ABSTRACTS

Program Abstract #1
STF-mediated transcriptional repression in leaf blade outgrowth
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STENOFOLIA (STF) is a WOX transcription factor required for leaf blade outgrowth in *Medicago truncatula* and other dicot species. STF promotes cell proliferation at the adaxial-abaxial juxtaposition of the leaf blade early in leaf development acting as transcriptional repressor. The C-terminal domain of STF (WUS-box and STF-box) recruits the co-repressor TOPLESS (TPL) to accomplish this transcriptional repression. But, how the STF-TPL complex mechanistically achieves transcriptional repression is still unclear. Our data suggests that one way to achieve this may be through chromatin modification of the target locus. STF interacts with histone deacetylases, HDA6 and HDA19, and this interaction is required to repress the expression of one of the STF targets, *AS2*. We found that the acetylation level of histone H4K16 at the *AS2* locus decreases with binding of STF to the *AS2* promoter. On the other hand, leaf blade outgrowth is antagonized by transcriptional activation mechanisms. STF interacts with transcriptional activators such as WOX9 or co-activators such as AN3. We observed that overexpression of WOX9 worsens the *stf* mutant phenotype and translational fusion of AN3 to the STFdel fragment that lacks repression activity worsens the *stf* mutant phenotype similar to fusion of an exogenous activator domain VP16 to STFdel, suggesting that activation activity is antagonistic to STF function. The fact that STF interacts to both co-activators and co-repressors suggests that may be activation and repression functions need to be balanced in the middle domain of the leaf blade for proper proliferation and differentiation functions that ultimately determine outgrowth and expansion of the blade. However, STF homologues are not found in monocot species, where *STF* function is taken over by a related gene *PRS/NS/WOX3*. We will discuss the evolutionary significance of this sub-functionalization in the monocot-dicot dichotomy. This research was supported by NSF grant IOS-1354422.

Program Abstract #2
STENOFOLIA Directly Recruits RPD3-type Histone Deacetylases to Repress ASYMMETRIC LEAVES2 in the nucleus
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WUSCHEL-related homeobox (WOX) transcription factors are key players for stem cell maintenance and lateral organ development in plants. The distinguishing feature of WOX family proteins is the specific Homeodomain (WOX-HD). Functions of WOX-HD, other than DNA binding, are poorly understood. Here we identified a KRRR motif, a 3’ extension in the HD of the WOX transcription factor STENOFOLIA, that prevents the distribution of STF protein into the cytoplasm, which is important for STF function in leaf development. The KRRR motif is also important for interaction with DNA. In addition, we identified RPD3-type of Histone Deacetylases as conserved STF interaction partners. Mutation of either the KRRR motif or FY residue greatly reduced the interaction with HDACs and showed reduced STF repressive activity that compromised leaf blade outgrowth. Binding of STF to two regions of the *ASYMMETRIC LEAVES2* (*AS2*) promoter reduced the histone acetylation level of both regions, suggesting that repression of *AS2* by STF involves chromatin modification. The interaction with HDACs is conserved among WOX proteins, which may shed some light on HD-mediated common mechanism in the function of repressive WOX transcription factors.

Program Abstract #3
The Walking Heads: Development and Evolution of the Tardigrade Body Plan
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We would like to understand how animal body plans arose. Panarthropods (arthropods, onychophorans, and tardigrades) have modular, segmented bodies, facilitating comparisons between body plans. How the body plan of tardigrades, which consists of a head and four leg-bearing segments, relates to that of other panarthropods, and how the tardigrade body plan evolved, are unclear. To address these issues, we developed *in situ* hybridization methods for tardigrades and investigated the expression patterns of Hox genes and other anteroposterior patterning genes using the tardigrade *Hypsibius dujardini*. We detected expression of the head gap gene orthodenticle across the developing head; *Hox3* in leg-bearing segments 2 and 3; *Deformed* in the posterior of leg-bearing segment 2 through segment 3; and *fushi tarazu* in the anterior part of the fourth leg-bearing segment. Based on comparisons with other panarthropods, these results suggest that the body plan of *H. dujardini* is primarily composed of segments homologous to arthropod head segments. Next, we detected expression of the Hox gene *Abdominal-B* and the ParaHox gene *caudal* in the posterior-most region of leg-bearing segment 4. Based on comparisons with other panarthropods, these results suggest that a posterior body region is conserved between *H.*
dujardini and other panarthropods. However, Hox genes that specify intermediate segments–trunk segments in arthropods–are missing in the H. dujardini genome, but phylogenetically reconstructed as ancestral for Panarthropoda. In sum, our results suggest that there has been a gap mutant-like loss of a region homologous to the arthropod trunk in the tardigrade lineage.

Program Abstract #4
Comparative genomics and reverse genetics support Bmp6 underlying evolved tooth gain in sticklebacks
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Understanding how changes to developmental genetic networks lead to morphological evolution remains an outstanding goal in developmental biology. The threespine stickleback fish offers several genomic and genetic advantages to dissect how morphology evolves in nature. Marine sticklebacks have repeatedly colonized and adapted to numerous freshwater environments throughout the Northern hemisphere. In response to new diets in freshwater habitats, changes in craniofacial pattern have evolved in derived freshwater populations. A freshwater population adapted to live on the bottom of Paxton Lake, Canada has evolved a near two-fold increase in tooth number that arises late in development. This evolved tooth gain is heritable, and largely controlled by a quantitative trait locus (QTL) on chromosome 21 that contains a cis-regulatory allele of Bone Morphogenetic Protein 6 (Bmp6). We fine-mapped the chromosome 21 tooth QTL, further supporting Bmp6 as underlying the QTL. Comparative genomics of marine and freshwater chromosomes with and without the tooth QTL identified a cluster of sequence variants in intron 4 of Bmp6 which surround a robust tooth and fin enhancer. During tooth development, this intronic Bmp6 enhancer drives partially complementary expression compared to a previously described 5’ Bmp6 tooth enhancer. Induced coding mutations in Bmp6 reduced tooth number, revealing a required role for Bmp6 during tooth development. Surprisingly, induced regulatory mutations in Bmp6 resulted in increased tooth numbers, revealing complex roles for Bmp6 in regulating tooth development and replacement. Collectively these data support a model where mutations around aBmp6 intronic tooth enhancer contribute to evolved tooth gain. Supported by the NSF-EDEN (L.G.) and NIH NIDCR R01 DE021475 (C.M.).

Program Abstract #5
Quantitative genetic analysis of evolved tooth gain in sticklebacks
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The goal of this project is to elucidate the genetic basis of morphological evolution of tooth patterning in the threespine stickleback fish, Gasterosteus aculeatus, which are well-suited for the dissection of genetic circuitry underlying evolved traits. Ancestral marine populations have repeatedly colonized inland water bodies, where they have undergone dramatic adaptive radiation. Adaptive radiations are often associated with changes in trophic morphology, as populations adapt to new diets in new environments. In the threespine stickleback adaptive radiation, several trophic differences evolve upon freshwater adaptation, including increased tooth number in some freshwater populations. Marine and freshwater populations are interfertile, and can be crossed to map chromosome regions controlling evolved differences. A previous study identified a chromosome 21 region, containing a cis-regulatory allele of Bmp6, associated with tooth gain in a large genetic cross between individuals from a high-toothed Paxton Lake freshwater benthic population and a low-toothed Japanese marine population. Our goal was to further elucidate the processes underlying evolved tooth gain with a combination of genetic approaches. Firstly, we hoped to determine whether other wild-derived chromosome 21 alleles from the Paxton Lake benthic population control tooth gain. We did this by performing Quantitative Trait Loci (QTL) analysis for an additional F2 cross between different Paxton Lake and Japanese marine individuals. Our QTL mapping data revealed two additional wild chromosome 21s from the Paxton benthic population that contain the tooth QTL. Finally, whole-genome sequencing of fish with and without the tooth gain QTL can be used to identify candidate mutations that correlate with the presence or absence of the tooth QTL, thereby identifying candidate mutations that contribute to tooth number variation. This work was made possible by support from the NSF Evo-Devo-Eco Network and Sigma Xi.

Program Abstract #6
Dogwoods as a model for evo-devo genetic study of inflorescence morphology - tools and progresses
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Variation in floral display (inflorescence) affects the success of plant reproduction and the yield of a crop by influencing
seed number and dispersal/harvest ability. Despite its importance, little progress has been made in understanding how developmental and genetic changes have shaped inflorescence architectures in angiosperm evolution, in part because existing model organisms exhibit little variation in these traits. Species of Dogwood (*Cornus* L.) are popular ornamental trees in American landscapes due to their spectacular inflorescences often associated with large showy (petaloid) bracts. The genus *Cornus* offers us a unique opportunity to address this important problem using a synergistic, multidisciplinary approach. Lineages within *Cornus* exhibit striking differences in inflorescence structures. Using the phylogeny as a framework, we aim to unravel the developmental and genetic changes associated with the origins of different inflorescence types. We successfully established a regeneration and transformation system for *Cornus Canadensis* for gene functional analyses and have identified the developmental changes on the phylogeny that were associated with the origins of head-like and umbel-like inflorescences. We test the roles of *Cornus* homologous of key inflorescence and petal regulators of *Arabidopsis* through gene expression analyses and genetic transformation experiments. We also search for new candidate genes using comparative transcriptome analyses. Our results suggested a possible role of *CorPI-B* in the origin of petaloid bract in *Cornus florida* and roles of *CorLFY*, *CorTFL1*, and *CorAPI* in flower and inflorescence development in *Cornus*. Genetic transformation and gene expression patterns together demonstrate evolutionary changes in expression level of *CorTFL1* and *CorAPI* during early inflorescence development are likely responsible for the evolutionary modification of inflorescence morphology in *Cornus*.

Program Abstract #7
Molluscan biominalization: Teaching old proteins new tricks
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Complex biological systems tend to be hierarchical in many ways. This hierarchy imposes a challenge in exploring the evolutionary trajectory of homologous traits. This is because evolution of particular phenotype can result from a multitude of underlying levels such as DNA, RNA, proteins, cells, tissues and organs. It is apparent from recent evo-devo work that these levels while interlinked might not follow the same evolutionary path. The question is then: given a certain complex trait, which level of biological complexity is appropriate to deem it homologous across taxa? And does homology in one level of biological complexity implies homology across all levels? To contribute to this discussion chose the process of biominalization in metazoa. Being dependent on well-defined processes such as transport, cell adhesion, and extracellular matrix organization (ECM), biominalization can be considered an emergent property. There has been quite a debate on whether biominalization evolved once or multiple times (*de novo*) across metazoan phyla. To answer this question we employ a systems-biology approach examining the various proteins involved in the process across all calcifying metazoan lineages using computational approaches and analysis of protein interaction networks. Our findings show that a core biominalization related toolkit is likely to be shared across metazoa, yet to be only conserved at the universal aspects of the process with multiple species innovations across lineages. The results show that while a trait can be considered homologous, when this homology breaks at a given level of complexity between taxa, it gives rise to evolutionary innovations. In addition we are trying to extend our analysis by experimental examination of biominalization genes in the snail * Biomphalaria glabrata*.

Program Abstract #8
Zelda promotes enhancer activity during the maternal-to-zygotic transition in *Drosophila*
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During the maternal-to-zygotic transition, a dramatic reprogramming of the early transcriptome occurs as many maternal RNAs are degraded and the zygotic genome is activated. Recent studies have shown that the transcription factor Zelda plays a global role in genome activation in *Drosophila* [1-4]. Zelda is uniformly distributed, and activates batteries of genes required for early development, including a set of microRNAs known to be involved in maternal RNA degradation. Zelda also works together with the graded morphogens, Bicoid and Dorsal, to ensure the correct temporal and spatial expression of their target genes. Since Zelda is in nuclei very early, and binds genomic regions prior to genome activation and the accumulation of histone modifications, it was proposed to function as a pioneer factor to increase chromatin accessibility for the patterning factors to bind [4-7]. To test this hypothesis, we compared genome-wide Dorsal binding and nucleosome occupancy between wild-type and zelda embryos. We found that in zelda Dorsal binding decreases at target enhancers and that this is accompanied by an increase in nucleosome occupancy. Strikingly, Zelda-bound regions, which are enriched for early embryonic enhancers, harbor DNA sequences that favor nucleosome formation and are thus intrinsically “closed”. We propose that enhancers requiring precise spatial and temporal regulation during early
development have high intrinsic nucleosome occupancy, and that Zelda acts to lower the nucleosome barrier, thus facilitating enhancer activity. Our results represent a general model for enhancer function and a paradigm for mechanisms of genome activation in higher organisms.


Program Abstract #9
Transcriptional memory in the Drosophila embryo
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Transmission of active transcriptional states from mother to daughter cells has the potential to foster precision in the gene expression programs underlying development. Such transcriptional memory has been specifically proposed to promote rapid reactivation of complex gene expression profiles following successive mitoses in Drosophila development. By monitoring transcription in living Drosophila embryos, we provide the first evidence for transcriptional memory in animal development. We specifically monitored the activities of stochastically-expressed transgenes in order to distinguish active and inactive mother cells and the behaviors of their daughter nuclei following mitosis. Quantitative analyses reveal that there is a 4-fold higher probability for rapid reactivation following mitosis when the mother experienced transcription. Moreover, memory nuclei activate transcription twice as fast as neighboring inactive mothers, thus leading to augmented levels of gene expression.We propose that transcriptional memory is an epigenetic mechanism of precision, which helps ensure coordinate patterns of gene activity during embryogenesis.

Program Abstract #10
Programmed DNA Elimination in Nematodes
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Genomes rarely change. However, a few organisms undergo a programmed process that eliminates specific DNA sequences from the genome during development. In the parasitic nematode Ascaris suum, we found that 13% of the genome is eliminated in somatic cell lineages during early embryonic divisions, while the germline genome remains intact. The eliminated DNA consists of specific repetitive and unique sequences, including ~700 genes. The eliminated genes are primarily expressed in the germline and early embryo leading us to suggest that DNA elimination in Ascaris is an essential, irreversible mechanism for silencing a subset of germline and early embryo expressed genes in somatic tissues. How specific Ascaris chromosomal regions are targeted and selected for elimination or retention remain unknown. The histone H3 variant CENP-A epigenetically defines centromeres and is required to nucleate kinetochore assembly for microtubule attachment to facilitate chromosome segregation. Nematodes have holocentric chromosomes with multiple centromeres distributed along the length of the chromosome. Our data suggest that only retained chromosome regions have extensive deposition of CENP-A during Ascaris DNA elimination; chromosome regions destined for elimination have little associated CENP-A. This suggests a mechanism for how specific portions of chromosomes are retained or eliminated. We will describe studies that define the genome-wide distribution of CENP-A and examine whether CENP-A deposition is regulated to facilitate differential chromosome segregation during DNA elimination. We recently found that worm specific Argonautes, WAGO-2 and -3, are highly enriched on retained (WAGO-2) or eliminated (WAGO-3) condensed chromosomes during DNA elimination mitoses. Experiments will be described that examine the potential contributions of these Argonautes and small RNAs to DNA elimination.

Program Abstract #11
Chromatin accessibility dynamics and gene expression changes driving vertebrate regeneration
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The capacity of vertebrate animals to regenerate complex tissues such as the spinal cord and limb varies widely among taxa, but the factors that govern regenerative competence remain poorly understood. The Xenopus tropicalis tail represents an excellent model for molecular and genomic investigations of regeneration. Following amputation, tail regeneration progresses rapidly through stereotyped stages to form new muscle, spinal cord, nerves, skin, and vasculature. To understand the gene regulatory landscape that enables this regenerative capacity, we have undertaken a genomic approach, defining both gene expression and chromatin accessibility over time during tail regeneration. Using RNA-Seq, we have identified clusters of coordinately regulated gene expression that change rapidly during the first 24 hours of regeneration.
These implicate rapid changes in cell morphology and communication immediately after tail amputation, followed by upregulation of multiple embryonic signaling pathways and widespread changes in RNA metabolism functions. These rapid global changes in gene expression suggested that the underlying chromatin landscape must also be rapidly remodeled. We have profiled this chromatin remodeling for the first time in regeneration by using an assay for transposase accessible chromatin followed by sequencing (ATAC-Seq). This has revealed extremely rapid global changes in chromatin accessibility in the first several hours of tail regeneration. By applying motif analysis to specific regions of chromatin that gain or lose accessibility, we have found enrichment for both expected and novel transcription factor binding sites. Perturbation of either chromatin remodeling enzymes or cell signaling upstream of these transcription factors results in impaired regeneration. These observations combine to create a profile of highly dynamic transcription factor access to chromatin that drives coordinated changes in cell fate and behavior during regeneration.

Program Abstract #12
Small RNA Pathways as Epigenetic Architects: Guarding the Genome and Defending Fertility
Julie Claycomb
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The germline is an immortal cell lineage that connects all generations and enables the viability of a species. A network of endogenous small RNA pathways related to RNA interference play key roles in regulating germline gene expression and transmitting these patterns of gene expression from one generation to the next to maintain germline totipotency. These regulatory activities occur both in the cytoplasm and nucleus and, remarkably, can act in both positive and negative capacities. My lab is investigating the molecular mechanisms by which small RNA pathways compete and cooperate to maintain proper patterns of germline gene expression over generations. In sum, these small RNAs can serve as potent epigenetic couriers and can guide the acquisition of key histone modifications that are transmitted from parent to progeny. These findings have implications proper genome regulation in animal germlines, stem cells, and during oncogenesis.

Program Abstract #13
Chromatin and transcriptional mechanisms of intestinal lineage plasticity
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Intestinal epithelial cells are rapidly self-renewed by stem cells located in the crypt, a pocket-like structure of the intestinal gland. Exposed directly to the harsh external environment, these epithelial cells exhibit remarkable plasticity upon injury. Lgr5 expressing stem cells differentiate into two major cell types, absorptive enterocytes and secretory cells, via Notch mediated lateral inhibition, an evolutionally conserved process, in which two mutually exclusive cell fates become specified. Of note, these cell fates are transiently reversible, but the underlying mechanisms were unknown. To investigate chromatin and transcriptional mechanisms of intestinal lineage plasticity, we enriched and isolated enterocyte and secretory progenitors by employing Notch signaling mutant mice. Our enhancer analyses of intestinal stem cells and lineage-specific progenitors have revealed that broadly permissive chromatin is established in stem cells and maintained throughout distinct intestinal lineages. Although lateral inhibition occurs within bona fide bipotential progenitors, little is known about this cell population; because they are likely transient, they have been difficult to isolate and characterize. To identify intestinal bipotential progenitors, we isolated Lgr5+ intestinal stem cells and analyzed them at single cell resolution by using microfluidic quantitative RT-PCR assays with 185 selected primers for stem and mature cell markers. Unsupervised k-means clustering of our gene expression data has revealed two distinct, mutually exclusive stem cell subpopulations: one population expresses stem cell markers, while another expresses both enterocyte and secretory lineage specific markers, identifying for the first time bipotential progenitors in intestinal stem cell populations. Multi-lineage priming and broadly permissive chromatin established in intestinal stem cells are likely responsible for lineage plasticity during lateral inhibition.

Program Abstract #14
Sex-determination mechanisms in diploid persimmon
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Similarly to approximately five percent of plant species, male and female flowers grow on separate trees in Caucasian persimmon (*Diospyros lotus*). This sexual system, called dioecy, is often associated with sex chromosomes. Dioecy has evolved multiple times independently in different plant taxa, but the molecular mechanisms underlying sex determination
remain poorly understood. We studied this problem using a segregating population of diploid persimmon. Using a combination of genomic and transcriptome sequencing, as well as genetics and evolutionary analyses, we were able to identify a potential master regulator of sex in this species, called \( OGI \). Further small RNA and sequence analyses indicate that \( OGI \) is located on the Y chromosome and produces small RNA that repress a homologous gene called \( MeGI \). Functional characterization of candidate genes in \( Arabidopsis \) and \( Nicotiana \) confirmed the repressive role of \( OGI \) on \( MeGI \), as well as the feminizing role of \( MeGI \). We have begun assembly of the persimmon Y-chromosome to understand the evolutionary history of sex determination in this genus.

Program Abstract #15
DNA methylation status in pre-differentiation pancreatic progenitors dictates \( \alpha \) and \( \beta \) cell production in the developing pancreas
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Understanding how seemingly equivalent progenitor cells adopt different cell fates remains a fundamental question. To this end, a group of embryonic pancreatic progenitors transiently activate transcription factor Ngn3 to specify endocrine islet cells, including insulin producing \( \beta \) cells and glucagon+ \( \alpha \) cells. It is known that early Ngn3+ cells give rise to largely \( \alpha \) cells, while later Ngn3+ cells give rise to both \( \alpha \) and \( \beta \) cells. We investigate the mechanisms/factors that dictate this temporal competence. We show that inhibition of DNA methylation of pancreatic progenitors promotes \( \beta \)-cell production without an intermediate \( \alpha \)-cell state, indicating direct differentiation rather than transdifferentiation. Interestingly, early pancreatic progenitors, before their endocrine commitment, display significantly different promoter methylation status from late progenitors, in several genes required for \( \alpha \)- and \( \beta \)-cell production, including Arx and Myt1. Moreover, the late pancreatic progenitors contain a mixed cell population with different degrees of CpG island methylation. Corresponding to this heterogeneity, we show that the transient Ngn3+ cells are a mix of subpopulations with different co-expression of \( \beta \) cell factors. These include a target of Ngn3, transcription factor Myt1 that is later detected in all endocrine cells, resulting in the simultaneous detection of transient Ngn3+Myt1+ and Ngn3+Myt1- progenitors. By utilizing a novel bipartite-Cre system for lineage tracing, we further show that the Ngn3+Myt1+ progenitors preferably give rise to \( \beta \) cells, demonstrating the functional significance of Myt1 co-expression with Ngn3in the endocrine progenitors. These overall findings, combined with the established role of Arx/Myt1 in \( \alpha \)-cell determination and \( \beta \)-cell maturation, suggest that the DNA methylation differences in individual progenitor cells could underlies the competence of endocrine progenitors to adopt different cell fate. Funding sources: NIDDK/JDRF.

