent of SLE-like syndrome. (Supported by CIHR)

Program Abstract #572
The Role of the Plexin A family in zebrafish eye development
Sarah Emerson, Sarah Light, Riley St. Clair, Bryan Ballif, Alicia Ebert
University of Vermont, USA
During development, migrating neurons navigate to their correct synaptic targets using a variety of transmembrane and secreted guidance cues in their environment. Plexins (Plxns) and Semaphorins (Semas) are a family of signaling factors that were initially discovered to be repulsive signals for migrating neurons by influencing actin dynamics at the growth cone. It is becoming widely appreciated that Plxns and Semas have a much broader role in development than simply axon guidance. Using a combination of Morpholino knockdowns*, in situ* hybridization and immunohistochemistry in zebrafish, we have uncovered a novel early role for PlxnA2 and Sema6A in maintaining proper cohesion and proliferation of migrating optic vesicles during early eye development. In a microarray using RNA extracted from un-injected control and morphant fish, we have generated a dataset of downstream genes that are differentially regulated by PlxnA2 and Sema6A signaling, and have focused on characterizing genes that are known to be involved in cellular migration and proliferation. We have shown that one such gene, *Rasl11b*, negatively regulates cellular proliferation. We have also shown that PlexinA1 has a compensatory role for PlexinA2 in this system. Further work will investigate the expression patterns and roles of the remaining Plexin A family members in eye development.
Funding- NIH 1456846

Program Abstract #573
Biochemical and Functional Characterization of PlexinA2 Tyrosine Phosphorylation in Semaphorin6A Signaling
Riley St. Clair, Marion Weir, Alicia Ebert
University of Vermont, USA
The precise wiring of neuronal processes during development is essential for every task of the nervous system, from movement and sensation to emotion and cognitive functioning. In order to make accurate connections and develop a healthy and functional nervous system, migrating neurons must respond appropriately to extracellular cues. Using the zebrafish as a model, we have shown that one such signaling transduction pathway, Semaphorin6A-PlexinA2 (Sema6A-PlxnA2), is critical for vertebrate eye development. However, the mechanisms underlying this pathway are not yet fully understood. Sema6A is a membrane-bound guidance molecule that regulates neuronal migration upon binding to the receptor PlxnA2. PlxnA2 is hypothesized to interact with the src-family tyrosine kinase Fyn to initiate downstream

signaling, ultimately destabilizing the cytoskeleton to induce growth cone collapse. We and others have shown that Fyn can phosphorylate PlxnA2. However, the mechanisms of this interaction are not well characterized. The objective of this study is to determine the functionally-important phosphorylation events between PlxnA2 and Fyn. Bioinformatics and mass spectrometry data identified potential residues that may be phosphorylated by Fyn. We show results testing these proposed phosphorylation sites on PlxnA2. We also show preliminary work using cellular assays and PlxnA2$^{-/-}$ CRISPR zebrafish to determine the functional relevance of these phosphorylation events in the development of the vertebrate visual system.

Program Abstract #574

Model of lymphedema and rescue by regulation of MEK/ERK activity

Joanne Chan[1,2], Amrita Dasgupta[1], Stanley Hazy[1], John Mably[2]

[1]Skin of Color Research Institute, Hampton University, Hampton, VA, USA; [2]Department of Biological Sciences, Hampton University, Hampton, VA, USA

The lymphatic system transports fluids and facilitates the return of extravasated cells and macromolecules back into the blood circulation. Obstructions in lymphatic vessel function, known as lymphedema, can occur as a primary or a secondary disorder. Milroy's disease is an example of a genetic or primary lymphedema where mutations in one allele of the VEGFR3 gene leads to pervasive lymphatic malfunction resulting in the accumulation of fluids in the lower limbs. In zebrafish, this disorder can be modeled by mutations in this receptor or its ligand, VEGF-C. In secondary lymphedema, an injury to lymphatic vessels can occur after surgery, causing a blockage in the lymphatic system and fluid accumulation in affected tissues. In both cases, pressure and massage have been the only treatments over the last 200 years. To understand the signaling pathways that might be involved in inducing lymphedema, we investigated the ability of preclinical cancer drugs targeting the VEGFRs, MAPK/ERK or PI3K/mTOR to disrupt lymphangiogenesis. We found that MEK1/2 inhibition over a 6-hour period at 3 days post fertilization provides a dramatic blockade of lymphatic function that cannot be restored by drug removal. To determine whether transgenic overactivation of these pathways may rescue this phenotype, we created 2 transgenic lines, using the fli1 promoter to drive endothelial expression. Treatment with the MEK1/2 inhibitor typically impeded the formation of the thoracic duct in greater than 80% of zebrafish larvae. In the transgenic fli1::MEK1DD line, this lymphatic vessel is effectively rescued. However, increased endothelial mTORC1 in a fli1::rhebS16H transgenic line had no effect. To provide further mechanistic insight into the signaling components required for proper lymphangiogenesis, we are currently examining the ability of a number of chemical compounds for their ability to rescue this phenotype.

This work was supported in part by the NIH, NIAID (AI102223, J.C.).

Program Abstract #575

Hyaluronan is an essential signaling molecular for vertebrate synovial joint development

Yingcui Li[1], David Rowe[2], Angela Lu[2], Tiffany Wang[2], Kimberly Deschenes[1], Kathleen McCarthy[1], Nikia Grant[1], Sheila Leite Baptista[1]

[1]University of Hartford, USA; [2]University of Connecticut Health, USA

Vertebrate synovial joints and articular cartilage play crucial roles in the skeletal function, but little is known about the signaling factors controlling its formation and its lifetime maintenance during development. Hyaluronan, a very large linear glycosaminoglycan, is a major component in the extracellular matrix of cartilage cells, where it is synthesized by primary hyaluronan synthase *Has2*. Due to its high water entrapping capacity it can function in a structural role in cartilage matrix through binding to other molecules to form large proteoglycan aggregates to stabilize and organize the matrix. Recent studies suggest that these aggregates can also affect intracellular signaling pathways that control chondrocyte behavior. In our studies, wefound that *Has2* as well as hyaluronan itself are highly localized to the developing joint interzone, an area of higher cell density mesenchymal cell layer forming at future joint sites that is believed to carry initial active signal for joint development. We examined the effects of genetic loss of hyaluronan through manipulating *Has2* gene expression in limb mesenchymal cells *in vivo*. The joint interzones were poorly formed, diffused and dramatically widened and poorly defined. The patterns of digit interzones are affected and the synovial joints failed to go through cavitation. In addition, we found that TGF-β might be a target of hyaluronan signaling activity in joint progenitors. In conclusion genetic loss of hyaluronan disrupts chondrocyte differentiation and causes joint malformation. Articular cartilage failed to form in these malformed joints. This study demonstrated that "hyaluronan is not just a goo!" but an essential signaling molecular for vertebrate synovial joint development.

*This work was supported by **ANRF** (Arthritis National Research Foundation), **Dean's Research Fund**, College of Arts and Sciences, University of Hartford; and **Women's Education and Leadership Fund** (WELFund) to YL.*

Program Abstract #576

Activin-Beta/TGF-Beta signaling in skeletal muscle controls insulin/TOR signaling, metabolism and final body size

Lindsay Moss-Taylor, Xueyang Pan, Michael O'Connor

University of Minnesota, USA

Inter-organ communication is essential for regulating development and homeostasis. Mutations in *Drosophila Activin-Beta* (*Act-Beta*) cause accelerated pupariation and reduced final body and organ size. To determine how Act-Beta affects size and timing, we first looked at which cells express Act-Beta and found expression in the Insulin Producing Cells (IPCs), neuroendocrine cells and motor neurons. Overexpression of *Act-Beta* in either neuroendocrine cells or motor neurons increases body size. Muscle-specific knockdown of the TGF-Beta signaling transducer/transcription factor dSmad2 reduces body size, indicating muscle is a target tissue of the Act-Beta signal. Additionally, levels of phospho-dSmad2 are reduced in skeletal muscle samples of *Act-Beta* mutants and increased in animals overexpressing *Act-Beta* from motor neurons. Levels of phospho-S6K in *Act-Beta* mutants are correlated with phospho-dSmad2 levels, suggesting TGF-Beta signaling regulates insulin signaling. Because insulin signaling controls metabolism, we used GC/MS analysis to identify and quantify levels of metabolites in whole-larval samples of *Act-Beta* mutants. We found intermediates of the energy-producing steps of glycolysis and lactic acid are reduced, indicating reduced flux through glycolysis. Overall, this indicates neuronally-derived *Act-Beta* signals to the skeletal muscle to regulate levels of insulin signaling and subsequent glycolysis. We have identified over 300 downstream targets of dSmad2 using RNA-seq of *Act-Beta* mutant skeletal muscle. We are testing *impL2*, an insulin binding protein, as a potential dSmad2 target gene regulating systemic insulin signaling. Funding sources: American Heart Association fellowship to LMT, National Institutes of Health R01 to MBO.