Program Abstract #16
Exploring renal branching morphogenesis in three dimensions
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Although kidneys of equal size can vary 10-fold in nephron number at birth, discovering what regulates such variation has been hampered by a lack of quantitative parameters defining kidney development. Here we report a comprehensive, quantitative, multiscale analysis of mammalian kidney development in which we measure changes in cell number, compartment volumes, and cellular dynamics across the entirety of organogenesis, focusing on two key nephrogenic progenitor populations: the ureteric epithelium and the cap mesenchyme. In doing so, we describe a discontinuous developmental program governed by dynamic changes in interactions between these key cellular populations occurring within a previously unappreciated structurally stereotypic organ architecture. We also illustrate the application of this approach to the detection of a subtle mutant phenotype. This baseline program of kidney morphogenesis provides a framework for assessing genetic and environmental developmental perturbation and will serve as a gold standard for the analysis of other organs.

Program Abstract #17
Hedgehog signaling constrains cell migration driving early eye morphogenesis
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Uveal coloboma, a defect characterized by a hole or cleft in the eye, is a significant cause of blindness worldwide. It results from disrupted development of the choroid fissure, a transient structure through which retinal axons exit and
vasculature enters the eye. The Hedgehog (Hh) signaling pathway is essential for choroid fissure development: in humans, mutations upstream, within, and downstream of Hh signaling can cause uveal coloboma. Notably, in both humans and zebrafish, loss-of-function mutations in patched, which lead to hyperactive Hh signaling, result in coloboma. Despite its importance, the cellular mechanisms underlying choroid fissure morphogenesis, and its disruption in coloboma, have been unknown. Here, using zebrafish and a combination of 4-dimensional timelapse imaging and computational cell tracking and visualization methods, we have determined the origin of and cellular mechanisms underlying choroid fissure morphogenesis under normal and colobomatous conditions. We find that an unexpected series of morphogenetic events drives choroid fissure formation: early polarized cell migration from the midline and subsequent tissue folding splits a single population of cells into the choroid fissure margins. Surprisingly, hyperactive Hh signaling, as found in patched2 mutant zebrafish, acts very early in eye development to disrupt choroid fissure formation by dramatically reducing cell migration from the midline. Cells that normally give rise to the choroid fissure adopt an aberrant multipolar morphology, cease migration, and become trapped in the optic stalk, prior to reaching the optic cup. These data indicate that uveal coloboma can be caused by very early disruptions to cell movements driving choroid fissure formation, and we are working to determine whether hyperactive Hh signaling acts via canonical or non-canonical pathways to control cell movement. This work was funded by grants from the Knights Templar Eye Foundation, March of Dimes, and NIH/NEI.

Program Abstract #18

Opposing temporal morphogens specify serially derived neuron types
Tzumin Lee
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To create a complex brain, a diverse array of neurons needs to be specified. This is accomplished by neural stem cells that are both heterogeneous and that have age-dependent developmental potentials. Drosophila neural stem cells, called neuroblasts, are individually programmed to make a distinct yet stereotyped series of neuronal types, termed a lineage. Here we present the identification of opposing temporal morphogens intrinsic to all neuroblasts that specify serially derived neuron types in a way analogous to the embryonic spatial patterning by the opposite gradients of Bicoid and Nanos.

Program Abstract #19

Specific regulation of retrograde mitochondrial transport in axons
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The cytoplasmic dynein motor is the primary motor protein complex responsible for retrograde cargo transport in axons. How this single motor moves disparate cargo in a regulated fashion remains largely unknown. We have utilized forward genetics, live imaging and biochemistry to identify a role for a dynein associated protein, Actr10, in the selective retrograde transport of mitochondria. In a forward genetic screen, we isolated a zebrafish mutant with mitochondria rich, swollen axon terminals. Using RNA-seq based analysis, we identified the causative mutation as a T to G change in the start site of the gene encoding Actr10. Actr10 is a component of the dynactin, a large accessory complex oftentimes associated with the cytoplasmic dynein. Actr10 is situated in a “pointed end” region of dynactin which is postulated to function as an adaptor for cargo transport. However, a specific role for Actr10 in retrograde cargo movement in axons has not been elucidated. Using in vivo analysis of mitochondrial motility in axons, we found a decrease in the retrograde transport of this organelle, whereas anterograde transport was not perturbed. The localization of dynein-dynactin components and other cargos, such as lysosomes, autophagosomes, and activated JNK (c-Jun N-terminal Kinase) was normal in actr10 mutants. Additionally, in vivo analysis of dynein motility demonstrated that movement of this motor is unaffected with loss of Actr10, arguing for a specific role for Actr10 in mitochondria retrograde movement. Finally, using mitochondrial fractionation and immunoprecipitation, we have shown that loss of Actr10 results in failed dynactin-mitochondrial coupling further supporting a role for this protein in attaching mitochondria to the retrograde motor complex. Together, our data support a model in which Actr10 serves as a unique adaptor which links mitochondria to the retrograde motor complex for transport and proper positioning in axons.

Program Abstract #20

Signaling by touch – morphogens on the move
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Development creates a vast array of forms and patterns with elegant economy, using a small vocabulary of pattern-
generating proteins such as BMPs, FGFs, Wg, and Hh in similar ways in many different contexts. Much theoretical and experimental work has investigated the mechanisms that disperse these morphogen signaling proteins, and there is now strong evidence that establishes a fundamental and essential role for cytonemes – specialized filopodia that make functional connections between signaling cells and that transport signaling proteins from producing to receiving cells. Cytoneme-mediated signaling is a dispersal mechanism that delivers signaling proteins directly at sites of cell-cell contact.

Program Abstract #21
Super-resolution imaging of chromatin nanostructure links epigenetic state and 3D genome packaging
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Metazoan genomes are packaged at multiple scales, and regulation of this spatial organization may play an important role in development and cell fate specification. Unfortunately, current methods provide little information about 3D organization of the genome at the length scale of genes (kilobases) and regulatory domains (hundreds of kilobases) in single cells. I will present a new super-resolution imaging approach to study the structural organization of the genome at the kilobase to megabase scale in individual cells at 20 nm resolution. These domains largely occupy diffraction limited volumes and thus their structures cannot be resolved with conventional imaging approaches. From thousands of images of dozens of epigenetic domains from across the *Drosophila* genome, we have discovered, within a single cell type, a substantial diversity of structural patterns: compact and diffuse domains, branched and linear domains, domains that are highly entangled with one-another and domains which are strictly segregated. These different structural features are closely correlated to certain differences in the epigenetic state of the chromatin. I will focus on the organization of Polycomb bound domains, which exhibit a surprising, entangled structure and length-dependent compaction. Computational models suggest this organization could contribute to the repressive nature of the domains. Preliminary comparisons between developing tissues suggests differential regulation of chromatin structure associated with developmental fate selection. This work suggests further super-resolution imaging studies of chromatin structure at this scale may greatly aid our understanding of the role of genome structural regulation in development.

Program Abstract #22
RNA organization in *Drosophila* germ granules
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Germ granules, specialized ribonucleoprotein particles, are a hallmark of all germ cells. In *Drosophila*, an estimated 200 mRNAs are enriched in the germ plasm, and some of these have important, often conserved roles in germ cell formation, specification, survival and migration. How mRNAs are spatially distributed within a germ granule and whether their position defines functional properties is unclear. We have used single-molecule FISH and structured illumination microscopy, a super resolution approach, to show that mRNAs are spatially organized within the granule whereas core germ plasm proteins, such as Vasa, oskar, Tudor and Aubergine are distributed evenly throughout the granule. Multiple copies of single mRNAs self-organize into “homotypic clusters” that occupy defined positions within the center or periphery of the granule. This organization, which is maintained during embryogenesis and independent of the translational or degradation activity of mRNAs, reveals new regulatory mechanisms for germ plasm mRNAs that may be applicable to other mRNA granules.

Program Abstract #23
Visualizing Cell Turnover During Epithelial Tissue Homeostasis and Repair Using Developing Zebrafish
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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 how these two processes influence each other to 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 target cells for ablation. Here we have used time-lapse imaging and reverse genetic techniques to characterize cell turnover under normal physiological conditions, after damage, and when extrusion is altered. 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 underwent apoptosis and were rapidly eliminated by extrusion. 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 new mechanisms controlling tissue homeostasis and the specific alterations that lead to pathologies and cancer.

Program Abstract #24
Transcriptional and hormonal regulation of gravitropisms in forest trees.
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Forest trees reinforce and even reorient their woody stems in response to gravitational and mechanical stresses. In angiosperm trees, this is achieved by the production of “tension wood,” which contains fiber cells with specialized cell wall layers that can generate strong tensile forces. We show here using the model forest tree, *Populus*, that the Class I KNOX homeobox gene ARBORKNOX2 (ARK2) affects graviresponse and gravibending. An endodermal cell layer is the point of gravity perception in woody stems, and the endodermal-localized auxin transport protein ptPIN3 shows polar relocalization as an initial response to gravity perception. Proper ptPIN3 relocalization is compromised in trees with lowered ARK2 transcript abundance. Surprisingly, trees with reduced ARK2 transcript levels also produce large numbers of tension wood fibers, but exhibit poor gravibending. Notably, wild-type trees normally upregulate the expression of genes encoding Fasciclin-like Arabinogalactan (FLAs) as well as xyloglucan endotransglycosylase (XET) proteins during tension wood development, and these proteins are thought to play important roles in generating the force displayed by tension wood fibers. Immunolocalization of FLAs and visualization of XET activity in vivo showed that the tension wood fibers of trees with reduced ARK2 expression levels have marked delay of these markers. Finally, we present gene co-expression networks for wood forming tissues of trees, including analyses identifying gene modules correlated with specific phenotypes, and identification of candidate genes regulating developmental traits. We conclude that ARK2 influences tension wood formation and gravibending by regulating both hormonal-related processes, as well as the expression of genes required for force generation in tension wood fibers. This project was supported by AFRI grant # 2011-67013-30062 of the USDA National Institute of Food and Agriculture.

Program Abstract #25
Engineering cellular interactions for synthetic development
Paul Grant,1 Neil Dalchau,2 James Brown,1 Fernan Federici,3 Timothy Rudge,1 Boyan Yordanov,2 Andrew Phillips,2 Jim Haseloff1
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The study of the development of multicellular organisms has yielded a multitude of mechanisms by which complex structures can arise from simple molecular and physical interactions. These self-organizing processes are a rich source of inspiration for the engineering of synthetic biological systems that arrange matter in ways that may prove useful for biomaterials, organized bioreactor communities, or medical applications. They can also serve as a testbed for the design rules needed to re-engineer multicellular systems such as crop plants to create novel morphologies and functionalities. In the short term, however, we can--by building synthetic systems based on the principles of development--test how well we understand those principles, explore the parameters under which they can function, and determine how generalizable they are to new contexts. To this end we have built a synthetic two-channel cell-cell communication system using two different quorum-sensing signals that can be sent and received by *E. coli* cells with minimal crosstalk. Because we have built this system from scratch we have full control over the components allowing us to measure the response in a wide variety of conditions. This large amount of quantitative data allows us to infer the parameters to a highly detailed mathematical model giving us the ability to predict the optimal expression levels of receptors to minimize crosstalk. We have combined these signaling circuits with a novel spatial assay system in which cells are grown on gridded membranes so that we can create arbitrary arrangements of populations. In this context we have built a circuit that can propagate
signals by positive feedback through mutual activation and a patterning circuit that creates mutually exclusive contiguous domains of gene expression with sharp boundaries by, analogously to the *Drosophila* gap gene network, using self-activation combined with mutual inhibition to create bistability at the population level.

**Program Abstract #26**

**Regulation of cardiopharyngeal cell fate and behavior in a simple chordate**

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How genetic programs coordinate cell identity and behavior in animal development remains a poorly understood problem. We study this question using the ascidian cardiopharyngeal mesoderm as a simple chordate model. As in vertebrates, mesodermal cardiopharyngeal progenitors produce both heart and pharyngeal muscles, which in ascidians form body wall and atrial siphon muscles (ASM). Because ascidian embryos and larvae develop with limited cell numbers, cell lineages and morphogenesis are extremely stereotyped, allowing straightforward clonal interpretation of cellular arrangements. Combining tissue-specific molecular perturbations, FACS, transcriptome profiling, fluorescent imaging and quantitative analyses, we explore the molecular and cellular mechanisms of cardiopharyngeal fate specification and collective cell migration. Following induction, the cardiopharyngeal progenitors migrate as polarized pairs of cells. The behavior of cardiopharyngeal progenitors is canalized towards collective polarity and directed migration by constraints from surrounding tissues. I will first discuss how the interplay between the environment and intrinsic properties determines the behavior of the cardiopharyngeal progenitors. Following migration, multipotent cardiopharyngeal progenitors divide twice asymmetrically to produce first and second heart precursors (FHP and SHP) and the atrial muscle founder cells (ASMF). Cardiopharyngeal progenitors display multilineage transcriptional priming, meaning that they express early regulators of both the heart- and ASM-specific programs. Following oriented asymmetric divisions, regulatory cross-antagonisms segregate the cardiac and ASM programs to their corresponding precursors. I will discuss the signaling and regulatory mechanisms that break the initial heart-ASM balance and the genome-wide transcriptional dynamics underlying transition from multipotency to distinct fate-restrict states.

**Program Abstract #27**

**Deployment of a retinal determination gene network drives directed cell migration**

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Gene regulatory networks (GRNs) provide a systems-level orchestration of an organism’s genome encoded anatomy. As biological networks are revealed, they continue to answer many questions including knowledge of how GRNs control morphogenetic movements. The migration of the small micromeres in the sea urchin embryo provides an exceptional model for understanding the genomic regulatory control of morphogenesis. Throughout gastrulation, small micromeres actively extend filopodia and lamellipodia at the tip of the archenteron until the end of gastrulation when they undergo an epithelial-mesenchymal transition (EMT). Once mesenchymal, the small micromeres migrate over the archenteron to the posterior halves of the forming coelomic pouches. Here, we show that ectopically placed small micromeres reach the coelomic pouches via a directed homing mechanism. When the coelomic pouch mesoderm GRN was perturbed, the small micromeres were unable to home. Transcription factor knockdowns within the small micromere and coelomic pouch lineages shed light on the regulatory underpinnings of that homing behavior. An assay using the robust homing potential of these cells reveals a “coherent feed-forward” transcriptional subcircuit composed of Pax6, Six3, Eya, and Dach1 that is responsible for the directed homing mechanism of these multipotent progenitors. The linkages of that circuit are strikingly similar to a circuit involved in retinal specification in *Drosophila* suggesting that systems-level tasks can be highly conserved even though the tasks drive unrelated processes in different animals. Support for this project was provided by NIH RO1-HD-14483 and NIH PO1-HD-037105.

**Program Abstract #28**

**Regulatory changes associated with larval regeneration in the echinoderm Patiria miniata**

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Some organisms have an extraordinary capacity to regenerate their body following traumatic loss of tissue. This capacity appears in species across the animal tree of life, presenting the possibility that homologous mechanism may be used to regenerate whole bodies in these disparate taxa. Sea stars are a class of echinoderm known for their great capacities for whole body regeneration, including of their larval forms. They are also model system for the study of developmental gene
regulatory networks. However, little is known of their mechanisms of regeneration, providing a gap in our knowledge of deuterostome regeneration. We will discuss how the regulatory profile changes during regeneration and the role of Wnt signaling in reestablishing body patterning during regeneration of the larval sea star *Patiria miniata*. We will also discuss the extent to which developmental gene regulatory networks are used during regeneration. Finally, we will discuss whether common mechanism might be used to re-specify tissues during regeneration.

Program Abstract #29
Dynamic Foxh1 binding and epigenetics controlling the early vertebrate gene regulatory program
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The development of animals is regulated by the output of the complex transcriptional signaling programs hardwired in the genome. This regulatory logic, encoded by the combinatorial interactions of transcription factors and the integration of cell signals, produces dynamic regulatory states controlling subsequent developmental events. Nodal signaling, of which the maternal transcription factor Foxh1 is a key mediator, is critical for the proper induction of mesoderm and endoderm. Here, we report on the dynamic contribution of Foxh1 to the gene regulatory network controlling germ layer specification, and extend beyond its previously characterized role as a Smad2/4 co-factor. First, pointing to a pioneering function, we present evidence of Nodal-independent Foxh1 binding in cleavage stage embryos, which precedes both active Nodal signaling and histone modifications. Second, Foxh1 ChIP-seq over a timecourse of *Xenopus tropicalis* development during the onset of Nodal signaling reveals highly dynamic Foxh1 binding changes from the blastula to the gastrula stage. Third, Foxh1 potential targets are components of major signaling pathways (e.g. BMP, Wnt), suggesting Foxh1’s involvement in signaling crosstalk. Fourth, the incorporation of epigenetic modifications demonstrates that Foxh1 associated regions correlate to active enhancers. Lastly, motif-finding analysis on the regions under the Foxh1 peaks implicates that Foxh1 partners with pluripotency factors (i.e. Oct, Sox) and regulates the transcription of early developmental programs. This work is funded by a U.S. Department of Education GAANN (P200A120207) fellowship to R. L. and NIH HD073179 to K. C.

Program Abstract #30
Gene regulatory interactions mediating neural crest formation along the body axis
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The neural crest is a population of multipotent, migratory progenitor cells that forms at the border of neural and non-neural ectoderm in vertebrate embryos. These cells then migrate from the neural tube along defined pathways, populate numerous sites and differentiate into diverse cells types including melanocytes, sensory and autonomic neurons, and the craniofacial skeleton. However, neural crest populations differ along the neural axis with respect to migration pathways and derivatives. For example, cranial neural crest cells give rise to cartilage and bone of the face, whereas those at trunk levels do not normally do so. Gene regulatory networks (GRNs) comprised of interacting transcriptional regulators and downstream effector genes are thought to confer properties like multipotency and migratory capacity to nascent neural crest cell populations. This has been best characterized for the cranial neural crest, but GRN differences along the body axis are not well understood. To elaborate the neural crest GRN at particular axial levels and gain insight into differences along the body axis, we are using axial level specific enhancers to isolate neural crest subpopulations and perform transcriptome analysis. We then test functional connections in the neural crest GRNS by systematically perturbing transcription factors to establish their role in specification and examining the effect of these perturbations on likely downstream genes in establish interrelationships. The results suggest that a series of regulatory circuits are involved in inducing the migratory neural crest cell population, maintaining its stem cell properties for a time and finally leading to progressive differentiation with both common and differential GRN components present at different axial levels.

Program Abstract #31
A Molecular Network Module Governing Sensory Neuron Diversification
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Sensory neuron diversity is required for organisms to decipher complex environmental cues. In *Drosophila*, 50 olfactory receptor neuron (ORN) classes are clustered in stereotyped combinations within distinct sensilla subtypes. Each sensilla subtype houses 1-4 ORNs with stereotypic ORN identities that arise through asymmetric divisions from a single
functions related to jasmonic acid, auxin biosynthesis, stress responses, and meristem development were disrupted in the protein plays a role in meristem function. RNA-Seq analysis of mutant and wild-type plants found that genes with leaves. Application of daily, low-level heat stress increased severity of the phenotype. These defects suggest the SR45a splicing. Mutants had fewer inflorescence structures and lower first nodes with rosette shaped leaves in place of cauline stress. To study SR45a function, we characterized an SR45a T-DNA insertion line with reduced expression and altered involved in splicing that produces at least four alternative splicing variants that change in relative abundance under heat stress. An alternative view is that remodeling the splicing machinery enables expression of alternative splice forms that many genes involved in splicing, suggesting that stresses remodel the splicing machinery to enable correct splicing under stress. An alternative view is that remodeling the splicing machinery enables expression of alternative splice forms that are better adapted to stresses. To investigate the role of alternative splicing in stress adaptation, we used RNA-Seq to survey gene expression, but established methods separate cells from their native spatial context. Here we present Seurat, a computational strategy to infer cellular localization by integrating single-cell RNA-seq data with in situ RNA patterns. We applied Seurat to spatially map 851 single cells from dissociated zebrafish (Danio rerio) embryos at 50% epiboly stage, and then inferred a transcriptome-wide atlas of gene expression. We confirmed Seurat’s accuracy by mapping manually extracted cells. Additionally, Seurat correctly localizes rare subpopulations, accurately mapping both spatially restricted and scattered groups. We used Seurat to identify and characterize both known cell types and a potentially new one. Seurat will be applicable to mapping cellular localization within complex patterned tissues in diverse systems. This work was supported by the Jane Coffin Childs Memorial Fund for Medical Research (J.A.F.), F32 HD075541 (R.S.), the NIH (A.F.S.), National Human Genome Research Institute, Center of Excellence in Genome Science 1P50HG006193, the Klarman Cell Observatory and Howard Hughes Medical Institute (A.R.).