Program Abstract #577

Members of the Rusc protein family interact with Sufu and inhibit vertebrate Hedgehog signaling

Jing Yang[1], Zhigang Jin[1], Tyler Schwend[2], Jia Fu[1], Jing Liang[1], Huimin Zhao[1], Wenyan Mei[1]

[1]Univ of Illinois At Urbana-Champaign, USA; [2]Hope College, USA

Hedgehog (Hh) signaling is fundamentally important for development and adult tissue homeostasis. It is well established that in vertebrates, Sufu directly binds and inhibits Gli proteins, the downstream mediators of Hh signaling. However, it is unclear how the inhibitory function of Sufu toward Gli is regulated. Here we report that the Rusc family of proteins, whose biological functions are poorly understood, form a heterotrimeric complex with Sufu and Gli. Upon Hh signaling, Rusc is displaced from this complex, followed by dissociation of Gli from Sufu. In mammalian fibroblast cells, knockdown of Rusc2 potentiates Hh signaling by accelerating signaling-induced dissociation of the Sufu-Gli protein complexes. In *Xenopus* embryos, depletion ofRusc1 or overexpression of a dominant negative Rusc enhances Hh signaling during *Xenopus* eye development, leading to severe eye development defects. Our study thus uncovers a novel regulatory mechanism controlling the response of cells to Hh signaling in vertebrates.

Program Abstract #578

Connexin43 maintains the ependymal cilia in the zebrafish spinal cord

Wenting Li

Chonnam National University, KR

Ependymal cells are glial cells in the central nervous system (CNS). The surfaces of ependymal cells are covered by microvilli and have a central cluster of long cilia. Ependymal cells have recently drawn a lot of attention as they were reported to have stemness in the subventricular zone of mouse brain. However, their development and origin remain elusive. To tackle this issue, we chose zebrafish as a model organism. We first performed transmission electron microscopy (TEM) on the developing spinal cord of zebrafish and observed ciliated cells lining the central canal from 2 days post-fertilization (dpf) onward. I next found that connexin43 (cx43) is not only specifically expressed in the cells lining the central canal of the spinal cord, but also required for the maintenance of ependymal cilia. Injection of dye into the ventricle of cx43 morphants showed significant decrease in the circulation of CSF compared to control. Treatment with IWR-1, a Wnt signaling inhibitor, from 12 to 48 hpf led to a reduction of cilia in spinal cord, while the Wnt activator BIO rescued the cilia defect in cx43 morphants. The heat-shock inducible dkk-GFP line and the pipetail (ppt, wnt5b) line revealed that Wnt signaling plays a critical role in the cilia maintenance. In addition, the injection of the thapsigargin into the larvae's ventricle elicited loss of cilia. The immunostaining with anti-Cx43 and anti-acetylated tubulin antibodies on human spinal cord harvested from a cadaver, revealed that Cx43 is expressed in human spinal ependymal cells. Finally, cx43 knockout mice displayed no spinal ependymal cilia. Taken together, these findings indicate that Cx43 maintains cilia of spinal ependymal cells in a Wnt signaling dependent manner.

Program Abstract #579

Long-distance communication by specialized cellular projections during pigment pattern development and evolution

Dae Seok Eom, Emily Bain, Larissa Patterson, Megan Grout
University of Washington, USA

Changes in gene activity are essential for evolutionary diversification. Yet, elucidating the cellular behaviors that effect modifications to adult form remains a profound challenge. Here, we use neural crest-derived adult pigmentation of zebrafish and its relative, pearl danio, to uncover cellular bases for alternative pattern states. We show that stripes in zebrafish require a novel class of thin, fast cellular projection that contribute to transducing a Delta-Notch signal over long distances from precursor cells of yellow-orange xanthophores to melanophores. By contrast, the uniformly patterned pearl danio lacked such projections owing to Colony stimulating factor 1-dependent changes in xanthophore differentiation, limiting the signaling available to melanophores. Our study reveals a novel mechanism of cellular communication, critical roles for differentiation state heterogeneity in pigment cell interactions, and an unanticipated morphogenetic behavior that contributes to translating a species difference in gene activity to a corresponding difference in cellular organization and adult form.

Program Abstract #580

Casting a line to trailing cells: A simple mechanism for polarizing signaling in the posterior lateral line primordium

Damian Dalle Nogare, Ajay Chitnis
Eunice Kennedy Shriver National Institute of Child Health and Human Development, United States of America

The zebrafish Posterior lateral line primordium (PLLp) is a group of ~150 cells which spearheads the development of the lateral line by migrating along the length of the embryo, periodically depositing epithelial rosettes which serve as sense organ precursors. The PLLp is patterned by juxtaposed and mutually inhibitory Wnt and FGF signaling systems. Wnt in leading cells drives the expression of both FGF ligands and FGF signaling inhibitors. FGF ligand therefore activates receptors in more trailing cells, promoting rosette formation. However, the mechanisms by which this polarity is established and then maintained are incompletely understood. Using high resolution timelapse imaging, we show that leading cells extend long vesicle-bearing fillopodial protrusions, similar to cytonemes, toward trailing cells. Vesicles released by these protrusions are taken up by trailing cells and rapidly transported apically, where FGF is known to accumulate in a microlumenal compartment of the epithelial rosette. What determines the polarized extension of these protrusions toward trailing cells? Our observations suggest a simple yet elegant mechanism. While the filopodial protrusions are initially in random directions, their extension is highly correlated with the direction and speed of cell migration. This suggests that the tips of initial protrusions are anchored in the surrounding matrix. Then as cells extending these protrusions move forward, the protrusions elongate, leaving these anchored protrusions in their wake, offering signals carried by vesicles to trailing cells that follow. Extension of these protrusions is sensitive to inhibition of HSPG sulfation, a manipulation also known to prevent an effective FGF response in trailing cells. In this manner, we propose that direct, filopodia-mediated signaling is, at least in part, responsible for delivering signals from leading cells to trailing cells to in a manner intrinsically tied to the direction of PLLp movement.

Program Abstract #581

Investigating Sonic hedgehog signaling in a polarized epithelium

Emily Kolenbrander, Tim Stearns
Stanford University, USA

The primary cilium is required for vertebrate Sonic hedgehog (Shh) signaling and is thought to have a role in both ligand sensing and signal transduction. However, the potential for regulation of Shh signaling by the location of the cilium with respect to Shh source in complex 3D environments has not been explored. For example, in the neural tube, Shh produced by the notochord presumably does not have direct access to the cilia on the apical surface of presumptive floor plate cells. This raises the question of whether Shh can be trafficked to the apical side, or whether it can activate signaling without accessing the primary cilium on the apical side. To address this issue, we are investigating Hedgehog signaling in polarized epithelia. Using 2D and 3D IMCD3 cell culture, we have tested whether basally-applied ligand is sufficient to activate Shh signaling in cells with apical cilia. We have shown that basally-applied Shh is not sufficient to activate target gene transcription, whereas basally-applied SAG, a cell permeable receptor agonist, is sufficient. We are currently investigating whether earlier events in the signaling pathway occur. Additionally, we are using tagged Shh to determine where the ligand binds in these polarized cells and whether we can detect transcytosis of Shh in this system. Our results

suggest that the location of the cilium in space is a potential regulator of Shh responsiveness, and that the classical developmental contexts in which Shh has been shown to function will require more work to understand the mechanism of signaling. Most importantly, these results reinforce the general concept that spatial distribution of ligand with respect to receptor apparatus is a critical feature of developmental signaling pathways. This work is supported by the Stanford Graduate Fellowship and the NIH.