Program Abstract #32
Spatial reconstruction of embryonic single-cell gene expression
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Rahul Satija and Jeffrey Farrell made equal contributions. Aviv Regev and Alexander Schier jointly supervised the work. Spatial localization is a key determinant of cellular fate and behavior, but spatial RNA assays traditionally rely on staining for a limited number of RNA species. In contrast, single-cell RNA-seq allows for deep profiling of cellular gene expression, but established methods separate cells from their native spatial context. Here we present Seurat, a computational strategy to infer cellular localization by integrating single-cell RNA-seq data with in situ RNA patterns. We applied Seurat to spatially map 851 single cells from dissociated zebrafish (Danio rerio) embryos at 50% epiboly stage, and then inferred a transcriptome-wide atlas of gene expression. We confirmed Seurat’s accuracy by mapping manually extracted cells. Additionally, Seurat correctly localizes rare subpopulations, accurately mapping both spatially restricted and scattered groups. We used Seurat to identify and characterize both known cell types and a potentially new one. Seurat will be applicable to mapping cellular localization within complex patterned tissues in diverse systems. This work was supported by the Jane Coffin Childs Memorial Fund for Medical Research (J.A.F.), F32 HD075541 (R.S.), the NIH (A.F.S.), National Human Genome Research Institute, Center of Excellence in Genome Science 1P50HG006193, the Klarman Cell Observatory and Howard Hughes Medical Institute (A.R.).

Program Abstract #33
Role of SR45a in alternative splicing and stress response
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Most protein-coding genes in higher eukaryotes contain introns, regions of the primary transcript that are removed during pre-mRNA splicing. Thanks to alternative splicing, one gene can produce multiple mRNA species encoding proteins with different but related functions. Alternative splicing is believed to affect more than 20% of all multi-exon genes in plant species. In plants, cold, heat, and water deprivation stresses activate expression and differential alternative splicing of many genes involved in splicing, suggesting that stresses remodel the splicing machinery to enable correct splicing under stress. An alternative view is that remodeling the splicing machinery enables expression of alternative splice forms that are better adapted to stresses. To investigate the role of alternative splicing in stress adaptation, we used RNA-Seq to survey gene expression and splicing in plants undergoing heat or drought stresses. This identified a large number of “super splicers,” genes with highly variable splicing patterns. One such “super splicer” was SR45a, an SR-like protein involved in splicing that produces at least four alternative splicing variants that change in relative abundance under heat stress. To study SR45a function, we characterized an SR45a T-DNA insertion line with reduced expression and altered splicing. Mutants had fewer inflorescence structures and lower first nodes with rosette shaped leaves in place of cauline leaves. Application of daily, low-level heat stress increased severity of the phenotype. These defects suggest the SR45a protein plays a role in meristem function. RNA-Seq analysis of mutant and wild-type plants found that genes with functions related to jasmonic acid, auxin biosynthesis, stress responses, and meristem development were disrupted in the
heat-stressed mutant in comparison to wild type controls. These results indicate that SR45a plays a role in both development and stress responses in *Arabidopsis*.

**Program Abstract #34**

**Somatic cell encystment promotes abscission in germline stem cells following a regulated block in cytokinesis**

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Stem cell behavior, from rates of cell division to the capacity for self-renewal, is regulated by the specialized microenvironment in which those stem cells reside. A critical function of such niches in many tissues is the coordination of behavior across multiple stem cell lineages. However, the means by which this coordination is achieved are largely unknown. We have identified delayed completion of cytokinesis in germline stem cells (GSCs) as a novel mechanism that regulates the production of stem cell daughters within the niche of the *Drosophila* testis. Through live imaging, we have characterized the delay within GSCs and identified two regulatory mechanisms layered on top of cytokinesis. Following contractile ring disassembly, a novel F-actin ring is formed through regulation of Cofilin activity to block further progression of cytokinesis. The duration of this block is controlled by Aurora B kinase activity. Importantly, both regulatory mechanisms are specific to GSCs and do not operate in their differentiating daughter cells. Additionally, we have identified a critical requirement for somatic cell encystment of the germline in promoting the final step of GSC cytokinesis. We suggest that this non-autonomous role exists to promote the coordination necessary between stem cell lineages within this niche to achieve robust production of sperm. Together, these findings shed significant insight into the mechanisms by which cytokinesis is inhibited and reinitiated in GSCs and why such complex regulation might exist within the stem cell niche.

**Program Abstract #35**

**Identification of factors critical for regeneration of the planarian nervous system**

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After injury, an organism faces numerous challenges for tissue replacement. New cells must be made in the appropriate number and mixture. New tissue must be patterned and integrated in the context of existing tissues; in the 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 molecules that drive robust CNS regeneration in planarians. We used high-throughput sequencing to identify differentially regulated transcripts after head amputation. We determined the expression patterns of upregulated genes and screened ~300 neural- and stem cell-expressed factors for functions in regeneration. We identified several key genes, including a *hey* homolog with a role in brain patterning, an *arrowhead* gene required for medial CNS structures, a signaling molecule related to CRELD, genes important for restoration of chemosensory behavior, and novel factors involved in stem cell maintenance. We further explored conserved binding partners of Arrowhead and we propose a LIM domain binding protein-hubbed transcriptional network essential for head regeneration. Finally, we 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. We find that planarian glia express genes for neurotransmitter reuptake and are visible in the neuropil by electron microscopy. 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.

**Program Abstract #36**

**A ciliary inhibitor is required for ciliogenesis - fine tuning Cp110 levels during development and function of multiciliated cells**

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Motile cilia are required for the establishment of directional fluid flows along epithelia. In mucociliary epithelia, multiciliated cells (MCCs) establish extracellular fluid flow, while secretory cell types release mucus and other substances, thereby contributing to mucociliary clearance and regulation of cell function. We are using the *Xenopus* embryonic epidermis to study vertebrate mucociliary development *in vivo*. Here we present how cell type-specific
transcriptional regulation, together with microRNA- (miR-ﬁsted post-transcriptional control contribute to the precise regulation of Cp110 levels in MCCs, and thereby to development and function of mucociliary epithelia. miRs from the 34/449 family are highly enriched in MCCs and are regulated through the MCC-speciﬁc transcription factor Multicilin. miR-34/449 promote multi-ciliogenesis through post-transcriptional downregulation of target mRNAs. A key target of miR-34/449 is cp110, a conserved inhibitor of ciliogenesis in mono- and multiciliated cells. Loss of miR-34/449 leads to increased levels of Cp110 in MCCs, which in turn causes defects in centriole-to-basal body transition, resulting in basal body docking defects and failed ciliogenesis. Loss of function and rescue data indicate that Cp110 levels in MCCs need to be precisely controlled: Increased levels of Cp110 lead to ciliogenesis defects, but Cp110-depleted MCCs also fail to establish extracellular ﬂuid ﬂow. Loss of Cp110 causes impaired formation of ciliary adhesion complexes and defective interactions of basal bodies with the actin cytoskeleton. Therefore, Cp110-deﬁcient MCCs show reduced numbers of cilia, as well as loss of ciliary motility and directionality. Taken together, our work reveals novel molecular functions of the ciliary inhibitor Cp110 in MCC development and exempliﬁes how post-transcriptional regulation can be used within a transcriptional program to precisely regulate protein levels. Funding: PW DFG Wa3365/1-1; RMH NIH GM42341.

Program Abstract #37
Altered cholesterol biosynthesis results in abnormal differentiation of neural progenitors in the developing mouse forebrain
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Proper cholesterol homeostasis is critical in organogenesis and inborn errors of cholesterol biosynthesis result in a number of CNS abnormalities in humans and mice. We previously reported a recessive hypomorphic mutation in the cholesterol biosynthesis gene hydroxysteroid (17-beta) dehydrogenase 7 (Hsd17b7rudolph) that results in prenatal lethality with dramatic phenotypes in the embryonic forebrain. Immunohistochemical (IHC) analysis for apical polarity shows loss of β-catenin, Phalloidin, and ZO-1 occurs by E13.5 in mutants. However, this phenotype appears secondary to proliferation and differentiation defects. IHC with mitotic markers reveals abnormal neuroprogenitor proliferation in the mutant telencephalon, with increasing numbers of mitotic cells displaced from the ventricular zone (VZ) by E11.5. Simultaneously, progenitor proliferation at the VZ is decreased. Phosphorylated Vimentin identiﬁes the displaced cells as dividing radial glia suggesting improper interkinetic nuclear migration (IKNM). EdU pulse-chase labeling also suggests IKNM defects. Additionally, staining for Pax6 and Tbr2 indicate decreased numbers of apical progenitors and an expansion of intermediate progenitors by E12.5. Regions of TuJ1-positive post-mitotic neurons appear expanded with a “double-cortex” evident by E14.5. In vitro primary neuron culture of E11.5 neuroprogenitors supports our model of accelerated differentiation in mutant cells. Consistent with a primary defect in cholesterol biosynthesis, combined administration of both statins and dietary cholesterol in utero resulted in partial rescue of multiple phenotypes in the Hsd17b7rudolph embryo, including the forebrain. Taken together, these results suggest that modiﬁcations in cholesterol biosynthesis can alter neural progenitor cell fate.

Program Abstract #38
Elucidating the role of Cadherin-6B soluble C-terminal fragments during chick cranial neural crest EMT.
Andrew Schiffmacher, Lisa Taneyhill
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At the onset of epithelial-to-mesenchymal transition (EMT), many cells proteolytically process their cadherins to disassemble cell-cell junctions and generate cleavage products with novel functions. These cadherin fragments modulate cell proliferation and migration, but their roles during EMT remain unclear. Chick premigratory cranial neural crest cells (NCCs) downregulate existing Cadherin-6B (Cad6B) levels through multiple mechanisms, including proteolysis, before completing EMT. Our prior work revealed that Cad6B is subjected to serial processing via ADAM metalloproteinases and γ-secretase to generate shed N-terminal ectodomains and soluble intracellular C-terminal fragments (CTF2). Interestingly, Cad6B CTF2 overexpression in premigratory cranial NCCs prematurely reduces Cad6B levels, leading us to hypothesize that CTF2 may participate in a regulatory loop to promote Cad6B-junction disassembly and EMT. Our ﬁndings suggest that β-catenin is the primary interactor of CTF2, with elevated levels of CTF2 in NCCs causing a redistribution of β-catenin from the membrane to the cytosol. Migratory NCCs generated from premigratory NCC explants overexpressing CTF2 display a CTF2-dependent nuclear localization of both CTF2 and β-catenin. In addition, in vitro co-immunoprecipitation experiments conﬁrm a CTF2/β-catenin direct interaction and nuclear localization. Furthermore,
transient overexpression of Cad6B CTF2 in NCCs significantly upregulates β-catenin, CyclinD1, and Snail2 expression. To determine if these results are due to CTF2-mediated modulation of Wnt/β-catenin signaling, we have designed constructs expressing mutant CTF2s that bind β-catenin at significantly lower affinities. Collectively, our findings reveal that Cad6B proteolysis in premigratory NCCs may regulate EMT effector genes, providing insight into the role of CTF2 in developmental EMTs. This work is supported by a grant to AS (F32DE022990) and LAT (NSF IOS-0948525).

Program Abstract #39
Tbx16 and Mesogenin regulate cell migratory persistence during completion of the EMT in zebrafish
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During early vertebrate embryonic development, mesoderm becomes organized progressively from anterior to posterior along the body axis to form blocks of muscle precursors, the somites. Mesodermal progenitor cells residing at the posterior end of the body undergo an epithelial to mesenchymal transition (EMT) as they begin migrating anteriorly and initiate differentiation. In zebrafish, the transcription factors Spadetail/Tbx16 (Spt) and Mesogenin1 (Msgn) are together essential for mesodermal cell morphogenesis and differentiation. In spt;msgn-deficient embryos, pre-mesodermal cells can initiate but not complete the EMT, and therefore accumulate at the posterior end of the embryo. This double mutant represents a unique system for uncovering the mechanisms used by cells to transition to a mesodermal state. A novel tailbud explant method combined with high spatial- and time-resolution imaging of individual cells reveals that actin-based protrusions form fairly normally in spt;msgn-deficient cells and that these cells are motile. However, spt;msgn-deficient cells have specific cell-autonomous defects in orientation and persistence of migration. RNA-sequencing of wild-type and spt;msgn-deficient tailbuds uncovered genes involved in various processes related to cellular morphogenesis which are differentially expressed in the two genotypes. Several of these genes have been implicated in regulation of Rho GTPase signaling, which is required for proper intracellular coordination of cell migration. Interestingly, many of these genes are expressed at higher levels in the spt;msgn-deficient tailbud than in wild-type and can induce significant migration changes when overexpressed in single mesodermal precursor cells. These results suggest that there are several Spt/Msgn transcriptional targets that interact to coordinate Rho GTPase signaling as cells move through the EMT, providing new insight into the changes required in vivo for cells to become mesenchymal.

Program Abstract #40
Roundabout Receptors Control Lung Sensory Function via Regulating Pulmonary Neuroendocrine Cell Development
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The lung epithelium, being expose to air, poses as a prominent interphase to sense the environment. However, the mechanism and impact of this sensory function are not well defined. The pulmonary neuroendocrine cells (PNECs) are the only innervated epithelial cells in lung, but their in vivo role remains elusive. Here we show that Roundabout receptor (Robo) genes Robo1 and Robo2 are expressed in PNECs. Inactivation of these genes in mice leads to PNEC development defects. These defects result in lung physiological defects and structural remodeling. Our data suggest that PNEC malformation may synergize with environmental triggers to underlie susceptibility to lung diseases.

Program Abstract #41
Whole or hole? Development of the Diaphragm and Congenital Diaphragmatic Hernias
Gabrielle Kardon, Allyson Merrell, Benjamin Ellis, Zachary Fox, Jennifer Lawson, Jeffrey Weiss
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The diaphragm is an essential mammalian skeletal muscle, and defects in diaphragm development are the cause of congenital diaphragmatic hernias (CDH), a common and often lethal birth defect. The diaphragm is derived from multiple embryonic sources, but how these give rise to the diaphragm is unknown and, despite the identification of many CDH-associated genes, the etiology of CDH is incompletely understood. Using mouse genetics, we show that the pleuroperitoneal folds (PPFs), transient embryonic structures, are the source of the diaphragm’s muscle connective tissue, regulate muscle development, and their striking migration controls diaphragm morphogenesis. Furthermore, Gata4 mosaic mutations in PPF-derived muscle connective tissue fibroblasts result in the development of localized amuscular regions that are biomechanically weaker and more compliant and lead to CDH. Thus the PPFs and muscle connective tissue are critical for diaphragm development and mutations in PPF-derived fibroblasts are a source of CDH.
Program Abstract #42
The left-right Pitx2 pathway drives organ-specific arterial and lymphatic development in the intestine
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After more than a 100-year debate over the origin of mammalian vascular lymphatics, genetic studies have confirmed that lymphatic endothelial cells sprout from veins. To our surprise, we recently discovered within the gut and associated mesenteries of chicken and mouse embryos a distinct population of lymphatic endothelial precursors arises locally at a great distance from veins, and is dependent upon prior arteriogenesis and the evolutionarily conserved left-right (LR) transcription factor Pitx2. Our findings reveal lymphatic progenitor heterogeneity in the embryonic dorsal mesentery (DM), the major conduit for blood and lymphatic vessels supplying the looping gut. Gut lymphatics serve as the sole and essential channels for absorption and transport of dietary lipids. However, the molecular mechanisms governing their specialized functions remain unknown. Here we show that gut arteries and lymphatics have a left-sided origin dependent on Pitx2, Cxcr4 (the G-protein-coupled chemokine receptor) and its ligand Cxcl12. Our immediate goals are to understand how Pitx2-driven Cxcl12/Cxcr4 signaling affects changes in cell behavior that affect lymphatics and whether this pathway serves as the mechanism linking asymmetric arterial and lymphatic patterning. Our unexpected findings bear critical therapeutic importance as distinct lymphatic endothelial subsets are thought to contribute differentially to intestinal disorders. Moreover, such lymphatic progenitor cells can be exploited to restore lymphatic function following cancer surgery or lymphedema.

Program Abstract #43
Genetic control of growth and patterning in the early plant embryo
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Both growth and tissue patterning are processes that occur continuously during plant life. A key question is how these are coordinated in space and time to generate plant shape and function. In plants, all patterning occurs in the absence of cell migration, and thus oriented cell division and local growth are key to generating 3D patterns and shapes. We use the early Arabidopsis embryo as a simple and highly predictable model in which cell identity specification, growth and patterning are intricately coordinated. I will discuss our recent work aimed at understanding the cellular basis for the establishment of multicellular patterns in 3D. Furthermore, I will present our progress towards understanding the definition of embryonic and tissue identity through transcriptional control.

Program Abstract #44
Tissue landscape impacts morphogen signaling during Drosophila oogenesis
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Morphogens control developmental processes in a concentration-dependent manner. Responses to these signals are impacted by parameters that govern morphogen concentration, including release, diffusion, uptake, and decay rates. Extracellular signals are often modeled as diffusing into open domains, but the physical structure of adjacent tissue could influence local amounts of signals, and thereby cell fates. We examined this issue in the Drosophila egg chamber, which has relatively simple tissue architecture: large germline cells are surrounded by a follicular epithelium. In mid-oogenesis, two anterior follicle cells secrete Unpaired (Upd) to activate the conserved factor Signal Transducer and Activator of Transcription (STAT) in neighboring cells. High STAT activity instructs a subset of cells to become motile. Our genetic analysis showed that all cells near the Upd source are able to respond to this signal. However, when viewed end-on, we found that STAT activation and resulting cell fates often arose in unexpected, asymmetric patterns, suggesting that some cells experienced different Upd levels than predicted by their proximity to the source. To characterize morphogen activity in the extracellular domain, we developed a three dimensional mathematical model of the spatio-temporal distribution of Upd. Simulation results supported the idea that irregular tissue domains can produce asymmetric distributions of activator, consistent with results in vivo. Mutants with altered cell architecture predominantly showed radially symmetric patterns of activation. Our model and mutant analysis support the idea that extracellular clefts generated by underlying tissue are sufficient to produce an asymmetric distribution of signaling molecules. This work illustrates a novel aspect to morphogen signaling within complex tissue landscapes, and sheds light on the acquisition of cell motility.
Program Abstract #45

Extracellular matrix dynamics, mediated by the secreted metalloprotease ADAMTS9 regulates vascular smooth muscle cell rotation and primary cilium orientation during growth of the umbilical cord

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The umbilical cord is the sole fetal-maternal link in placental mammals, conducting blood from the fetus for exchange in the placenta. Excessively long or short cords or cord vascular anomalies are associated with high fetal morbidity, and a risk of stillbirth. Astonishingly, nothing is known about the pathway governing the growth of the cord or the cellular events involved in umbilical vessel development. Here we show that vascular smooth muscle cells (VSMCs) of mouse umbilical vessels undergo an orthogonal reorientation between E11.5 and E14.5. We establish that cytoskeletal dynamics mediated by the Shh and PDGF-B signaling pathways are critical for regulating this process, and show that extracellular matrix (ECM) remodeling by the secreted metalloprotease ADAMTS9 is required for propagating these signals. Using conditional mutagen and gene trap mouse mutants, we show that ADAMTS9 is crucial for versican proteolysis and matrix turnover in the umbilical cord. Accompanying ECM accumulation, mutant umbilical vessels do not show VSMC reorientation and fail to elongate, causing intra uterine growth retardation and fetal death by E15.5. The excessive ECM found in Adams9 mutant umbilical cords also has an adverse affect on primary cilium orientation. Primary cilia play important roles in Shh, PDGF-A, and Wnt signaling and are also known to work as ECM sensors in connective tissues since ECM molecules such as collagen and proteoglycans interact with the cilium membrane. We show that ECM interacting molecules integrins α5, and β1 localize to the primary cilium of umbilical cord mesenchymal cells. The primary cilia of wild type cells are oriented radially whereas Adams9 mutant umbilical cords have an altered orientation possibly due to reduced matrix remodeling. We conclude that ADAMTS9 is a key regulator of umbilical cord morphogenesis critical for maintenance of cell signaling, polarity, and cilium orientation.

Program Abstract #46

Identification of receptor-like kinases with polar localization and roles in tissue patterning

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Development often relies on extrinsic, non-cell-autonomous cues for positional information and as determinants of cell fate. Transmembrane and membrane-associated receptor-like kinases (RLKs) are implicated in perception of intercellular signals. In plants, the RLK protein family is greatly expanded and while several RLKs have established roles in plant development, the majority remains functionally uncharacterized. Using high-resolution spatial and temporal gene expression data, an in silico screen was conducted for signaling genes predicted to function in root tissue patterning. Several RLKs were identified and a subset, which show cell type-specific polar localization to lateral plasma membrane domains, were designated POLARLY LOCALIZED KINASEs (PLKs). One of these proteins, PLK1, is localized to the outer polar membrane domain of endodermal cells in the root meristem when expressed under its presumptive promoter. Intriguingly, upon ectopic expression in an adjacent cell layer, PLK1 remains polarly localized, but its orientation is inverted and it is now localized to the inner polar domain. plk1 mutants exhibit altered root cell morphology that appears to arise from abnormal positioning of cell division planes. These observations reveal the presence of polarized, RLK-mediated signaling domains in the root and suggest these domains function in perception of extrinsic cues involved in developmental patterning.

Program Abstract #47

Cadherin interactions in developing neural crest cells

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Numerous cadherin proteins, most notably N-cadherin (Ncad), E-cadherin (Ecad), Cadherin-6B (Cad6B) and Cadherin-7 (Cad7), are expressed in neural crest cells as they delaminate from the newly closed neural tube and acquire migratory properties. Whether the proteins function independently or coordinately remains unknown. To clarify their respective contributions, we have performed a comprehensive characterization of cadherin proteins in the neural tube of post-neurula chick embryos. The results reveal an unexpected degree of overlap of Ncad protein with other cadherins in the ventrolateral neural tube, raising the intriguing possibility that some cadherins may interact heterophilically during development. Using a novel “proximity ligation” assay to test this hypothesis, the results show that Ncad appears to form heterophilic complexes with both Ecad and Cad7, but not Cad6B, in the developing neural tube. In contrast, Ecad, but not Ncad, is expressed in the dorsal-most neural tube containing neural crest precursors, as well as on early migrating neural
crest cells where it partially overlaps with Cad7. Interestingly, Ncad remains absent in the migratory neural crest of chick embryos. In addition, perturbing Ncad and Ecad using gain and loss of function assays leads to alterations in protein localization of the remaining cadherins in the neural tube and neural crest cells. These findings suggest that different cadherin proteins may function in concert in the developing neural tube, but that they also maintain unique functions and cell type-specific localization in the premigratory and migrating neural crest.