Program Abstract #582
Understanding Patterning One Stem Cell at a Time
Anastasiia Nemashkalo, Aryeh Warmflash
Rice University, USA
Embryonic stem cells (ESCs) represent a promising system to dissect spatial patterning in vitro. During stem cell differentiation, cell fates are specified by a combination of exogenously supplied ligands and paracrine signals between the cells – effects that are difficult to parse. Here we disentangle these effects using a bottom-up approach: by growing stem cells confined to very small colonies (microcolonies, 1-8 cells/colony) using micropatterning techniques. This approach allows us to study the effects of exogenously supplied ligands in isolation and precisely modulate the level of paracrine signaling. We differentiated microcolonies of human embryonic stem cells with BMP4, a ligand critical for primitive-streak formation and gastrulation in vivo. Our results indicate that BMP4 acts as a simple switch rather than as a morphogen: pluripotent ($Sox2^{+}$) stem cells completely transition to extraembryonic ($Cdx2^{+}$) above a threshold BMP4 ligand concentration. To study cell-cell interactions in detail, we analyzed the results of signaling and differentiation as a function of the number of cells in the colony. Interestingly, under pluripotent conditions, a fraction of isolated cells spontaneously downregulate Sox2 and express the trophectodermal marker Cdx2, but as the colony size grows to 4 cells/colony or larger, spontaneous differentiation is not observed. We argue that this is the manifestation of the community effect, the enforcement of a common fate in groups of cells, in our microcolonies. We show that in pluripotent conditions, the community effect is mediated by paracrine FGF signaling that prevents spontaneous differentiation. When cells are differentiated with BMP4, the community effect is enforced by the signaling response itself. This work was supported by Cancer Prevention Research Institute of Texas (CPRIT) grant RR140073 and NSF grant MCB-1553228.

Program Abstract #583
Novel Roles for Hedgehog Co-Receptors During Craniofacial Development
Martha Echevarria-Andino, Benjamin Allen
University of Michigan, USA
The Hedgehog (HH) signaling pathway plays essential roles during vertebrate embryogenesis, regulating diverse processes such as patterning and growth of the central nervous system, digit specification in the limb, and craniofacial development. HH ligands signal through the canonical receptor Patched1 and 3 co-receptors, GAS1, CDON and BOC. These co-receptors are essential for proper levels of HH signaling during embryogenesis, where they are thought to promote HH pathway function. However, while these co-receptors share similar expression patterns during development, recent genetic studies suggest tissue-specific, antagonistic roles for these proteins during HH-dependent embryogenesis. GAS1 restricts HH pathway during mouse tooth development, while BOC antagonizes HH signaling during jaw formation in zebrafish. To explore possible antagonistic roles for GAS1, CDON and BOC in HH signal transduction, we used in vitro cell signaling assays, chicken in ovo electroporations and mouse genetics. Our data indicate that GAS1, but not CDON or BOC, can restrict HH pathway function in HH-responsive NIH/3T3 fibroblasts. This inhibition appears to be downstream of HH ligand, and can be mediated by multiple structural domains of GAS1. During craniofacial development in mouse, *Gas1* promotes HH pathway activity. Genetic deletion of *Gas1* results in significant craniofacial defects, consistent with reduced HH signaling. The deletion of *Boc* in a *Gas1* mutant background rescues the craniofacial defects observed in *Gas1* single mutants, indicating a potential antagonistic role for BOC in HH-dependent craniofacial development. These findings suggest that GAS1 and BOC can function as novel, context-specific HH antagonists and are not simply redundant HH co-receptors. Instead, these data indicate that GAS1, CDON and BOC perform dynamic, distinct, and tissue-specific functions in the control of HH signal transduction. **Funding sources:** NSF-GRFP, RGSR Grant, Bradley Merrill Patten Fellowship.

Program Abstract #584
The nucleoporin Nup98 sets the size-exlcusion limit for soluble molecule diffusion at the base of the cilium
Joseph Endicott
Yale University, USA

Cilia are found on nearly every vertebrate cell type, playing essential roles in mediating (or modulating) several signaling pathways involved in vertebrate development and tissue homeostasis. Defects in cilia biogenesis or function cause a number of human congenital diseases with broad pleiotropic phenotypes. The cilium maintains a unique composition of membrane-bound and soluble proteins, allowing it to concentrate signal receptors and their downstream effectors into a well-regulated space. Much remains unknown about the gating of soluble protein diffusion into and out of the cilium. We and others have found many nucleoporins localize to the base of the cilium, where they are hypothesized to form a size-exclusion permeability barrier. Moreover, we have identified several human patients with nucleoporin mutations and symptoms of ciliopathies, including abnormal development of left-right asymmetry. We have shown that FG-repeat containing nucleoporin Nup98 localizes to the ciliary base in a phosphorylation-dependent manner. We now report that knockdown of Nup98 by siRNA results in an increase in the size-exclusion limit at the base of the cilium. Using a diffusion trap, we have found that knockdown of Nup98 leads to faster diffusion of molecules larger than 70kDa into the cilium, without affecting the diffusion rates of molecules smaller than 70kDa. Nup98 knockdown also results in a decrease in cilium length; moreover, cilia to lose their ability to lengthen in response to serum starvation. Cells treated with Nup98 siRNA regenerate normal numbers of cilia after deciliation, indicating biogenesis machinery is intact, despite the decrease in cilia length. These data show for the first time that the knockdown of a nucleoporin alters the diffusion barrier for soluble molecules into the cilium, and suggests that the diffusion barrier for soluble molecules is required to regulate cilia length. Funding: NIH NHLBI

Program Abstract #585

The BMP Signaling Pathway is a Programmable Multi-Ligand Signal Processing System

Yaron Antebi[1], James Linton[1], Heidi Klumpe[1], Bogdan Bintu[3], Mengsha Gong[1], Christina Su[1], Reed McCardell[1], Michael Elowitz[1,2]

[1]Caltech, USA; [2]HHMI, USA; [3]Harvard, USA

The bone morphogenetic protein (BMP) signaling pathway comprises multiple ligands and receptors that interact promiscuously and appear in combinations. This feature is often understood in the context of redundancy and tissue specificity, but it has remained unclear whether it enables specific signal processing capabilities. We show that the BMP pathway performs a specific set of computations, including sums, ratios, and balance and imbalance detection, across the multi-dimensional space of ligand concentrations. These computations can arise directly from receptor-ligand interactions without requiring transcriptional regulation. Furthermore, cells can re-program the type of computation performed on specific ligands through changes in receptor expression, allowing different cell types to perceive distinct signals in the same ligand environment. These results may help explain the prevalence of promiscuous ligand-receptor architectures across pathways and enable predictive understanding and control of BMP signaling.

Program Abstract #586

Efficient selection of antibody fragments using phage display and exhaustive yeast two-hybrid screening

Brent Passer[1], Pat Tylek[1], Sandrine Moutel[2], Selma Djander[2], Vincent Collura[3], Alexis Arrial[3], Aurelien Olichon[5], Franck Perez[4], Jean-Christophe Rain[3]

[1]Hybrigenics Corp., USA; [2]Translational Research Department, Institut Curie, Paris, France; [3]Hybrigenics Services, France; [4]CNRS UMR144, Institut Curie, Paris, France; [5]INSERM, CRCT, Toulouse, France

Antibodies represent central tools in most biological studies to analyze protein localization and function. One of the remaining limitations is the challenge to make them work inside a living cell. For this purpose intrabodies can be selected as powerful tools to answer complex biological questions, as has been shown for example with a conformational intrabody recognizing specifically the GTP-bound form of the small GTPase Rab6 (1), GTP-tubulin (2), or farnesylated PSD95 (3). So far, the access to intrabodies was limited to highly trained lab specialists in this field. We have therefore set up a new platform for intrabody screening and designed for this purpose a fully synthetic humanized naïve Llama VHH library containing 3x10exp9 antibodies, based on a unique scaffold with random complementary determining regions (CDRs). We use a combination of phage display and subsequent yeast two-hybrid (Y2H) screening to identify antibodies against native antigens and eventually intrabodies. The VHH clones are directly accessible and the recombinant antibodies can be produced as fusions to either a human, mouse or rabbit Fc domain (4). We successfully selected from this library VHH against a variety of antigens including large proteins, haptens and receptors directly selected from cell surface expression. The affinity of our VHH is similar to the affinity of antibodies selected after animal immunization. Using only a single round of phage display followed by one round of Y2H screening we were able to significantly enrich the selection in intrabodies. In addition, we took advantage of yeast genetics to further study and characterize the selected intrabodies. Here this technique will be exemplified with the selection of intrabodies against GFP, p53 and USP7.

1) Nizak C. et al. Science 2003, 300: 984
2) Dimitrov A. et al. Science 2008, 322: 1353
3) Fukata Y et al. J. Cell Biol. 2013, 202: 145
4) Moutel S. et al. BMC Biotechnology 2009, 9: 14

AUTHOR INDEX

C

D

E

F

G

K

L

M

N

Q

R

T

W