Program Abstract #48
Shifts in the Expression of Developmental Regulatory Genes Involved in the Evolution of a Novel Life History Difference
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Here we investigate dramatic evolutionary changes within a well-defined developmental gene regulatory network (GRN) underlying a significant shift in life history strategy: the switch from feeding (planktotrophic) to nonfeeding (lecithotrophic) development in sea urchins. While the ancestral developmental mode of sea urchins is planktotrophic, lecithotrophic development has evolved independently multiple times. In order to identify evolutionary changes in gene expression underlying this ecologically significant shift in life history, we used Illumina RNA-seq to measure expression dynamics across 7 developmental stages in three sea urchin species: the lecithotroph Heliocidaris erythrogramma, the closely related planktotroph Heliocidaris tuberculata, and an out-group planktotroph Lytechinus variegatus. Our analyses draw on a well-characterized developmental GRN in sea urchins to understand how the ancestral developmental program was altered during the evolution of lecithotrophic development. Our results suggest that changes in gene expression profiles were more numerous during the evolution of lecithotrophy than during the persistence of planktotrophy, and this contrast is even stronger when only GRN genes are considered. We found evidence for both conservation and divergence of GRN linkages in H. erythrogramma, as well as significant changes in the expression of genes with known roles in patterning the larval skeleton and gut, which are greatly modified in lecithotrophs. We further explored network dynamics between species using weighted gene co-expression analysis, which allowed us to identify novel players likely involved in sea urchin neurogenesis and endoderm patterning. Collectively, these results indicate that the transition from planktotrophic to lecithotrophic development involved numerous changes to key developmental processes. Funding sources: National Science Foundation and Australian Research Council.

Program Abstract #49
Timing the developmental origins of mammalian limb diversity
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From bat wings to whale flippers, the achievement of diverse limbs has enabled mammals to infiltrate almost every habitat. We seek to determine the developmental processes that have been modified to produce new limb forms, and the degree to which the nature of these processes predisposes the production of certain limb forms instead of others. To pursue these goals we quantified the transcriptome and morphology of developing bat, mouse, pig and opossum limbs. Results suggest that limb development is initially conserved but diverges shortly thereafter. This finding is consistent with earliest limb outgrowth being developmentally constrained. To test this hypothesis we built and perturbed gene interaction networks for early (EN) and late (LN) limb development, and found that the EN is more robust. We then quantified levels of the same genes within populations, and found that they vary less at earlier limb stages. Population-level expression variation is also correlated positively with a gene’s sensitivity to network perturbation, and negatively with a gene’s effect on the network. Finally, we quantified variation in gene expression among species, and found that levels vary less at earlier limb stages. These results suggest that EN robustness buffers population-level variation in gene expression early in limb development, and constrains the evolution of early limb development among species.

Program Abstract #50
A molecular shift in limb identity underlies the convergent evolution and development of feathered feet
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The genetic and developmental mechanisms that control the decision between scale and feather growth – two profoundly different epidermal appendages, and an important developmental shift in the evolution of birds from their dinosaurian ancestors – remain poorly understood. Domestic pigeons display dramatic variation in foot epidermal appendages within a
Evolution of miRNA regulation in neural development
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miRNAs are particularly promising candidates for the study of developmental regulatory changes in evolution. Because an individual miRNA potentially targets a large number of genes, miRNAs are capable of broad manipulation of signaling pathways or development processes. miRNAs are well established as key regulators of development in model systems, and while miRNAs are broadly conserved and their expression correlates with morphological complexity, evolutionary-scale changes in their developmental roles are poorly understood. We are investigating the evolution of miRNA signaling in development using the direct-developing hemichordate Saccoglossus kowalevskii. As an invertebrate sister phyla to vertebrates, Saccoglossus is an important comparative model for deuterostome evolution. We have profiled miRNA expression across development in Saccoglossus and validated methods for in vivo functional perturbations to determine their developmental role. Our current focus is on the role of conserved miRNAs in neural development. Individual miRNAs are known to function in neural development across many species and analysis of nervous systems has been central to evolutionary comparisons. For this project we are characterizing the spatial expression and predicted targets of neural miRNAs in Saccoglossus. A number of these miRNAs have also been perturbed in Saccoglossus embryos, resulting in phenotypes that indicate a diversity of roles during development. Our functional data in particular will provide insight into how miRNAs are integrated into regulatory networks in different animals. Determining the evolving functions of conserved neural miRNAs will help us understand how miRNAs can be used in the developmental patterning of different nervous systems and provide insight into the evolution of miRNA regulation in neural development. Support for this project was provided by NIH R01HD042724.

Program Abstract #52
Butterfly color vision: stochastic patterning mechanisms and increased sensory receptor diversity
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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 more specifically, to determine how much of the retinal patterning network from Drosophila is reused or modified. Using genome sequencing, gene expression, and CRISPR gene knock out 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. In Drosophila, a complex regulatory network combined with cell-cell signaling specifies photoreceptor subtypes within each ommatidium. Then, a stochastic decision of whether to express the transcription factor Spineless in R7 photoreceptors determines which of two types of ommatidial is specified. We find that the R7 marker Prospero is expressed in two photoreceptors per ommatidium in butterflies. CRISPR knock-out of Spineless shows that this gene also controls stochastic choice in each of the two R7s in butterflies, suggesting there is deep evolutionary conservation of stochastic patterning mechanisms. Having two stochastically distributed R7s allows for the specification of three ommatidial types instead of two, which in turn allows 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 #53
Quantitative profiling of single-embryo resolution reporter expressions from many mosaically incorporated reporter constructs permits multiplex spatial cis-regulatory analysis
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We have formulated a new method for cis-regulatory analysis that can simultaneously measure both quantitative and spatial activities of many cis-regulatory modules (CRMs) in a single experiment. In a somewhat counter-intuitive way, our method takes advantage of stochastic, mosaic incorporation of linear reporter constructs in embryos in conjunction with the law of large numbers, the fundamental theorem of probability. According to the theorem, when a large number of incorporation events are considered, the overall patterns of mosaic incorporations between different CRMs will become virtually identical. Therefore, while reporter expression in each embryo is governed by both random clones of cells harboring the CRM::reporter construct and the intrinsic activity of the CRM under investigation, the quantitative profile of single-embryo resolution reporter expressions in a large number of mosaic embryos is solely determined by the intrinsic activity of the CRM, which can be used as the "fingerprint" of specific cis-regulatory activity. To validate this theory, we developed a high-throughput method for single-embryo resolution reporter assays and conducted ~54,000 independent assays for four known CRM::reporter constructs in sea urchin embryos. Using this analysis, we show that quantitative profiles of the four CRM::reporter constructs are consistent with their known spatial activities. Since our new method has the potential to process up to thousands of CRMs in a single experiment, it will facilitate fully utilizing quantitative and spatial gene regulatory information contained in a large number of CRMs.

Program Abstract #54
Enhancer synergy and interference in living Drosophila embryos
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Metazoan genes are embedded in a rich milieu of regulatory information containing numerous enhancers with overlapping regulatory activities. Here we employ quantitative live imaging methods to assess the function of primary and shadow enhancers in the regulation of key patterning genes—knirps, snail and Hunchback—in developing Drosophila embryos. Contrary to simple predictions, such pairs often fail to function in an additive fashion. The knirps enhancers function additively, whereas the Snail enhancers interfere with one another. These paradoxical observations are reconciled by the analysis of Hunchback. The enhancers function additively only in regions where there are diminishing levels of the Bicoid activator gradient. Quantitative modeling of enhancer-promoter interactions suggests that weak enhancers function additively while strong enhancers behave sub-additively due to competition with the target promoter. We propose that multiple enhancers need not function in a simple additive fashion, and suggest that their contributions may be revealed only under stress.

Program Abstract #55
Cell-ECM adhesion is required for force transmission during morphogenesis
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Tissue morphogenesis requires force-generating mechanisms to drive the organization of cells into complex structures. Changes in cell and tissue morphology are achieved by coordinated action of the cytoskeleton and cell adhesion to neighbouring cells and to the extra-cellular matrix (ECM). Integrin-mediated cell-ECM adhesion is known to transduce traction forces in spreading and migrating cells in vitro. The work we will present illustrates that cell-ECM adhesion also transduces forces in vivo in the context of animal morphogenesis. To study the mechanisms by which cell-ECM adhesion regulates tissue mechanics, we use the model system of Dorsal Closure (DC), an integrin-dependent morphogenetic process in the Drosophila embryo. We show that failure to regulate Cell-ECM adhesion results in abnormal levels of tension in the amnioserosa (AS), an extraembryonic epithelium required for DC. Using mutations to either increase or decrease Cell-ECM adhesion, we show that DC is defective and that AS cell dynamics are altered in both cases. Quantitative image analysis and in-vivo laser ablation experiments reveal a relationship between cell deformation and the level of tension in the AS. We propose a mechanism in which cell-ECM adhesion regulates cell deformation, which in turn influences the magnitude and distribution of tension required to complete DC. Our results suggest that Cell-ECM adhesion controls the transmission of forces across developing tissues in order to promote specific outcomes.
How Cells React to Noise: Direct Measurements Reveal Noise Attenuation Mechanisms in Retinoic Acid Signaling
Julian Sosnik, Likun Zheng, Christopher Rackauckas, Enrico Gratton, Qing Nie, Thomas Schilling
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The discovery that biological noise can generate diversity in genetically homogenous cell populations exposed to the same environmental cues, introduces the provoking possibility that noise is a tangible and important property of developing systems. Noise is ubiquitously present in developing organisms and stems from stochastic variations in gene transcripts, protein copy number, molecular interactions, protein activity, diffusion, etc. Genes expressed in developing rhombomeres in the embryonic zebrafish hindbrain arise with rough boundaries, which we have proposed sharpen, in part, due to noise-induced switches in gene expression. In this study we investigate the amplitude of noise in RA signaling, and how modulation of this variance affects the patterning of hindbrain boundaries. We have employed a new technique based on fluorescence lifetime imaging microscopy (FLIM) and phasor analysis to measure for the first time endogenous free intracellular concentrations of retinoic acid (RA) directly in vivo, and its variance both in space and time. Using this technique we show that noise is large in magnitude. We also show that cells use at least two molecular mechanisms to interpret their position within the morphogen gradient by regulating the average and variance of available RA in vivo. Cyp26a1, the main enzyme responsible for the degradation of RA, regulates mean levels of RA without altering the variance. In contrast, the cellular retinoic acid binding protein 2a (Crabp2a) regulates the variance (noise) in RA without altering the mean. Perturbing this regulation results in increased roughness in the rhombomeric boundaries when they first arise as well as increased variance in the level of expression of downstream genes from cell to cell. These findings are among the first to show how cells regulate noise and how manipulating noise in the RA signaling pathway affects the patterning of the developing hindbrain.

How to Communicate Your Science: Dos and Don'ts
William Anderson, Jennifer Zeitzer, Chanda Jefferson, Nicola Barber, Karen Weintraub
1FASEB, USA; 2C.A. Johnson High School, USA; 3University of Utah, USA; 4freelance journalist, USA; 5Harvard University, USA
While scientists are trained to communicate findings to their peers, they most often are not prepared to communicate their findings with those outside of academia. Many funding agencies now require scientists to describe the "broader impact" of their work in terms of training, education, public dissemination, etc. This symposium will discuss how scientists could best share the meaning and implications of their work to a variety of constituents. We will focus on how best to communicate science to those in Congress, K-12 educators and students, and the public at large.

Temporal and spatial patterning of neural stem cells
Claude Desplan, Claire Bertet, Ted Erclik, Xin Li
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We study neurogenesis in the Drosophila optic lobes. Sequential expression of five transcription factors (TFs) in each of the medulla neural stem cells as they age determines the type of neurons produced by these neuroblasts (NBs). Loss of either of these genes blocks further progression of the temporal sequence. Together with Notch-dependent binary fate choice, this controls the diversification of the neuronal progeny. Although a temporal sequence of TFs had been identified in fly embryonic NBs, this illustrates the generality of this strategy, with different sequences of TFs being used in different contexts. Further distinction of neurons results from the intersection of the temporal sequence of TFs in NBs with spatial patterning of the medulla neuroepithelium, which is sub-divided into six subdomains. While temporal patterning is identical in each region, the types of neurons that are generated by a given NB are region-specific. Generic temporal patterning generates unicolumnar neurons that exhibit a 1:1 stoichiometry with photoreceptor columns while spatial genes modify the output of this temporal patterning to generate locally many fewer broad field neurons. In another region of the OPC that produces neurons for other neuropils, a unique temporal series of TFs not only governs the sequential production of distinct neuronal subtypes, but also controls the mode of progenitor division as well as the selective apoptosis of NOFF or NON neurons. Within a single lineage, intermediate precursors initially do not divide and generate only one neuron; subsequently, precursors divide but their NON progeny systematically die through Reaper activity whereas later, their NOFF progeny die through Hid activity. These mechanisms dictate how this part of the neuroepithelium produces neurons for three different optic ganglia. Therefore, temporal patterning generates neuronal diversity by specifying both the identity and survival/death of each unique neuronal subtype.
Program Abstract #59

Mechanical control of airway branching morphogenesis
Victor Varner, Celeste Nelson
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Space-filling, branched networks form the basic architectures of several organs, including the lung, kidney and mammary gland. In the early embryo, these complex structures originate as epithelial tubes, which are molded by a sequence of branching events, patterned in both space and time. In most cases, branching involves reciprocal signaling between the epithelium and surrounding mesenchyme. In the developing mouse lung, airway branching is highly stereotyped and regulated in part by fibroblast growth factor (FGF) signaling. New epithelial branches emerge at locations adjacent to focal expression of FGF10 in the mesenchyme. This pre-pattern of FGF10 is thought to specify the locations of new branches. However, when the mesenchyme is removed, and reciprocal signaling is disrupted, isolated epithelial explants still branch in culture. In the absence of a mesenchymal FGF template, it is unclear how the epithelial branching pattern is specified. Here, using a combination of ex vivo culture experiments and mathematical modeling, we show that a growth-induced mechanical instability defines the relative locations of branches within the developing murine airway epithelium in the absence of mesenchyme. Time-lapse culture of mesenchyme-free explants revealed that new epithelial branches form simultaneously, with the airway epithelium cast into a folded geometry with a characteristic wavelength. To quantitatively investigate the mechanics of this process, we constructed a simple theoretical model for the growing airway epithelium embedded within a viscoelastic gel. Our results indicated that a viscoelastic folding instability underlies epithelial branching in the absence of mesenchyme. The dominant wavelength of the instability defines the branching pattern, and is controlled by epithelial growth rates. Taken together, these results suggest that purely physical interactions can drive the biological patterns, which underlie tissue morphogenesis in the embryo.

Program Abstract #60

Notochord vacuoles function as a hydrostatic scaffold for spine morphogenesis
Michel Bagnat
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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 are uncovering mechanism by which notochord vacuoles function during spine formation. Live imaging of individuals in which notochord vacuoles are disrupted in a mosaic fashion revealed that kinking of the axis is associated with vertebral bone growth. To gain new insights into the processes that lead to CS, we and others have 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 around the notochord. These studies suggest that lack of a hydrostatic scaffold for bone deposition in fish with disrupted notochord vacuoles leads to aberrant spine morphogenesis. We propose that fully inflated notochord vacuoles are necessary to evenly distribute the compressive force of growing vertebrae during spine development. These studies offer mechanistic insights into spine morphogenesis and the etiology of CS.

Program Abstract #61

Patterning the microtubule cytoskeleton during development
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The centrosome acts as the microtubule organizing center (MTOC) during mitosis in most animal cells. Microtubules are nucleated and anchored by γ-tubulin ring complexes (γ-TuRCs) embedded within the centrosome’s pericentriolar material (PCM). The PCM is required for the localization of γ-TuRCs and both are steadily recruited to the centrosome, culminating in a peak in MTOC function in metaphase. In differentiated cells, the centrosome is often attenuated as an MTOC and MTOC function is reassigned to non-centrosomal sites such as the apical membrane of epithelial cells, the nuclear envelope in skeletal muscle, and down the lengths of the axons and dendrites of neurons. Hyperactive MTOC function at the centrosome is associated with several types of epithelial cancers and has recently been linked to invasive behavior in tumor cells. Little is known about the mechanisms that limit MTOC activation at the centrosome. Here, we find that MTOC function at the centrosome is completely inactivated during cell differentiation in C. elegans embryonic...
intestinal cells and MTOC function is reassigned to the apical membrane. In cells that divide after differentiation, the cellular MTOC state switches between the membrane and the centrosome. Using cell fusion experiments within living embryos, we find that the centrosome MTOC state is dominant and that the inactive MTOC state of the centrosome is malleable; fusion of a mitotic cell to a differentiated or interphase cell results in rapid reactivation of the centrosome MTOC. We show that this conversion of MTOC state involves the conserved centrosome protein SPD-2/CEP192 and CDK activity from the mitotic cell.

Program Abstract #62
Bimodal roles of the adhesion GPCR Gpr126 in Schwann cell development
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Myelin, a multilamellar structure that supports and protects axons, is formed in the peripheral nervous system by Schwann cells (SCs). During development, SCs radially sort axons in a 1:1 relationship and then wrap their membrane to form the myelin sheath. We recently found that the adhesion GPCR Gpr126 has domain-dependent functions in radial sorting and myelination. Gpr126 is autoproteolytically cleaved into an extracellular N-terminal fragment (NTF) that remains associated with the 7-pass transmembrane-containing C-terminal fragment (CTF). Using an allelic series of zebrafish, we found that Gpr126-NTF is necessary and sufficient for radial sorting independent of the CTF, which is required for cAMP elevation and terminal differentiation. Furthermore, we characterized a tethered agonist sequence (“Stachel”) within Gpr126-CTF that is required for myelination. Our studies suggest that the Stachel is physically embedded within the NTF, which must be removed for receptor activation. Additionally, we found that Laminin-211 (LN-211), a major constituent of the SC basal lamina, is a novel interacting partner of Gpr126-NTF. As SCs develop, they secrete a basal lamina that matures via polymerization of LN-211. We therefore speculated that LN-211 polymerization mechanically removes Gpr126-NTF from the CTF to activate signaling. Our findings suggest that, during radial sorting, LN-211 stabilizes an NTF-bound CTF that masks the Stachel. Later, LN-211 polymerization removes the NTF, exposing the Stachel and promoting signaling and SC myelination. Thus, we have found that LN-211 mediates bimodal roles of Gpr126 in SC development. This work was supported by NIH F32 NS087786 to SCP and NIH R01 NS079445 to KRM.

Program Abstract #63
BMP signaling modulates physical forces to control intestinal coiling
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During embryogenesis, the dramatic transformation from a mass of cells into the fully patterned adult form necessitates stereotyped regulation of physical forces at the genetic and molecular level. However, this is a very poorly understood aspect of morphogenesis. Here, we address this in the context of looping morphogenesis of the chick midgut, a process by which the initially straight gut tube coils to form the small intestine. Looping is essential to decouple gut length (hence absorptive capacity) from organismal length. Previous work from our group demonstrated that gut looping results from compressive forces generated by differential growth between the rapidly elongating gut tube and its attached mesentery. Loop number and curvature result entirely from physical properties of the two tissues. The present work focuses on understanding how these physical properties are specified at the molecular level. A candidate screen by in situ hybridization revealed an enrichment of bmp2 expression in the dorsal mesentery and its attachment to the gut tube. Functional studies relying on viral misexpression revealed that modulation of BMP signaling directly controls the number of intestinal loops by controlling compressive forces exerted by the mesentery on the gut tube. For example, inhibition by noggin overexpression resulted in a loss of loops, while overexpression of bmp2 resulted in supercoiling and an overall increase in the number of intestinal loops. Accordingly, we propose a model whereby BMP signaling in the mesentery suppresses elongation of the tissue with respect to the rapidly elongating gut tube. Loss of BMP activity in the mesentery therefore results in a loss of differential growth, eliminating the buckling forces that drive gut looping. In ongoing work, we are investigating whether evolutionary modulation of physical forces through the BMP signaling axis can explain natural variation in looping morphology. Funded by R01HD047360 (CJT) and F32HD069074 (NLN).
**Program Abstract #64**

**Human Pluripotent stem cell-derived tissues as new models to study development, digestive diseases and diabetes**

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Successful efforts to direct the differentiation of human embryonic and induced pluripotent stem cells (PSCs) into specific organ cell types in vitro have largely been guided by studies in embryonic development. We have used signaling pathways that control early endoderm organ specification and morphogenesis in vivo to generate complex, three-dimensional organ tissues with improved functionality from human PSCs in vitro. We identified that by modulating FGF, Wnt and BMP signaling pathways, we were able to control anterior-posterior patterning PSC-derived definitive endoderm as well as gut tube morphogenesis in vitro. The resulting three-dimensional gut tube tissues resembled either foregut or mid/hindgut. These gut tube tissues could be further directed into specific organ tissue types by additional manipulation of embryonic signaling pathways. For example we have been able to use a temporal series of growth factor manipulations that mimic embryonic intestinal development to generate three-dimensional human small and large intestinal organoids (HIOs). We have also generated foregut-derived organoids including fundic and antral gastric organoids. Organoids contain epithelial structures diverse cell types that are unique to their representative organ. Moreover, we are able to manipulate specific cell lineages using genetic gain- and loss-of-function approaches. We have also engineered additional complexity into organoids, for example we have incorporated a functional enteric nervous system into HIOs and generated intestinal tissue that is capable of peristaltic-like motility. Lastly, we are using organoids to model diseases caused by genetic or infectious agents. For example we have modeled epithelial repair induced by infection with *Helicobacter pylori* and established a new human model for neonatal diabetes and congenital malabsorption.

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**Program Abstract #65**

**Sensing Hh: Apical Par proteins affect ciliogenesis and Hh signaling in neural precursors**

Laura Hudish

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During neural development, precursors both divide to expand the precursor population and differentiate as neurons and glia. This balance of proliferation and differentiation is crucial to the proper development of the central nervous system. Intriguingly, the Hedgehog (Hh) signaling pathway, which is well known for patterning the ventral neural tube, also promotes precursor proliferation. How Hh signaling is modulated to regulate the transition from proliferation to differentiation is poorly understood. In dividing cells, primary cilia are disassembled prior to mitosis and reassembled shortly after. Because cilia are the sites of Hh signal detection and processing, we hypothesized that differential reassembly of cilia following precursor division results in different levels of Hh signaling, thereby determining whether a precursor remains proliferative or differentiates. Apical Par polarity proteins, which are required to maintain neural precursors, can promote ciliogenesis, raising the possibility that Par proteins regulate proliferation and differentiation by regulating ciliogenesis and Hh response. We tested this possibility using live imaging coupled with genetic and pharmacological manipulation in zebrafish embryos. We found that overexpression of Par proteins caused formation of longer cilia and elevated Hh signaling whereas embryos lacking par gene functions had shorter cilia and decreased Hh signaling. Additionally, sustained expression of Par proteins maintained neural precursors and blocked their differentiation. Inhibition of Hh signaling reversed the effect of elevated Par protein expression, providing strong evidence that the effect of Par proteins on neural precursors is mediated by the Hh pathway. Our data are therefore consistent with the idea that Par protein regulation of cilia formation helps modulate Hh responses within neural precursors, maintaining the balance between proliferation and differentiation during early neural tube development.

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**Program Abstract #66**

**Cytoplasmic mRNA degradation: roles for VCS and SOV in development and beyond**

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University of Utah, United States

Roles of cytoplasmic mRNA degradation in *Arabidopsis* development were initially suggested by finding development mutants with defects in genes encoding components of the mRNA decapping complex. Three general pathways for cytoplasmic mRNA decay are initiated by deadenylation, and include decapping (followed by 5’ to 3’ digestion), 3’ to 5’ decay by the RNA exosome, and 3’ to 5’ decay by SOV. Previously, we found that these three complexes were not simply redundant, but that some mRNAs degrade by specific combinations of pathways. To further explore the specificity of mRNA degradation pathways, we are carrying out a genome-wide mRNA decay study. Our strategy uses four *Arabidopsis* genotypes which differ in the presence/absence of VARICOSE and SOV. VARICOSE encodes the scaffold
for DCP1 and DCP2, which decap mRNAs, and SOV encodes an enzyme that carries out 3’ to 5’ decay. Using mathematical modeling, we have calculated mRNA decay rates in each genotype for more than 16,000 genes. Some general principles are emerging from this analysis. First, most—but not all—mRNAs require VCS (decapping) for normal degradation rates. Second, some mRNAs degrade independently of VCS or SOV, and so are presumably substrates for the exosome. Third, when VCS (decapping) is intact, SOV contributes to degradation of a small fraction of mRNAs. A cluster analysis identified 15 major groups of genes based on their decay parameters. Identification of decay pathways for mRNAs that encode important developmental regulators might allow us to understand how developmental signals intersect with the cellular machinery that directs mRNAs to be targeted to specific mRNA decay machinery.

Program Abstract #67
Signal Induced Switch in Dicer Function Regulates Oocytes to Embryo Transition
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Few examples exist wherein extracellular cell signaling pathways exert direct regulatory control over Dicer function during cell biological and developmental events. We show that MPK-1/ERK2 directly phosphorylates Dicer at two distinct sites in its RNase IIIb and dsRNA binding domains that are conserved from C. elegans to humans. Dicer phosphorylation at these sites is necessary and sufficient to target Dicer to the nucleus, and this cellular dynamic is evolutionarily conserved from the C. elegans to mouse and human cells. In the C. elegans germline phosphorylation alters Dicer function in two ways: phosphorylation of the dsRNA binding domain confers on Dicer the ability to negatively regulate ERK activity; and, phosphorylation of the RNase IIIb domain blocks Dicer function. Moreover, Dicer function is inhibited during meiosis I of oogenesis and then reactivated just before embryogenesis for normal oocyte-to-embryo transition. Since phosphorylation dynamics of Dicer mirror this functional transition, we propose that ERK-mediated phosphorylation of Dicer constitutes a regulatory switch that inhibits Dicer function in oocytes and helps reactivates it prior to embryogenesis.

Program Abstract #68
BMP release is regulated by pulses of electrical activity
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Loss of embryonic ion channel function can cause craniofacial and limb abnormalities in mammals, but the underlying reason for these defects has remained elusive. We show that depolarization of epithelial cells governs a novel mechanism of morphogenesis. We show that cells of the Drosophila wing disc undergo pulses of electrical activity. We find that changing the pulses of electrical activity by inhibition of an inwardly rectifying potassium channel, Irk2, alters morphogenesis and disrupts BMP signaling. We find that the channel is required for regulated and pulsatile release of BMP in the developing fly wing, and that the inwardly rectifying conductivity of Irk2 is required for this function. Our results lead to a new model of controlled BMP release based on the activity of ion channels.

Program Abstract #69
Wnt5a-Ror2 antagonizes β-catenin to regulate proliferation of migrating primordial germ cells
Andrea Cantu, Svetlana Keylin, Boris Reznik, Michael Kissner, Kevin Ebata, Diana Laird
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Propagation of the genome depends on the expansion of a small number of primordial germ cells (PGCs) in the early embryo as well as their migration to the forming gonads. The proliferation of mammalian PGCs is concurrent with their movement through changing microenvironments; however, the mechanisms coordinating these conflicting processes remain unclear. Here we find the rate of PGC proliferation varies by location rather than by embryonic age, suggesting microenvironmental regulation of the cell cycle. In Wnt5a and Ror2 mutants, which have aberrant PGC migration, we find that PGC proliferation is disrupted in the hindgut and surrounding mesentery, where Wnt5a is most highly expressed. Global expression analysis of Ror2 PGCs likewise shows dysregulation of cell cycle genes as well as the canonical Wnt target Axin2 during early migration. We use ex vivo culture to show that somatic cells from the hindgut suppress β-catenin-mediated canonical Wnt signaling in PGCs and to validate WNT5a as a key mediator of this effect. Examination of β-catenin in PGCs in vivo confirms that canonical Wnt activity increases as PGCs progress along their migratory route. Genetic over-activation of β-catenin in PGCs to bypass the suppression by somatic microenvironment induces a proliferative increase in the hindgut, similar to that seen in Wnt5a and Ror2 mutants. Our results suggest that the balance
between expansion of migratory PGCs and their movement is fine-tuned by the opposing β-catenin-dependent (canonical) and Ror2 (non-canonical) pathways through Wnt5a as well as other regulators produced in various somatic compartments. Thus, differing levels and compositions of Wnt ligands along the PGC migratory route comprise the changing “traveling niche.” Funding: NSF GRFP Fellowship; NIH 1DP2OD007420

Program Abstract #70
Transcriptional timers regulating mitosis in early Drosophila embryos
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Embryonic development is characterized by precise regulation of cellular behaviors both in space and time. Several experiments have elucidated the mechanisms underlying the precise spatial regulation of development. However, the mechanisms of temporal patterning throughout development remain poorly understood. In this study, we aim to understand how Drosophila embryos achieve the precise temporal sequence of mitoses observed during gastrulation. This pattern is regulated by the transcription of the cdc25 homolog, string (stg), which drives mitosis in 25 highly reproducible mitotic domains (MD). We hypothesize that accumulation dynamics of a small number of rate-limiting regulators control the time of activation of stg transcription. To test this model, we examine whether changing the dosage of different chromosomal regions using heterozygous deficiency embryos alters the temporal pattern of mitosis in MD1 and 2. Using a whole-genome screen we have identified several effectors that encode the underlying clock. Heterozygote embryos develop with normal spatial pattern. In theory, a rate-limiting factor might act in all mitotic domains to set up timing globally throughout the embryo. However, we have observed very few such global regulators. Instead most of the effectors we have identified act in domain-specific manners, shifting the relative timing of mitosis in MD1 and 2. These regulators are made up of both activators and repressors. For example, the timing of MD2 is delayed in buttonhead heterozygotes, but advanced in hairy heterozygotes. We conclude that in temporal regulation, the rate of accumulation of the regulators set up the developmental clock. In spatial patterning, like eve stripe 2, repressors are largely absent from the expression regions. In contrast, in temporal patterning of mitotic domains, regulation occurs in the presence of high levels of repressors and the expression occurs when the activators can overcome the repressors to activate stg expression.

Program Abstract #71
Ontogeny of metabolic rate and mitochondrial physiology in Drosophila
Kristi Montooth, Cole Julick, Omera Matoo
University of Nebraska-Lincoln, United States
While scaling laws may well predict metabolic rate as a function of mass across taxa that span large differences in body size, metabolic scaling relationships within species can vary across development and as a function of the environment (Greenlee, Montooth, Helm. 2014 ICB 54:307). Moreover, there is considerable variation in metabolic rate within species that is independent of variation in mass. For example, we have shown that the scaling of metabolic rate as a function of mass depends on the developmental thermal environment in Drosophila, and that mitochondrial-nuclear genotype significantly affects larval mass-specific metabolic rate. This latter genetic effect is itself conditional on the developmental thermal environment, and we have found that interactions between genotype and developmental environment affect metabolic rate plasticity (i.e., the $Q_{10}$ for metabolic rate) (Hoekstra, Siddiq, Montooth. 2013 Genetics 195: 1129). To better understand the ontogeny of metabolic rate and the underlying mechanisms that govern the development and plasticity of energetic processes, we have characterized larval metabolic rate and aspects of mitochondrial physiology across development for a number of natural D. melanogaster genotypes, as well as for mitochondrial-nuclear genotypes that combine single nucleotide polymorphisms from different species to generate energetic inefficiencies. We discuss how changing energy demand in insects that undergo complete metamorphosis, as well as changing tissue-body size allometries, across development may contribute to ontogenetic patterns in whole-organism metabolic rate. In this way, development generates a dynamic internal environment that will impact the fitness effects of energetic mutations and, thus, the evolution of metabolic systems. Funded by NSF IOS-1149178 to K.L.M.

Program Abstract #72
Retinoic acid is continuously required in Spemann’s organizer and in the head-inducing prechordal mesoderm
Abraham Fainsod, Michal Gur, Graciela Pillemer
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Retinoic acid (RA) is a central signaling molecule regulating multiple important processes during embryogenesis. RA excess induces head malformations, primarily a reduction in anterior head structures. Despite these teratogenic effects, several syndromes exhibiting microcephaly, such as DiGeorge/VeloCardioFacial, Vitamin A Deficiency and Fetal Alcohol syndromes have been attributed to reduced RA, suggesting a requirement for normal head development. To study this proposed role of RA we used Xenopus frog embryos focusing on the early events in head formation while manipulating RA levels. Using the secondary axis induction assay and RA loss-of-function, we show that RA is indeed needed for proper head formation. We mapped this requirement to early gastrula stages. This function corresponds to the biosynthesis of RA in Spemann’s organizer. Manipulation of the endogenous Spemann's organizer RA production resulted in microcephalic embryos in agreement with the twined axis results. To study the function of retinoic acid in head induction we examined different aspects of gastrulation and concluded that RA inhibition leads to abnormal morphogenetic movements and delays in the migration of the leading edge mesendoderm/prechordal mesoderm, the inducer of the rostral neuroectoderm. Focusing on RA biosynthesis we studied two RA-producing enzymes expressed early in gastrulation, RALDH2 and RALDH3. RALDH3 knock-down shows that it is the key enzyme for RA production for head formation. These observations suggest that, in addition to its potential teratogenic effect on head development, RA also has a positive regulatory role in rostral neuroectoderm induction during early gastrula identifying a novel activity in agreement with the proposed reduction of this signal in syndromes exhibiting microcephaly. Funding: Canadian Friends of the Hebrew University, the Manitoba Liquor Control Commission and the Israel Ministry of Health to AF.

Program Abstract #73

Developmental Regulation of Metabolic Transitions by Drosophila Nuclear Receptors
Carl Thummel, William Barry, Jason Tennessen, Keith Baker, Janelle Evans, Geanette Lam
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The Drosophila genome encodes 18 nuclear receptors, providing an ideal model system for studying roles for nuclear receptors in maintaining metabolic homeostasis. Our recent studies of two nuclear receptors, dERR and dHNF4, have shown that they coordinate widespread metabolic switches during development. dERR directly activates a transcriptional program during mid-embryogenesis, up-regulating the genes that encode enzymes in glycolysis, the pentose phosphate pathway, and lactate production. Combined with metabolomic analysis, this work indicates that dERR establishes a metabolic state related to the Warburg effect, which is normally associated with proliferating cancer cells. This dERR-regulated mid-embryonic transition anticipates the metabolic needs of the next stage in development, establishing an aerobic glycolytic state that supports the remarkable 200-fold increase in larval mass. Interestingly, our functional studies of dHNF4 have revealed a subsequent nuclear receptor-regulated metabolic transition at the pupal-adult transition. Through a series of studies we have shown that dHNF4 mutants display adult-onset hyperglycemia, glucose intolerance, and defects in glucose-stimulated insulin (DILP2) secretion. These phenotypes mirror those of MODY1 patients, who carry mutations in the human ortholog of dHNF4, HNF4α, providing an animal model to study this disorder. dHNF4 is required in both the fat body and insulin-producing cells to maintain glucose homeostasis by supporting a developmental switch toward oxidative metabolism and glucose-stimulated insulin secretion at the transition to adulthood, supporting the energetic needs of the mature animal. The long-term goal of these studies is to exploit our ability to link Drosophila nuclear receptors with defined transcriptional cascades and specific biological responses as a means of furthering our understanding of how nuclear receptors regulate metabolism and contribute to human disease.

Program Abstract #74

Insulin and glucagon regulate development and regeneration of the pancreatic islet in zebrafish.
Ryan M. Anderson, Lihua Ye, Morgan A. Robertson
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Insulin and glucagon are counter-regulatory peptide hormones released from pancreatic beta and alpha cells, respectively, that are crucial for the control of glucose metabolism. Insulin promotes glucose uptake and storage during nutrient abundance, while glucagon promotes release of glucose via gluconeogenesis and glycogenolysis during fasting. The failure of the islet to meet the physiological demands for insulin via increased beta cell function or number results in the morbid metabolic disease diabetes. The augmentation of beta cell mass through in situ stimulation of beta cell production is a tantalizing therapeutic approach; however, it is limited by our knowledge of the molecular players that regulate endocrine fate. We recently reported a role for glucagon in the regulation of islet differentiation during zebrafish pancreatogenesis, which reflects a highly similar role in mammalian development. Additionally, we found that glucagon regulates the conversion of alpha cells to beta cells after beta cell loss. Here, we report that using multiple approaches in
zebrafish, we have uncovered a novel negative regulatory role for insulin signaling during differentiation of pancreatic progenitors. Moreover, insulin signaling promotes alpha cell fate at the expense of beta cell fate, both during islet development, and during islet regeneration. Finally, using novel blastomere-to-larva transplantations, we found that loss of insulin signaling in stem cell-like blastomeres drove their differentiation into beta cells. Furthermore, the extent of this differentiation appeared to be dependent on the function of the host beta cell mass. Together, our results indicate that modulation of insulin signaling will be crucial for the development of β cell regeneration therapies for diabetics; further clarification of the mechanisms of insulin signaling in β cell progenitors will reveal therapeutic targets for both in vivo and in vitro β cell generation.

Program Abstract #75
The brain-gut axis, inter-organ signalling and epithelial plasticity (all inside one fly)
Irene Miguel-Aliaga
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Our work explores how the gastrointestinal tract senses and responds to nutritional information. Over the past few years, we have begun systematic characterization of enteric neurons in Drosophila melanogaster, and have developed new technology for the study of their functions. Our work has uncovered evolutionarily conserved mechanisms of autonomic control in Drosophila1, and has also identified novel and physiologically relevant communication between the gut, its neurons and other internal organs2,3. More recently, we have been exploring the physiological plasticity of the intestinal epithelium: an obvious cellular target of the enteric neurons. I will present some of our ongoing work, which is revealing unexpected sexual dimorphisms and intestinal contributions to reproductive success.


Program Abstract #76
Microcephaly Disease Gene Wdr62 Regulate Mitotic Progression of Embryonic Neural Stem Cells and Brain Size
Jian-Fu Chen1, Si-Lu Yang1, Lee Niswander2
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Ours is a new neural developmental biology laboratory at the University of Georgia (UGA). A major focus of my lab is to understand mechanisms regulating neural tube closure (NTC) and cortical development. Malformations of cortical development (MCD) represent a major cause of developmental disabilities. Human genetic studies have established a link between a class of centrosome proteins and MCD. However, the in vivo functions of these centrosome proteins during normal cortical formation and the pathophysiological mechanisms of MCD diseases remain obscure. Mutations in WDR62 are associated with microcephaly and other cortical abnormalities in humans. We created a mouse model of Wdr62 deficiency and found that the mice exhibit reduced brain size due to decreased neural progenitor cells (NPCs). Wdr62 depleted cells show spindle instability, spindle assembly checkpoint (SAC) activation, mitotic arrest and cell death. Mechanistically, Wdr62 associates and genetically interacts with Aurora A to regulate spindle formation, mitotic progression and brain size. Current studies of microcephaly focus on defective centrosome/spindle orientation. Our results suggest that Wdr62 interacts with Aurora A to control mitotic progression, and loss of these interactions leads to mitotic delay and cell death of NPCs, which provides a novel model for the potential cause of human microcephaly.

Program Abstract #77
Different unilateral insults to limb growth trigger distinct mechanisms of long bone size regulation
Alberto Rosello-Diez1, Linda Madisen2, Hongkui Zeng2, Alexandra Joyner1
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A poorly understood property of metazoans is the regulation of organ size, necessary to attain the correct body proportions during development and to regain normal size after transient growth impairment. To develop effective animal models of growth regulation, we focused on the vertebrate limbs. Being paired organs, limbs are well suited for the problem, as by altering growth unilaterally, it is possible to maintain an internal control, which can help distinguish between systemic and local size regulation mechanisms. We developed two new mouse models that allow induction of transient growth insults at any desired stage in the left hindlimbs. In model 1, acute embryonic induction of cell death leads to a rapid growth delay of the left limb, but leg length is nearly fully regained during postnatal growth. Most of this recovery takes place at the final stages of growth, as the left limb keeps growing after right limb growth stops. This suggests that the progressive proliferative decline that characterizes the growth plates (GPs, transient cartilage regions driving bone growth) can be regulated (delayed) in response to a size defect. This possibility was not predicted by current models of bone growth,
which assume the GP is composed by a fixed number of progenitors with a fixed proliferative potential. One of the regulatory mechanisms we are testing is the recruitment of new progenitor cells to the GP. In model 2, embryonic misexpression of p21 in ~60% of the left GP cells reduces their proliferation but, unexpectedly, growth of the left bones is not delayed. Interestingly, nonautonomous compensatory proliferation is observed in left WT cells, which expand at the expense of the mutant p21+ cells. We are now testing if this response is due to a putative stress signal produced by p21+ cells. In summary, our novel mouse models reveal that long bones can recover from transient cell death or reduced proliferation, allowing the study of different growth regulation mechanisms.

Program Abstract #78
Pathfinding, with Passion
Kathryn W. Tosney
The University of Miami, USA
Winning the Viktor Hamburger Outstanding Educator Prize is such an honor because it construes educational excellence in the broadest sense, that happily coincides with my passion for teaching both science and professional skills. The “pathfinding” in the title of my talk touches on how these passions fueled my own career path, from studying the pathfinding of neural crest cells and axons, to leading a successful conservation program for marine iguanas in the Galapagos Islands, to helping people navigate their own career paths successfully. The major focus will be on career strategies, abstracted from a longer talk I give nationally, “Survival in Academia.”

Program Abstract #79
Choose Development! - A long-term and continuing mentoring program to increase the diversity of undergraduates entering research careers in developmental biology
Ida Chow¹, Karen Bennett², Graciela Unguez³
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Built upon a culture of inclusiveness, Choose Development! is a three-year (2013-2016) NSF/IOS-funded program through which the Society for Developmental Biology (SDB) aims to increase the number of undergraduate students from underrepresented (UR) groups and students with disabilities entering into doctoral programs in fields related to developmental biology. Recent studies have shown that despite the interest in science, technology, engineering and mathematics by UR students in their freshman year, a dismal number actually graduates with majors in these areas. Data show an attrition that might be due in part to insufficient mentoring, peer support, low personal or societal expectations, and an unwelcoming environment; as well as “one-time fixes” have yielded low returns. Choose Development! emphasizes professional development and research training under the continuing supervision by research faculty, advanced graduate students and postdocs, a multi-level mentoring approach. A total of 15 SDB Fellows were selected and matched to research laboratories of active SDB faculty members across the country (http://www.sdbonline.org/choose_development) for three summers (2013-15). The Fellows present their previous summer data at the SDB Annual Meetings, and are introduced to the SDB community. Five are in their final years, three have been accepted to graduate programs at Stanford, NYU and Lehigh, one is doing a research internship in Germany, one is in the Harvard Post-baccalaureate program, one will work as a lab assistant in a research lab, three are preparing for GRE/MCAT and one has withdrawn from school. Preliminary data using SURE III Survey advocate our approach to provide several mentors to the Fellows throughout the years that cover their multiple intensive summer research experiences. Remarkably, all participating mentors reported a positive impact on their lab environment and excitement toward student mentoring in the lab. Supported by grant NSF-IOS 1239422.

Program Abstract #80
Talk Matters: An Analysis of Explicit Instructor Talk in a Large Introductory Biology Course
Laura Burrus¹, Shannon Seidel¹, Amanda Reggi¹, Jeffrey Schinske², Kimberly Tanner¹
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Teachers not only facilitate concept learning, but also design learning environments, which influence student motivation, resistance, and self-efficacy. Social psychology research on instructor immediacy shows that decreased social distance between instructors and students is correlated with increased learning. Despite the importance of how instructors create learning environments, little research has been conducted about what instructors say and do to create learning environments in college biology classrooms. We hypothesize that effective instructors talk about more than biology concepts in their classrooms. To test this hypothesis, we systematically investigated Explicit Instructor Talk, which we define as a language used by an instructor that does not directly relate to the course content. Our research addresses the
following questions: In an introductory biology course, 1) What types of Explicit Instructor Talk are used? 2) When does Explicit Instructor Talk happen? 3) To what extent do co-instructors differ in their use of Explicit Instructor Talk? Using a mixed-methods approach, we generated transcripts of 30 class sessions of a semester-long, co-taught introductory biology course (n=270 students) at an urban, public university. Transcripts were analyzed to identify emergent categories of Explicit Instructor Talk. Five categories of Explicit Instructor Talk emerged from our analysis and include: 1) Establishing Classroom Culture, 2) Building Instructor/Student Relationships, and 3) Explaining Pedagogical Choices. All five categories were represented in over 85% of analyzed class sessions with an average of 37 instances per class session. Developing a framework for analyzing Explicit Instructor Talk may yield insight into varying levels of instructor effectiveness, reveal origins of student resistance, and serve as a valuable faculty development tool. Acknowledgements: Funded by NSF grant #1226361 and HHMI grant #52007556.

Program Abstract #81
Implementation of Scientific Teaching in a Large Biology Department
Carmen Domingo, Gloriana Trujillo, Shannon Seidel, Kimberly Tanner
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Nation-wide reports such as NSF’s Vision and Change for Undergraduate Biology Education have advocated for changes in teaching practices to better prepare undergraduates in the sciences. In addition, the need to improve the retention rates for undergraduate science majors, which is less than 40% nationally (Seymour, 1997), has inspired many to attempt pedagogical reform on their campuses. Unfortunately, the perception has been that many higher education reform efforts have a limited impact due to that fact that only a small fraction of a single department participate in the efforts. With funding from an HHMI Undergraduate Science Education grant, we are in the process of a department-wide effort to introduce scientific teaching to all instructional staff in the Department of Biology including tenured/tenure track faculty, lecturers, and graduate student instructors. We provide data that shows that engagement of a large proportion of biology department faculty is possible. By Year 3 of the grant, 88% of eligible faculty (including tenured, tenure-track faculty and lecturers) have participated in a week-long institute referred to as Biology FEST: Biology Faculty Explorations in Scientific Teaching. Following their training in this institute, 73% of the eligible faculty participated in post-institute, semester-long teaching collaborations. A large survey of biology faculty and students revealed significant differences in their perceptions of the frequency in use of innovative teaching strategies in lecture courses taught by HHMI alumni faculty compared to non-HHMI faculty. While investigation of the impact of this professional development on classroom practice and student learning is in progress, these initial findings suggest that systemic professional development efforts are possible, and that they do translate to the adoption of more innovative teaching strategies across the department.

Program Abstract #82
Devidetorials: Making a Case to Flip Developmental Biology
Michael Barresi1, Scott Gilbert2, Kathryn Lee1
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Science pedagogy has long debated how to balance the opposing demands of content delivery with active learning approaches. The immense diversity of scientific disciplines that influence Developmental Biology exacerbates this problem. Traditionally, instructors have relied on the lecture for content delivery and left problem solving for students to toil over on their own outside of class. However, the recent use of online video resources provide a novel mechanisms to flip this scenario, and engage students with relevant content outside of class to create an opportunity for more interactive student problem solving in the classroom. We are in the process of building an online video resource of developmental biology tutorials, called “Devidetorials”. These relatively short videos are professionally edited to capture the experienced Developmental Biology Teacher describing a core concept in the field of Developmental Biology paired with images, videos, and animations to engage student learning. In connection with Sinauer Associates, each Devidetorial will have an accompanied Case Study problem set that can serve as the basis for the active learning module in the classroom. Flipping a Developmental Biology classroom with these resources was piloted Fall of 2014, and student perceptions of this experience were assessed. A vast majority of students found that the use of these Devidetorials helped to deepen their understanding of course content, to become a more active participant in class, and to better investigate and solve problems in Developmental Biology. Over 75% of the students preferred the classes with an associated Devidetorial and case study as opposed to class periods without them. The overwhelming positive response from students and measured outcomes support the pedagogical approach of “flipping” Developmental Biology with Devidetorials and case studies to achieve deep learning in students ready and willing to attack tomorrow’s problems in science.
Program Abstract #83
How to Read a Science Paper: a novel discussion-based undergraduate course resulting in gains in student confidence and training
Rebekah Le1, Charles Yi1, Anne Phan1, Cristian Aguilar1, Vaishali Jayashankar1, Seong Kim1, Michelle Mattson-Hoss1, Rabi Murad1, Justin Shaffer1, David Gardiner1, Brian Sato2, Pavan Kadandale2, Debra Mauzy-Melitz1
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Undergraduate students majoring in the biological sciences at large universities receive most of their formal coursework through large lectures. For students with career interests in the health sciences and biological research, it is imperative to acquire the ability to read, critically evaluate, and communicate primary literature — skills that require training beyond traditional lectures. To satisfy the need to train students in these skills, senior graduate students in the department of Developmental and Cell Biology at UC Irvine have designed a new course, Bio Sci D140: How to Read a Science Paper. This small, discussion-based course integrates guest research lectures and undergraduate student-led journal clubs to provide guided instruction on the critical analysis and communication of primary scientific literature. Through guest lectures given by Ph.D. students on current topics in their respective fields, undergraduates also had the opportunity to interact with cutting-edge researchers. In turn, Ph.D. students were given an opportunity to present a lecture and receive feedback on their teaching skills. We will present the structure of this course, and student assessments of the course components. Through the use of pre- and post-course surveys, Bio Sci D140 students indicated an increase in confidence in learning, performing, and communicating biological research. Importantly, learning assessments confirmed that students gained skills in data analysis and critical evaluation. Finally, we will include a discussion of interactive activities that can be incorporated into any existing course. Our findings and experiences with this course will be important in improving scientific training for biological sciences majors at large research universities.

Program Abstract #84
Authentic research experiences in Developmental Biology for introductory-level undergraduates result in significant gains for students
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Grinnell College, United States
Undergraduate research experiences result in important gains for students (SURE survey; Lopatto, 2004), but are usually restricted to a few majors because of limited resources. Including authentic research in undergraduate classes may provide an alternative that reaches a broader audience, but questions remain about whether significant gains are made in such courses. Grinnell College has been using authentic research experiences in Bio150 Introductory Biology courses. The Bio150 course uses a single focused research topic to teach the scientific process. For example, one section focuses on embryonic development, using C. elegans as a model system. Students read primary papers, design experiments, and perform authentic experiments using mutant strains and RNA-mediated interference. Students report their findings to a larger community in scientific format papers, presentations, and college-wide poster sessions. Since nearly half of all Grinnell students take Bio150 and two-thirds major in other programs, this course introduces the scientific process to a broad audience. Using the CURE assessment survey (Lopatto, 2008), Bio150 students report significant gains in “high engagement” areas that correlate with higher learning gains and a greater likelihood to declare a science major. These gains exceed those reported by students in 100-level courses taken elsewhere and also exceed those reported by REU students (SURE; Lopatto, 2004). While these gains may seem to come at the expense of content, the research emphasis asks students to learn about and connect the key biological principles outlined in the NSF Vision and Change report. Our analysis indicates inquiry-based undergraduate classes are effective for progression in the major, can reach a broad audience, and produce significant gains similar to research experiences, indicating they are a good use of resources. NSF CCLI grants, HHMI grants, and internal funding supported development of the Bio150 course.

Program Abstract #85
Infusion of research in modules promotes interest in Genetics and Development
Grace Okuthe
Walter Sisulu University, South Africa
BSP1202 group project, is a research platform that provides laboratory based learning experience for 1st year Biological Science students. Group projects was implemented through the use of zebrafish and other emerging indigenous model systems. The project was aimed at fostering research and interest in Biological sciences specifically in Developmental Biology by substituting some lecture hours for research work. Students are given an opportunity to work in groups, have
hands on approach in planning and executing research problems using available research protocols and equipment. Literature search constitutes one of the key components of training. Using indigenous species was useful, since they are readily available and easy to maintain under laboratory conditions. This not only makes student appreciate science, but also helps visualize development from an organism they can identify with. The exercise helps students recollect ideas learnt during laboratory sessions easily compared to those learnt during routine lecture sessions. The exercise has increased the number of students opting to pursue careers in Biological Sciences and provides an opportunity to care and maintain animals under laboratory conditions, design experiments, record observations and analyse data. Further, each group is provided with relevant laboratory consumables and glassware for basic histological analyses for the full semester. Since its inception, other lecturers have initiated similar projects in their modules. The idea of introducing research at undergraduate level is encouraged, but the large number of 1st year students is often discouraging. It is too early to evaluate the overall success, but student enthusiasm is overwhelming and encouraging. Roll out of the idea at all levels of undergraduate study is to be anticipated. Feedback from students who have participated in the program for the past two years will be made available.

Program Abstract #86
A Classroom-Based RNAi Screen for Regeneration Genes in Planarians
Alexandra Abbate, Derek Starkey, Casey Kimball, Amber Poirier, Stuart Nelson, Jason Pellettieri
Keene State College, United States
In addition to playing an important role in stem cell and regeneration research, planarians are ideally suited to inquiry-based science education projects. The easily observable regenerative response in species such as Schmidtea mediterranea provokes strong curiosity amongst nearly all students, and the availability of genome sequencing information and tools like RNAi enables rapid molecular genetic analyses. We took advantage of these attributes to develop a discovery-based research project for an undergraduate developmental biology course. During this semester-long lab, each of the 16 students in the class identifies and clones at least one novel S. mediterranea gene and uses RNAi to look for a possible regeneration phenotype. To date, we have screened over 50 genes via this approach and identified 3 required for formation of the regeneration blastema, a mass of new tissue that forms at the wound site of amputated animals. These include a mago nashi homolog previously shown to be expressed in the ovaries of the S. mediterranea sexual strain. We find that silencing mago nashi in asexual planarians causes head regression, ventral curling, and lysis, in addition to failed regeneration. These phenotypes are hallmarks of stem cell loss. Intriguingly, Drosophila mago nashi regulates germline stem cell differentiation and the related mouse gene magoh is required for maintenance of neural progenitor cells, suggesting evolutionarily conserved functions for this gene family in stem cell regulation. We are currently using whole-mount in situ hybridization to characterize the mago nashi expression pattern in asexual planarians, and phospho-histone H3 staining to address a possible requirement for this gene in planarian stem cell maintenance; results from these analyses will be reported at the meeting. Our work illustrates the simultaneous educational and research impacts of integrating discovery-based planarian research projects into the undergraduate biology curriculum.

Program Abstract #87
Pigeonetics: the power of the pigeon in an online genetics game
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Computer-based educational games are engaging, fun, and can be a welcome change from didactic learning, particularly when they build on new discoveries in the lab. We are teaching basic principles of genetics and inheritance through Pigeonetics, a highly interactive virtual pigeon breeding game designed for students in grade 5 and above as well as adults. Thousands of years of selection in the domestic rock pigeon (Columba livia) have produced striking phenotypic differences among over 300 recognized breeds. Pigeonetics challenges players to understand the genetic basis underlying some of this diversity by establishing crosses to produce offspring with specific combinations of traits, including pigment and plumage variants. In the process, players learn the concepts of dominant and recessive alleles, epistasis, independent assortment, and genetic linkage. Importantly, the game builds on our laboratory discoveries about the molecular and developmental basis of traits as well as discoveries by ancient and recent hobbyists, to develop novel, interactive, and sustainable teaching materials. Pigeon breeding is an enormously popular hobby worldwide, and we expect players of all ages to take advantage of this interactive and challenging learning opportunity that utilizes an animal model that is familiar to a broad diversity of people. Pigeonetics and associated learning materials are freely accessible with other educational resources on the Genetic Science Learning Center’s website at
Program Abstract #88
Assessment of the impact of various bisphenols on primordial germ cell migration in zebrafish
Edward Freeman, Bridget Babich, Nicole Weigert, Kevin Callahan
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Animal development is a complex process that generates and shapes the varied tissues and organs of the adult animal. This process is driven by countless proteins, cellular interactions and migratory events. As might be expected, if developmental processes are perturbed the impact on the developing adult forms can be dramatic. Of specific interest is the formation of the gonads, which require the migration of Primordial Germ Cells (PGCs), to the genital ridge. This movement is facilitated by chemical cues elicited by the somatic cells of the genital ridge. Exposure to chemicals which disrupt the endocrine system (Endocrine Disrupting Chemicals, EDCs) has been shown to impede this process and interrupt the normal development of the gonads through as yet undetermined mechanisms. The effects of EDCs have been studied extensively in various animal models, including the zebrafish (*Danio rerio*), where proper formation of the gonad requires the presence of a specific number of PGCs. Failure of proper PGC migration in the zebrafish has been demonstrated when EDC exposures occurred in the 24 hours post fertilization (24 hpf) but again specific genes or mechanisms have not been identified. Our experiments are aimed at studying PGC migration following exposure to one specific type of EDC, bisphenols, during the initial 24 hpf. Quantitative PCR is used to evaluate gene expression from exposed embryos. *Nanos* and *Deadend* are currently being studied based on their known roles in zebrafish PGC migration. *In-situ* hybridization (ISH) with the *Vasa* gene, a known PGC marker, is also being performed to visualize gross changes in the migration pattern of PGCs.

Program Abstract #89
Organogenesis: The intersection of the bioelectric code and genetic programming
Emily Pitcairn, Kelly McLaughlin, Michael Levin
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Understanding how embryos transform from a single fertilized cell to complex three-dimensional organisms is one of the driving questions of developmental biology. Classically, developmental biologists have focused on biochemical signaling, genetic regulatory networks, and mechanical forces as underlying control parameters for embryogenesis. However, in order to fully understand the complexity of development, a new control paradigm must be included: bioelectric signaling. Bioelectric signaling regulates important cellular behaviors such as proliferation, migration, and differentiation through the establishment of voltage gradients across cell populations. These voltage gradients are not the fast-spiking action potentials generated in the nervous system but rather are slow, long-lasting currents in non-excitable cells. Previous research has shown that disruption of voltage gradients during embryogenesis can alter cell fate decisions and even induce the formation of ectopic organs such as eyes. Currently, one of the biggest challenges in the field of bioelectricity is understanding how and when bioelectric signaling interacts with gene cascades to change cell fate and patterning. Using *Xenopus laevis* animal cap explants, we have characterized how particular ion fluxes lead to phenotypic, physiological, and genetic changes in an isolated environment. Interestingly both hyperpolarization and depolarization of cells, through manipulation of individual ion channels, induce specific phenotypes and mediate the activation of a panel of global patterning genes. Our study serves to extend the initial findings in whole embryos as we begin to unravel the complexities of bioelectric signaling. Funding: NDSEG (EP), NSF (KM), AHA (ML/KM)

Program Abstract #90
OVOL2 is a critical regulator of ER71/ETV2 in generating FLK1+, hematopoietic, and endothelial cells from embryonic stem cells
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In this study, we report that OVOL2, a C2H2 zinc finger protein, is a novel binding protein of ER71, which is a critical transcription factor for blood and vessel development. OVOL2 directly interacted with ER71, but not with ETS1 or ETS2, in the nucleus. ER71-mediated activation of the Flk1 promoter was further enhanced by OVOL2, although OVOL2 alone failed to activate it. Consistently, coexpression of ER71 and OVOL2 in differentiating embryonic stem cells led to a significant augmentation of FLK1+ endothelial, and hematopoietic cells. Such cooperative effects were impaired by the short hairpin RNA-mediated inhibition of Ovol2. Collectively, we show that ER71 directly interacts with OVOL2 and that such interaction is critical for FLK1+ cell generation and their differentiation into downstream cell lineages.

Program Abstract #91
Asynchronous single-cell fate decision events drive lineage commitment in the mouse blastocyst
Nestor Saiz Arenales, Kiah Williams, Anna-Katerina Hadjantonakis
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Lineage specification in the mouse blastocyst occurs in two sequential steps that lead to the segregation of the extraembryonic trophoderm (TE) and primitive endoderm (PrE) from the embryonic epiblast (EPI). Differential activation of the FGF4-MAPK signaling axis results in the establishment of distinct gene expression patterns within the inner cell mass (ICM), restricting GATA6 to PrE cells and NANOG to the EPI. We have applied a single-cell, quantitative approach to analyze PrE and EPI specification with high spatiotemporal resolution. Functional assays and time-lapse analyses revealed a gradual loss of plasticity within the ICM, arising from individual, asynchronous cell fate decision events. Individual cells become irresponsive to FGF signaling as they commit to either EPI or PrE, thus ensuring the stability of both lineages. We also observed that the ratio of PrE to EPI cells needs to be tightly regulated for the embryo to be viable. We propose that lineage specification within the ICM of the mouse blastocyst is a cumulative process, where incremental allocation of precursors to either lineage creates a window of opportunity to achieve balanced proportions of PrE and EPI cells. [Supported by NYSTEM contract #N13G-236]

Program Abstract #92
Trophectoderm formation is initiated in the absence of CDX2 in rabbit embryos
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During preimplantation mammalian development first differentiation event sets apart pluripotent inner cell mass from extraembryonic trophoderm (TE) lineage. This process have been extensively studied in the mouse, but little is known about its mechanism in other mammalian species. CDX2 has been shown previously to be one of the main transcription factors specifying TE fate in the mouse embryos. Here we demonstrate that in the rabbit preimplantation embryos TE initially becomes specified in the absence of CDX2, resembling pattern described earlier in human embryos. Moreover, early to mid-blastocyst stage rabbit embryos express pluripotency-associated factors OCT4 and NANOG throughout trophectoderm. In summary, these results suggest that other, CDX2 independent mechanism might be involved in specifying TE lineage in the rabbit. This work is supported by National Science Centre (NCN) grant number 2011/03/D/NZ3/03992

Program Abstract #93
MEx reporter mouse: a novel tool for fate mapping stem cells and their progeny
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Cell lineage analyses have been greatly facilitated by the advent of genetic fate-mapping tools that utilize recombinase-dependent systems such as Cre/lox and FLP/FRT to mark genetically-defined progenitor cells and their offspring with a reporter gene. However, the permanent nature of these systems limits their utility when tracing lineages that exhibit complex temporal expression patterns of the genetic locus driving the recombinase. For example, if Cre is actively expressed both by progenitor and postmitotic cells, it is difficult to determine which reporter-positive cells derived from labeled progenitors, versus those that turned on the reporter postmitotically. Inducible recombinase systems have partially overcome this limitation by providing temporal control over recombination, but they often suffer from inefficiency that does not permit labeling of the entire lineage. We have designed a novel reporter system that provides both spatial and temporal information of lineages by using Cre- and FLP-mediated recombination in a mutually exclusive (MEx) manner.
The MEx system marks cells with distinct reporter gene combinations depending on whether they expressed Cre alone (tdTomato/βGal), Cre before FLP (tdTomato/GFP), or FLP before Cre (GFP). Thus, by combining the MEx reporter system with different Cre- and FLP-expressing mouse lines, we can fate map lineages not only at the intersection of Cre and FLP expression, but also distinguish sublineages defined by the complex temporal expression patterns of the two recombinases. We have begun testing the utility of the MEx system to fate map subtypes of neural progenitors in the developing cerebral cortex by in utero electroporation. Ultimately we will generate a MEx reporter mouse line by targeting the reporter to the modified (CAG promoter-driven) Rosa26 locus to trace the entirety of complex cell lineages.

Program Abstract #94
Retinoic acid restricts caudal FGF and Wnt signals to control axial stem cell differentiation
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Axial stem cells residing in the caudal epiblast during late gastrulation generate neuroectodermal (Sox2+) and presomitic mesodermal (Tbx6+) progeny in a coordinated manner to drive vertebrate body axis extension, but the mechanism that balances these two fates is not fully understood. FGF and Wnt signals in the caudal epiblast are required to maintain the axial stem cell pool, while also required during mesoderm formation and differentiation. Retinoic acid (RA) generated in axial stem cell progeny restricts caudal FGF to control somite formation, although the influence RA may exert on axial stem cell differentiation remains unclear. Here, we found that mouse mutants deficient in RA synthesis exhibited an imbalanced axial stem niche, with increased Tbx6+ progeny. By utilizing RA loss-of-function studies and CRISPR/Cas9 genome editing of a conserved RA response element upstream of Fgfl, we demonstrate that RA directly represses Fgfl transcription in the neural plate as neural progeny exit the caudal epiblast during body axis extension. We also investigated Wnt8a mutants, and found that Wnt8a is required in the axial stem cell niche, alongside Wnt3a, for maintenance of Fgfl expression in the axial stem cell pool and to restrict premature Sox2 upregulation during early somite stages. Wnt8a function is thus conserved throughout the vertebrate lineage. Similar to Fgfl, RA synthesis mutants display increased Wnt8a and we describe a Wnt8a RA response element that binds nuclear RA receptors that may also function repressively. These studies demonstrate that RA signals generated in axial stem cell progeny act as a rheostat to dial in the correct levels of FGF and Wnt signals back to the axial stem cell pool to correctly coordinate axial stem cell differentiation required for body axis extension, providing mechanistic insight into robust control of stem cell fate in vivo.

Program Abstract #95
Regulation of Nodal signals during endoderm differentiation
Yukio Saijoh, Ranajeet Saund
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Nodal signals are known to play an important role in endoderm and mesoderm differentiation during gastrulation. After these two cell types emerge through the primitive streak, Nodal expression ceases. Although mechanisms of induction of the Nodal gene have been studied, we still do not know how Nodal expression is turned off after differentiation of endoderm and mesoderm. Interestingly, we previously found that Sox17 mutant embryos continue to express Nodal in foregut endoderm. Normally, Nodal is expressed in the primitive streak and endoderm cells near the primitive streak and the Nodal expression is turned off by the early bud stage. In the Sox17-/- embryos, endoderm cells still continued to express Nodal at early somite stages. Thus, the ectopic expression of Nodal in Sox17-/- embryos appears to be caused by the failure to downregulate transcription at an early stage of endoderm differentiation. Using transgenic analysis, we found that Sox17 appears to repress Nodal expression in endoderm through an enhancer located in the distal portion of the Nodal genomic region. Furthermore, Lefty1, an inhibitor of Nodal signals, is also expressed specifically in endoderm cell lineage during gastrulation and we found that Lefty1 mutant embryos showed upregulated Nodal expression in endoderm cells, suggesting that Sox17 and Lefty1 might cooperatively regulate Nodal activities in endoderm. We will discuss how Nodal signals is regulated during endoderm differentiation.
Program Abstract #96
Identification of temporal identity factors in *Drosophila* type II neuroblasts using RNA-Seq
Mubarak Hussain Syed
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Development of the central nervous system (CNS) requires both spatial and temporal patterning mechanisms, which generate an enormous number of diverse neuronal and glial subtypes from a relatively small pool of neural progenitors, defects in these processes lead to severe neurological disorders. *Drosophila* type II neuroblasts (NBs) and mammalian OSVZ progenitors share many similarities, both bud off self renewing intermediate neural progenitors (INPs) and send diverse array of neurons and glial cells to the higher order brain centers. Addressing how this extraordinary diversity in the brain is generated is very interesting and fundamental question. The main aim of my study is to identify temporal programs of gene expression in type II NBs, to characterize their function in generating neural diversity. In order to discover temporal identity factors, we performed RNA-sequencing analysis on the Type II NBs. We utilized TU tagging methodology to purify newly transcribed RNA from type II NBs at different time points (48h ALH, 72h ALH and 96h ALH) during development. Upon comparing the RNA sequencing data across different time points, we found differentially enriched genes for each developmental time point. Our primary focus is to study transcription factors from this differentially expressed gene list. From RNA-Seq and antibody expression data we have found Chinmo, Broad, Ecdysone receptor and Ecdysone induced protein 93 (E93) to be expressed temporally in type II NBs. Furthermore we were able to show Ecdysone as cell extrinsic cue regulating temporal expression of cell intrinsic transcription factors like Chinmo and Broad. Currently we are exploring the role of ecdysone signaling in regulating temporal identity of type II NBs. Funding: HHMI

Program Abstract #97
Early neural ectodermal genes are activated by Siamois and Twin during blastula stages
Sally Moody, Steven Klein
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BMP signaling distinguishes between neural and non-neural fates by activating epidermis-specific transcription and inhibiting neural-specific transcription. Neural ectoderm forms in the absence of BMP-mediated repression accomplished by the secretion of antagonists from the Organizer. However, it is not known whether neural genes also are transcriptionally activated. Therefore, we tested the ability of nine Organizer transcription factors to ectopically induce in the epidermis four neural ectodermal genes. We found evidence for two pathways: *Foxd4* and *Sox11* were only induced by Sia and Twn, whereas *Gmnn* and *Zic2* were induced by Sia, Twn, as well as seven other Organizer transcription factors. The induction of *Foxd4*, *Gmnn* and *Zic2* by Sia/Twn was both cell non-autonomous (requiring an intermediate protein) and cell autonomous (direct), whereas the induction of *Sox11* required Foxd4 activity. Since direct induction by Sia/Twn could occur endogenously in the dorsal-equatorial blastula cells that give rise to both the Organizer mesoderm and the neural ectoderm, we knocked down Sia/Twn in these cells. This prevented the blastula expression of *Foxd4* and *Sox11*. These studies demonstrate that at least some neural genes are directly activated by Sia/Twn in the blastula prior to the separation of the Organizer mesoderm and NE lineages.

Program Abstract #98
Differences in the Plasticity of Neural Ectoderm Cells in *X. Laevis* Embryos
Talia Hart, Krissie Tellez, Brigette Jong, Carmen Domingo
*San Francisco State University, USA*

During early embryogenesis, cells have the ability to differentiate into many different cell types. However, the role of in-vivo dynamics on cell plasticity and differentiation remain poorly understood. Our lab is using a cell transplantation approach in which fluorescently labeled neural ectoderm progenitor cells are transplanted to a muscle fated region on an unlabeled host embryo to examine the cell plasticity. In this system, cell fate change (neural to muscle) is used as a readout of cellular plasticity. We have shown that neural ectoderm cells remain responsive to muscle-inducing signals through mid neurulation. Specifically, cells from the prospective anterior neural region retain their ability to change their cell fate until the end of gastrulation (stage 12.5), whereas cells from the posterior neural ectoderm retain their ability to change their fate until mid neurulation (stage 18). By varying the age of the host embryo, we show that prospective neural cells are most likely to form muscle when grafted to the dorsal blastopore region of stage 12.5 embryos (end of gastrulation). To examine the behavior of these prospective neural cells in a muscle-inducing environment, we transplanted both rhodamine-labeled neural cells along with fluorescein-labeled mesoderm cells to the same blastopore region of unlabeled host embryos. Our results show that posterior neural ectoderm cells form muscle cells at the same
time and place as prospective mesoderm cells whereas anterior neural cells are delayed in their ability to form muscle and are found in more posterior regions of the axis in comparison to their neighboring mesoderm cells. Together these results reveal differences between the plasticity of posterior and anterior neural ectoderm cells in response to muscle-inducing signals. This study provides insights on possible mechanisms that regulate the in-vivo plasticity of cells during vertebrate development. Funded by NIH MARC and NIH MBRS 1SC3GM111118-01.

Program Abstract #99
Anterior-Posterior Neural Axis Plasticity in the Developing Central Nervous System of Xenopus laevis
Lyuba Bolkhovitinov, Vollter Anastas, Ryan Huyck, Margaret Saha
The College of William and Mary, United States
The establishment of an anterior-posterior axis is an essential step in the early development of the central nervous system. In the model vertebrate organism Xenopus laevis, neural induction and the A-P patterning of the nervous system begin during the late blastula stage and continue until the end of gastrulation. While the molecular mechanisms of the establishment of the A-P axis have been extensively studied, less is known about the plasticity of this process. This project investigates the ability of Xenopus laevis embryos to compensate for and recover from an inversion of their A-P axis during early neural development. During either the mid- or late-gastrula stage, presumptive neural ectoderm from a fluorescently labeled donor embryo was transplanted onto an unlabeled host embryo, and the orientation of the axis was either maintained during the transplant or rotated 180 degrees. Phenotypic observations, including the expression of regional marker genes (XCG-1, Otx2, En-2, and Krox20), indicate that embryos can recover from A-P rotation at the mid-gastrula stage and develop normally by the hatching stage, while late-gastrula rotated embryos exhibit morphological abnormalities. Embryos with non-rotated transplanted tissue recover fully at both stages, demonstrating that a loss of healing ability is not the underlying cause for the inability of late-gastrula embryos to recover from rotation. This study identifies a window of plasticity between the mid- and late-gastrula stages during A-P neural axis determination in Xenopus laevis. Funding Sources: The College of William and Mary Roy R. Charles Center Monroe Grant to LB; The Howard Hughes Medical Institute Science Education Program Grant to the College of William and Mary; NIH funding to MS (NIH 1R15HD077624-01).

Program Abstract #100
Cdx4 regulates the onset of spinal cord neurogenesis.
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Spinal cord neurogenesis at the caudal most end of the vertebrate embryo critically depends on the balance of two processes: primordial cell proliferation and differentiation. The caudal neural plate has a limited number of stem cells that self-renew while giving rise to the entire spinal cord via continuous generation of neural cell progenitors. Changes in the rate of cell-renewal and differentiation can result in spinal cord malformations with compromised function. While the molecular components driving spinal cord stem cell proliferation and differentiation are being elucidated, the molecular components driving the proliferation-to-differentiation switch are poorly understood. Here we show that Cdx4, a transcription factor essential for neural tube anteroposterior patterning, regulates the proliferation-to-differentiation switch in the spinal cord neural primordia. In gain and loss of function experiments in chickens, we show that Cdx4 functions as a differentiation switch by regulating three distinct temporal events. Cdx4 (1) inhibits pluripotency in young caudal stem cells by repressing the stem cell factor Cash4, while (2) promoting neural identity in early maturing transition zone cells by activating expression of the neural factor Pax6, as it (3) prevents their premature differentiation by repressing expression of the neurogenic factor Ngn2. Bioinformatics analysis supports a direct regulation of Pax6 by Cdx4, through two clusters of Cdx4 binding sites that are evolutionarily conserved among vertebrates. Moreover we also show that Cdx4 regulate the Notch signaling in the transition zone of the spinal cord by inhibiting expression of the cell proliferation marker Hes1/5, indicating that Cdx4 regulates cell cycle progression/exit. Together our findings suggest that, in addition to its previous known role in patterning, Cdx4 have a key function in regulating onset of spinal cord neurogenesis.

Program Abstract #101
The indirect role of Fgf8 in defining the Vomeronasal/GnRH-1 neurogenic area.
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FGF8 signaling plays a pivotal role in development of craniofacial structures, vomeronasal-olfactory system and GnRH-1 neurons. Bone morphogenetic protein-4 (BMP4) and fibroblast growth factor-8 (FGF8) have been reported to play opposite roles in defining epithelial versus neurogenic fate in the developing olfactory/vomeronasal system. FGF8 has been implicated in specification of olfactory and gonadotropin releasing hormone-1 (GnRH-1) neurons, as well as in controlling putative olfactory stem cell survival and neurogenic potential. We analyzed Fgf8 expression and cell lineage in the developing nose in relation to the expression of Bmp4 and its antagonist Noggin (Nog). Fgf8 genetic lineage tracing showed that FGF8 is expressed by cells that acquire epidermal and respiratory cell fate and not by cells giving rise to olfactory or vomeronasal neurons. Ectodermal and mesenchymal sources of BMP4 control the expression of BMP/TGFβ antagonist Nog. Mesenchymal sources of Nog were found to define the neurogenic borders of the olfactory pit. Fgf8 hypomorphic mouse models, Fgf8 (neo/neo) and Fgf8 (neo/null), displayed severe craniofacial defects together with overlapping defects in the olfactory pit including (1) lack of neuronal formation ventrally, where GnRH-1 neurons normally form, and (2) altered expression of Bmp4 and Nog, with Nog ectopically expressed in the nasal mesenchyme and no longer defining the GnRH-1 and vomeronasal neurogenic border. Our data show that (1) FGF8 is not sufficient to induce ectodermal progenitors of the olfactory pit to acquire neural fate and (2) altered neurogenesis and lack of GnRH-1 neuron specification after chronically reduced Fgf8 expression reflected dysgenesis of the nasal region and loss of a specific neurogenic permissive milieu that was defined by mesenchymal signals.

Program Abstract #102
The Effects of Maternal Alcohol Exposure on the Development of the Precerebellar System
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Exposure to alcohol during gestation can lead to fetal alcohol syndrome (FAS), a disease characterized by a range of cognitive and physical disabilities including mental retardation and impaired gross motor control. Studies using mouse models of FAS investigating the loss of motor coordination have traditionally focused on the cerebellum and in particular, the effects of alcohol on the granule and Purkinje cells. These studies have shown that embryonic alcohol exposure causes large numbers of cerebellar cells to undergo apoptosis by postnatal day 7. Subsequent analysis of brainstem structures from these neonates, such as those of the precerebellar nuclei (which function to relay inputs from the cortex and spinal to the cerebellum), also reveal large numbers of apoptotic cells. Unclear from these studies, is whether the apoptosis of precerebellar neurons is due to a primary effect of alcohol on precerebellar neuron development or to a secondary failure of these neurons to properly establish synaptic connections with cells in the cerebellum. To distinguish between these two possibilities, a mouse model of FAS was established. Analysis of neonates from this model has identified abnormalities in the precerebellar system at developmental time points prior to the initial formation of synapses between precerebellar and cerebellar neurons. This study suggests that the teratogenic effects of alcohol on precerebellar development are due to a primary insult to the developing cells as opposed to apoptosis resulting from a failure to form synapses with cerebellar neurons.

Program Abstract #103
BLM-s, a BH3-only apoptosis sensitizer/derepressor, mediates apoptosis in the developing peripheral nervous system
Pei-Hsin Huang, Wei-Wen Liu
Graduate Institute of Pathology, College of Medicine, National Taiwan University, Taiwan
Programmed cell death (apoptosis) attunes the number of neurons in the developing peripheral nervous system to match their target size by competing the limited quantities of trophic factors released by the target. Without these trophic factors, the developing neurons are committed to apoptosis. The central players mediating the life-or-death decision of cells are the BCL-2 family, among which several members including the prosurvival BCL-2 and the proapoptotic BIMEL are essential for apoptosis in the developing peripheral nervous system. We have recently identified a new member of the BCL-2 family genes- Blm-s (Bcl-2-like molecule, short form), which functions as a BH3-only apoptosis-sensitizer/derepressor and causes BAX-dependent mitochondria-mediated apoptosis in postmitotic neurons of the developing cerebral cortex (Liu et al. 2014). In the developing peripheral nervous system, we found that BLM-s transcripts are expressed in the sympathetic cervical ganglion (SCG) of the mouse newborn and the dorsal root ganglions (DRG) of the mouse embryos in a temporally regulated way. Via in vitro dissociated SCG, DRG, and PC12 cell culture, we revealed that NGF withdrawal induces the expression of Blm-s transcripts concomitant with neuronal apoptosis. In
addition, the level of phosphorylated BLM-s protein is increased in neurons under NGF withdrawal, suggesting post-translational regulation of BLM-s could play a role in the apoptosis of the peripheral nervous system. Interestingly, upregulation of Blm-s transcript upon NGF withdrawal is not inhibited by blockage of the JNK- or of the MAPK-signaling pathways, suggesting BLM-s transcriptional regulation via a pathway different from those involved in the regulation of BIMEL. This work is supported by a fund granted to P-H Huang (MOST100-2320-B-002-089-My3) supported by Ministry of Science and Technology, Taiwan.

Program Abstract #104
Integrated analysis of brain patterning and morphogenesis with ultrashort pulse microscopy (UPM) and image registration
Arne Lekven, Holly Gibbs, Brian Kelly, Alvin Yeh
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The embryonic brain is subdivided by unique patterns of gene expression prior to morphogenesis, but the mechanisms linking these phenomena remain poorly understood in part due to the lack of imaging techniques that can capture molecular, cellular, and tissue-scale dynamics simultaneously in 3-D. To address the need for imaging technologies that capture both tissue morphology and cell lineage information, we have established the use of Ultrashort Pulse Microscopy in zebrafish. This imaging platform utilizes the high peak power and short coherence length of broadband ultrashort optical pulses to generate two-photon excited fluorescence (from cellular autofluorescence and fluorescent reporter proteins) and high-resolution optical coherence signals (from NBT/BCIP). Combining these signals through 3-D image registration enables integrated imaging and analysis of morphogenesis, genetic cell lineage reporter dynamics, and gene expression domains, which we applied to analyze early steps in brain development. Using these tools, we characterized wnt1 lineage dynamics during midbrain-hindbrain boundary (MHB) formation and found differences in the refinement of gene expression domains along the dorsoventral axis at the boundary where wnt1 and fgf8a form an interface. We then examined these dynamics in fgf8a loss of function embryos and showed that initiation of the morphogenetic program at the boundary does not require fgf8a, but that anteriorly expanded hindbrain ventricle opening and failure to restrict the posterior limit of wnt1 expression at the dorsal MHB results in a cerebellar to tectal transformation and termination of constriction morphogenesis.

Program Abstract #105
Genetic regulation of hypothalamic neurogenesis by Lef1
Richard Dorsky, Yuanyuan Xie, Anna Clark, Ed Levine
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Neurogenesis in the vertebrate hypothalamus continues postembryonically and regulates behavior. We have previously shown that Wnt signaling through the transcriptional effector Lef1 is required for hypothalamic neurogenesis in zebrafish and mice. However, the specific cellular and molecular functions of Lef1 remain unknown. We have performed a comprehensive analysis of Lef1 mutants to address these questions and to determine the evolutionary conservation of Lef1 function in vertebrates. We find that in zebrafish, lef1 cell autonomously promotes the differentiation of multiple ventricular neuronal subtypes from Wnt-responsive progenitors, and in mutants failure of this process leads to apoptosis and decreased tissue size. RNAseq analysis of lef1 mutants supports these results, showing decreased expression of known Wnt target genes as well as markers for defined neuronal subtypes, and mediators of other signaling pathways with potential roles in neurogenesis. Many of these genes respond rapidly to global activation and inhibition of Wnt signaling, suggesting that they may be direct transcriptional targets of Lef1. Analysis of a conditional hypothalamic Lef1 knockout mouse shows similar morphological defects that are most apparent postembryonically, as well as decreased expression of orthologous genes that are co-expressed with Lef1 in the premammillary nuclei, which control aggression and social dominance. Together, these data suggest a conserved role for Wnt signaling in regulating the continuous generation of specific neuronal populations, and identify potential behaviors that may be mediated by this process. Funded by NIH R01 NS082645

Program Abstract #106
Habenular precursors require Wntless-dependent Wnt signaling
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The habenular nuclei of the dorsal forebrain have been implicated in neurological conditions, such as bipolar disorder and
FGF8 signaling in cochlear development: Hearing lost and found

Suzanne Mansour, Chaoying Li, Lisa Urness

University of Utah, USA

FGF signaling plays dosage-sensitive roles during inner ear development. Genetic studies show that differentiation of cochlear pillar cells requires inner hair cell-expressed FGF8 signaling to FGFR3 expressed by prospective supporting cells (SCs). We studied hearing in a mouse model of Muenke syndrome (Fgfr3P244R/+). These mice have dominant hearing loss (HL) that spares the high frequencies, similar to, but more extreme than the HL of Muenke syndrome subjects.

FGFR3b and FGFR3c such that both acquired responsiveness to FGF10. Moreover, Fgf10 rescue cochlear SC fate and function. Accordingly, we found that the FGFR3P244R mutation changed the specificity of FGF signaling in cochlear development: Hearing lost and found

Program Abstract #107
Ascl3 is a common marker for secretory cell precursors in different organs

Pei-Lun Weng, Mridula Vinjamuri, Catherine Ovitt

University of Rochester, United States

The olfactory epithelium undergoes continuous turnover throughout the life of the organism. Normal homeostasis and regeneration of the olfactory epithelium is dependent on the globose and horizontal basal stem cells. Ascl3, a member of the Achaete-scute transcription factor family, is expressed in secretory organs such as salivary and lacrimal gland. Previously we showed that Ascl3-expressing cells are bipotent progenitors of both acinar and duct cells in the salivary glands. In addition, we have found that Ascl3 is expressed in a population of apical cells in the olfactory epithelium. By genetic fate mapping, we demonstrate that Ascl3-expressing cells are progenitors of microvillar cells, which secret neuroproliferative factor Neuropeptide Y stimulating basal cell proliferation, and Bowman’s glands, which generate mucus preventing dehydration and infection. Cell specific ablation of Ascl3-expressing cells confirms that they generate all microvillar cell types, as well as Bowman’s glands. In addition, the thickness of the neuronal layer is decreased in the cell ablation model, suggesting that loss of microvillar cells and Bowman’s glands may affect the basal stem cells. To investigate the requirement for Ascl3-expressing cells in adult olfactory epithelium, we examined regeneration after injury. Within 3 days after methamazole injection, Ascl3-expressing cells reappear followed by microvillar cells and Bowman’s glands. In the Ascl3-cell ablation model, we observed impaired neuronal regeneration and significantly lower numbers of microvillar cells and Bowman’s glands after injury. Thus, in addition to marking progenitors in the salivary and lacrimal glands, Ascl3-expressing cells are progenitors of microvillar cells and Bowman’s gland in the olfactory epithelium. NIDCR grant R01 DE018896

Program Abstract #108
GFG signaling in cochlear development: Hearing lost and found

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University of Rochester, United States

FGF signaling plays dosage-sensitive roles during inner ear development. Genetic studies show that differentiation of cochlear pillar cells requires inner hair cell-expressed FGF8 signaling to FGFR3 expressed by prospective supporting cells (SCs). We studied hearing in a mouse model of Muenke syndrome (Fgfr3P244R/+). These mice have dominant hearing loss (HL) that spares the high frequencies, similar to, but more extreme than the HL of Muenke syndrome subjects. Fgfr3P244R/+ HL is associated with a cochlear SC fate transformation of two Deiters’ cells to two pillar cells; similar to, but more extreme than that seen in mice lacking the negative feedback regulator of FGF signaling, Sprouty2. In addition, these mice have ectopic sensory outer hair cells (OHCs). We found that in Fgfr3P244R/+ mice, prospective Deiters’ cells switch to an outer pillar cell-like fate sequentially between E17.5 and P3. Unexpectedly, the Fgfr3P244R/+ HL and SC fate transformation were not rescued by genetically reducing FGF8, the FGFR3c ligand required for pillar cell differentiation. Rather, reducing Kolliker’s organ-expressed FGF10, which normally activates FGFR2b or FGFR1b, was sufficient to rescue cochlear SC fate and function. Accordingly, we found that the FGFR3P244R mutation changed the specificity of FGFR3b and FGFR3c such that both acquired responsiveness to FGF10. Moreover, Fgf10 heterozygosity did not block
the $Fgfr3^{P244R/+}$ SC fate transformation, but instead, allowed a gradual reversion of fate-switched SCs towards the normal phenotype between P5 and at least P14. Thus, Deiters’ and pillar SCs can reversibly switch fates in an FGF-dependent manner over a prolonged period of time. This property might be exploited for the regulation of sensory hair cell regeneration from SCs. Recent studies of the rescue by Fgf10 heterozygosity of the excess OHCs in the Muenke model and the effects of blocking FGFR2b signaling at different stages of Muenke model development will be presented.

**Program Abstract #109**

**Otic Sensory Lineage Development Revealed Through Fbxo2**

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The otic sensory lineage arises from the otic placode and harbors potential to produce sensory cell populations including mechanosensory hair cells and supporting cells. Here we investigate developmental mechanisms of this lineage using the highly otic-specific gene $Fbxo2$. We have found that $Fbxo2$ activation is a specific transcriptional feature of the developing otic sensory lineage and we hypothesize that the $Fbxo2$ locus can be utilized to characterize, isolate, and propagate otic progenitors. We engineered $Fbxo2$-VenusHC reporter mice and stem cell lines by targeting the locus with a multifunctional tricistronic reporter cassette (expressing H2BVenus, HygroR, and CreERt2) to enable imaging, drug selection, and tracing of the $Fbxo2$ lineage. Current experiments include characterization of Venus as a reporter for $Fbxo2$, tracing the $Fbxo2$ lineage with tamoxifen injections and propagation of otic progenitors from embryos and stem cells. We seek to enable a stem cell model of otic sensory development using $Fbxo2$-VenusHC for lineage reporting/selection coupled with experimental manipulations in signaling pathways and transcription factor expression to test developmental hypotheses. We are also using $Fbxo2$-VenusHC fibroblasts to access the potential for transcription factor based reprogramming approach to convert non-otic cells to an otic sensory fate. We also seek to identify mechanisms regulating $Fbxo2$ expression in the otic sensory lineage. Phylogenetic and transcription factor binding site analyses suggest that one or more cis-regulatory modules may regulate $Fbxo2$ by integrating activity of multiple transcription factors, such as Pax2/5/8, Sox2/10, and Gata3. We have generated transgenic mice with tdTomato under the control of several candidate regulatory elements and are currently identifying the minimal elements required to recapitulate $Fbxo2$ expression.  

Funding: NIH F32 DC013210, NIH Pediatric LRP, NIH DC006167 and DC012250 to S.H., P30 DC010363

**Program Abstract #110**

**Probing Retinal Progenitor Heterogeneity**

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The seven major neural cell types of the vertebrate retina are produced in a sequential but overlapping order from a pool of retinal progenitors derived from an outgrowth of neuroepithelium. Single-cell transcriptome analysis has revealed the incredible diversity of these progenitors, and retroviral lineage tracing has demonstrated that retinal progenitors with restricted fate potentials can be identified by marker gene expression. However, the lack of genetic tools to manipulate specific progenitor populations has prevented a full characterization of this diversity. Utilizing the ease of electroporation of the postnatal murine retina, a number of cis-regulatory elements have been found adjacent to genes expressed in retinal progenitors that drive expression in specific subsets of these progenitors. The mature cell types that these progenitors give rise to were then characterized using recombinase-dependent lineage tracing, demonstrating that many enhancers label highly fate-restricted progenitor subsets. These enhancers can also be utilized as tools to characterize the response of these progenitor subsets to various genetic perturbations.

**Program Abstract #111**

**Dissection of Gene Regulatory Networks that control retinal cell fate specification**

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Gene regulatory networks (GRNs) regulate critical events during development. In complex tissues, such as the mammalian central nervous system (CNS), networks likely provide the complex regulatory interactions needed to direct the specification of the many CNS cell types. To understand these GRNs, we started by dissecting a GRN that regulates a binary fate decision between two siblings in the murine retina, the rod photoreceptor and bipolar interneuron. A simple strategy was employed and developed to dissect the rod-bipolar GRN. First, we identified enhancers of key transcription factors (TFs) that regulate the specification of rod and bipolar cells. Second, by analyzing enhancers, we uncovered
upstream regulatory TFs that regulate these enhancers. Third, we established the relationships among different TFs, and mapped TFs into GRNs via performing gene perturbation assays. Specifically, the rod-bipolar GRN centers on Blimp1, one of the TFs that regulates the rod versus bipolar cell fate decision. We identified a cis-regulatory enhancer, B108, which mimics Blimp1 expression. Deletion of genomic B108 by CRISPR/Cas9 in vivo using electroporation abolished the function of Blimp1. Otx2 and RORβ were found to regulate Blimp1 expression via B108, and Blimp1 and Otx2 were shown to form a negative feedback loop that regulates the level of Otx2, which regulates the production of the correct ratio of rods and bipolar cells. Furthermore, based on this study, we developed novel enhancer screen assays and TF pull-down assays to identify enhancers and regulatory TFs in a more profound and large-scale fashion to facilitate GRN studies. This work was supported by HHMI.

Program Abstract #112
Aplnr and its ligand Apela have opposite effects on Nodal signaling during cardiac development
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The Apelin receptor (Aplnr) and its early ligand Apela are essential for heart development by controlling the migration of cardiac progenitors to the anterior lateral plate mesoderm, but how this happens is unclear. In this study we demonstrate that Aplnr signaling modulates Nodal/TGFbeta signaling during gastrulation, a key pathway essential for mesendoderm induction and migration. Loss of Aplnr function leads to a reduction in Nodal target gene expression whereas activation of Aplnr increases the expression of these same targets. Furthermore, loss of Aplnr results in a delay in the expression of the cardiogenic transcription factors mespaa/ab. By elevating Nodal levels in aplnr mutant embryos we are able to fully restore cardiac differentiation. We find that loss of Aplnr attenuates the activity of a point source of the Nodal ligands Squint and Cyclops in part by regulating their extracellular processing. Most unexpectedly, we find that in the context of Nodal modulation the loss of Apela results in increased Nodal signaling, pointing to the possibility that Apela may work as an inhibitory ligand to Aplnr. We favour a model in which the antagonism between Apela and its receptor Aplnr is able to fine tune Nodal output to initiate the migration of lateral margin cells to the heart forming region. We propose that the Apela-Aplnr signaling cascade may therefore act as a specific rheostat for the Nodal/TGFbeta pathway during the earliest stages of cardiogenesis.

Program Abstract #113
Tbx6, Mesp-b and Ripply1 regulate the onset of skeletal myogenesis in zebrafish
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During embryonic development, the paraxial mesoderm becomes segmented into somites, within which proliferative muscle progenitors and muscle fibers establish the skeletal musculature. Here we demonstrate that a gene network previously implicated in somite boundary formation, involving the transcriptional regulators Tbx6, Mesp-b, and Ripply1, also confers spatial and temporal regulation to skeletal myogenesis in zebrafish. We show that Tbx6 directly regulates mesp-b and ripply1 expression in vivo, and that the interactions within the regulatory network are largely conserved among vertebrates. Mesp-b is necessary and sufficient for the specification of a subpopulation of muscle progenitors, the central portion of the Pax3+/Pax7+ dermomyotome. Conditional ubiquitous expression indicates that Mesp-b acts by inhibiting myoD and myf5 expression and by inducing the dermomyotome marker meox1. In contrast, Ripply1 induces a negative feedback loop by promoting Tbx6 protein degradation. Persistent Tbx6 expression in Ripply1 knockdown embryos correlates with a deficit in dermomyotome and myotome marker gene expression, suggesting that Ripply1 promotes myogenesis by terminating Tbx6-dependent inhibition of myogenic maturation. Together our data suggest that Mesp-b is an intrinsic upstream regulator of skeletal muscle progenitors and that in zebrafish, the genes regulating somite boundary formation also regulate the development of the dermomyotome in the anterior somite compartment.

Program Abstract #114
Cell fate choices at the intersection of genetics and epigenetics
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We observe a gain of bone cells and a corresponding loss of ligament cells in mef2ca mutant zebrafish, motivating the
hypothesis that mef2ca is a fate switch gene. In support of this hypothesis, time-lapse imaging studies demonstrate that ectopic bone forms in mutants at the expense of ligament and arises from transfated ligament progenitors, rather than by cell migration from some other source. An interesting feature of the ligament-to-bone fate switch is its extreme phenotypic variation. To understand this variation we carried out a genetic experiment tracking and selecting phenotype penetrance for several generations. This experiment demonstrates heritability of the phenotypic variation, and suggested that relatively few heritable factors modify the fate switch. Curious transgenerational inheritance patterns in this experiment motivated the hypothesis that heritable epigenetic factors modify mef2ca mutant phenotypes. To test this hypothesis we developed an epigenetic silencing reporter paradigm, providing a visual quantitative readout of DNA methylation-based transcriptional silencing in live animals. We discovered that this reporter is completely silenced in some bone cells, but not their close neighbors. Excitingly, the silenced cells are located where the bone expansion occurs in mef2ca mutants. Moreover, the level of epigenetic silencing is trans-generationally heritable and correlates with the penetrance of the ligament-to-bone switch phenotype in mef2ca mutants. These findings support the hypothesis that heritable epigenetic silencing modifies the mef2ca mutant phenotype. Most examples of transgenerational epigenetic inheritance involve parasitic repetitive elements, which modify transcription of nearby host genes. One such endogenous retrovirus resides immediately upstream of the mef2ca locus and motivates a model in which heritable DNA methylation-based silencing of a nearby repetitive element modifies the mef2ca ligament versus bone fate decision.

Program Abstract #115
Twist1 contributes to cranial bone initiation and dermal condensation by maintaining Wnt signaling responsiveness.
Radhika Atit, L. Henry Goodnough, Gregg DiNuoscio
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During development, proper spatiotemporal patterning and specification of tissues requires precisely orchestrated changes in gene expression, which are regulated by transcription factors in response to instructive cues from cell signaling pathways. Wnt/β-catenin signaling acts as a molecular switch in the cranial mesenchyme by simultaneously affecting specification of cranial bone and dermal lineages and repressing cartilage cell fate. The mechanism underlying how these cells interpret instructive signaling cues and differentiate into these two lineages is unclear. Twist1 is a target of the Wnt/β-catenin signaling pathway and is expressed in cranial bone and dermal lineages. Now, we show that onset of Twist1 expression in the mouse cranial mesenchyme is dependent on ectodermal Wnts and mesenchymal β-catenin activity. We have created a novel conditional Twist1 mouse mutant without compromising cell survival and neural tube closure in the head. Conditional deletion of Twist1 in the supraorbital arch mesenchyme leads to cranial bone agenesis and hypoplastic dermis, as well as craniofacial malformation of eyes, teeth, and palate. Twist1 is preferentially required for cranial bone lineage commitment by maintaining Wnt responsiveness. Twist1 mutant cranial dermis fails to condense and expand apically leading to extensive cranial dermal hypoplasia with few and undifferentiated hair follicles. Conditional absence of Twist1&2 leads to failure of dermal specification. RNA expression profiling of Twist1 mutant cranial mesenchyme show changes in genes predicted to associate with skeletogenic development, extracellular matrix interactions, Wnt signaling, and skeletalgenic/skin diseases. Thus, Twist1, a target of canonical Wnt/β-catenin signaling, also functions to maintain Wnt responsiveness and is a key effector for cranial bone fate selection and dermal condensation. Funding provided by NIH NIDCR R01 DE01870 (RA), NIDCR F31 DE020220 (LHG).

Program Abstract #116
Wnt/β-catenin activity-dependent Polycomb repression in craniofacial development
James Ferguson, Radhika Atit
Case Western Reserve, United States
Proper craniofacial development involves complex spatial and temporal genetic interactions in the cranial neural crest cells (CNC) to allow for differentiation into various lineages. Within the CNC derived supraorbital cranial mesenchyme, Wnt/β-catenin signaling is required for proper skull bone and dermal fate selection. In the absence of β-catenin, multiple chondrogenic genes are up-regulated followed by ectopic cartilage formation. How β-catenin activity suppresses the cartilage fate is unclear, however. Recently, we discovered that multiple genes up-regulated in the absence of β-catenin, such as Sox9 and Col2a1, are also targets of the Polycomb Repressive Complex (PRC2) leading to the hypothesis that β-catenin requires PRC2 to regulate cell fate selection. In the mesenchyme, the components of PRC2 are present and active. However, the global expression level of these components does not change following conditional deletion of β-catenin indicating PRC2 and β-catenin are required on a gene specific level. Following chemical inhibition of EZH2 function in vitro, both Sox9 and Col2a1 are up-regulated further supporting gene specificity. Additionally, the PRC2 repressive mark,
H3K27me3, is enriched on the Sox9 locus in the cranial mesenchyme by ChIP-sequencing. Current studies are investigating to whether Wnt/β-catenin activity is required for recruitment of PRC2 to its target genes. These findings provide new mechanistic insight into the mechanism by which β-catenin regulates lineage restriction and provides new insights into how epigenetic silencing and Wnt/β-catenin signaling interact to promote proper lineage identity of the supraorbital cranial mesenchyme.

**Program Abstract #117**

**Neural crest-specific deletion of Lrp1 results in craniofacial and vascular abnormalities**

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Low density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional receptor that processes signals from >40 ligands, acting through endocytosis and signal transduction activation to regulate various signaling pathways. LRP1 is thought to play an essential role in regulating pericyte and vascular smooth muscle cell (vSMC) function through modulation of PDGF-beta signaling. Pericytes are an essential component of the brain vasculature, required for the maintenance of the blood-brain barrier and for normal function of the neurovascular unit. In the mouse, Lrp1 hypomorphic and null mutants are embryonic lethal, displaying numerous defects including, open body wall, cleft palate, and vascular abnormalities. Recent work has shown that the vasculature of Lrp1-null embryos lacks normal vSMCs, resulting in vessel leakage and hemorrhage. To better understand the cell autonomy of the Lrp1 phenotype, we employed conditional deletion using a series of Cre drivers, including the neural crest-specific Wnt1-cre line (Lrp1-cko). Lrp1-cko embryos were lethal with a less severe phenotype than a complete null (surviving to E15.5), showing cleft palate, vascular defects and epithelial blebbing. Because most pericytes of the forebrain and face are derived from the cranial neural crest, we explored the differentiation, recruitment and retention of pericytes in these structures in both lineage marked and Lrp1-cko embryos. This work will provide a more thorough understanding of the role of Lrp1 in embryonic development and pericyte biology.

**Program Abstract #118**

**Determining the Effect of BMP Signaling on Thymus Fate Specification and Differentiation of the Third Pharyngeal Pouch in the Mouse**

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The third pharyngeal pouch is an endodermally derived epithelium that differentiates into thymus and parathyroid cells. Foxn1 (transcription factor) is a thymus-specific marker, which is required for thymic epithelial cell (TEC) proliferation and differentiation. Gcm2 is a parathyroid-specific marker, which is essential for parathyroid survival and differentiation. Bone morphogenetic protein (BMP) is a member of the TGF-β family of signaling molecules. Loss of function genetic analysis of BMP4 showed that BMP4 is required for organ morphogenesis but not initial thymus fate; however, repression of BMP signaling by transgenic expression of ectopic Noggin inhibitor suppressed Foxn1 expression. Based on these data, we hypothesize that BMP signaling is necessary for normal thymus fate specification during initial organogenesis with BMP signaling promoting thymus fate while inhibiting parathyroid fate. We are testing this hypothesis using inducible Gremlin transgenic mice in a tissue-specific Cre-Lox induction system, as well as gain and loss of function experiments in embryo cultures. Current data suggest delayed expression of Foxn1 when Gremlin is ectopically expressed. Explant cultures with dorsomorphin, a BMP inhibiting drug, showed a reduction of both Gcm2 and Foxn1. Also, cultured embryos with a BMP4 bead implant showed a reduction of Gcm2. Surprisingly, these data suggest inhibition of BMP signaling is suppressing Gcm2, while supporting the hypothesis that BMP signaling is necessary for initial thymus fate specification. Research reported in this poster was supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under award number R01AI107096.

**Program Abstract #119**

**Investigating the molecular determinants for anterior-posterior polarity during sea urchin embryogenesis**

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The Dishevelled (Dsh) protein in the Wnt signaling pathway is essential for endomesoderm specification in sea urchin embryos. Dsh is asymmetrically enriched in a vegetal cortical domain (VCD) of the unfertilized egg, and several lines of evidence indicate that activation of Dsh in the canonical Wnt (cWnt) pathway during endomesoderm specification
requires the VCD. First, while Dsh is required for activation of cWnt signaling in vegetal cells, overexpression of Dsh is not sufficient for ectopic endomesoderm formation in mesomeres. Second, a differentially modified form of Dsh accumulates in the VCD and this domain is selectively inherited by the vegetal blastomeres that nuclearize beta-catenin in early embryos. Moreover, embryological extirpation of the VCD from eggs blocks activation of cWnt signaling in embryos developing from VCD minus eggs. All of these observations indicate Dsh must work with some molecules at the VCD to regulate cWnt signaling activity and endomesoderm specification. To identify such candidate molecules, we carried out two separate molecular screens. To identify RNAs asymmetrically enriched in the egg cortex in general, we did a RNA-seq screen using eggs, egg cortices and different blastomeres from 16-cell stage embryos. To identify candidate proteins that regulate Dsh activity directly, we performed Dsh co-immunoprecipitation using lysates from eggs and isolated cortices. These screens have identified several intriguing candidates. Overexpression of one candidate, DIXDC1, produced severely vegetalized embryos indicating that this protein may play a critical role in the local activation of Dsh in the VCD during endomesoderm specification. We will discuss our ongoing studies to characterize the respective roles of these candidates in regulating cWnt pathway and endomesoderm specification in sea urchin embryogenesis. Funding source: Lingyu Wang was supported by a grant from NSF (IOS 1257967) to Athula H Wikramanayake.

**Program Abstract #120**

**Genome-Wide Assessment Of Differential Effector Gene Use In Embryogenesis**

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Six different populations of cells were isolated by FACS from late blastula and disaggregated gastrula stage sea urchin embryos according to the regulatory states expressed in these cells, as reported by recombineered BACs producing fluorochromes. Transcriptomes recovered from these embryonic cell populations revealed striking, early differential expression of large cohorts of effector genes. The six cell populations were presumptive pigment cells, presumptive neurogenic cells, presumptive skeletogenic cells, cells from the stomodeal region of the oral ectoderm, ciliated band cells, and cells from the endoderm/ectoderm boundary that will give rise both to hindgut and to border ectoderm. Transcriptome analysis revealed that each of these domains specifically expressed several hundred effector genes at significant levels. Annotation indicates the qualitative individuality of the functional nature of each cell population, even though they were isolated from embryos only 1 to 2 days old. In no case was more than a tiny fraction of the effector genes expressed specifically in one population also expressed specifically in any other of the six populations studied. As was particularly clear in the cases of the presumptive pigment, neurogenic, and skeletogenic cells, all three of which represent precociously differentiating cell types of this embryo, most specifically expressed genes of given cell types are not significantly expressed at all in the other cell types. Thus at the effector gene level a dramatic, cell type specific pattern of differential gene regulation is established well before any significant embryonic morphogenesis has occurred.

**Program Abstract #121**

**The ATP-binding cassette (ABC) transporter ABCC5a controls cyclic nucleotide mediated invagination in sea urchins**

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Development is choreographed by secreted signals. The ATP-binding cassette (ABC) transporters are a large family of conserved membrane pumps whose diverse substrates include signaling molecules. However, the role of ABC transporters in animal embryogenesis remains largely unexplored. Here we show that a C-type (MRP) ABC transporter in the mesoderm directs movement of the endoderm in sea urchin embryos. This transporter, Sp-ABCC5a (C5a), is expressed in pigment cells and their precursors, which are a subset of the non-skeletogenic mesenchyme (NSM) cells that become immunocytes. C5a is activated by Delta-Notch signaling from the skeletogenic mesenchyme and is downstream of gcm in the aboral NSM gene regulatory network. C5a knockdown embryos successfully gastrulate, but they develop a prolapse of the hindgut by the late prism stage (~eight hours after C5a protein expression normally peaks). Defective hindgut invagination occurs in ~90% of knockdown embryos. Since previous studies showed that (1) mammalian ABCC5 effluxes cyclic nucleotides, (2) cAMP-dependent protein kinase is expressed in pigment cells, and (3) peak expression of soluble adenylyl cyclase (sAC) is coincident with that of C5a, we hypothesized a link between cAMP signaling and C5a. We find that exposing embryos to the sAC-specific inhibitor KH7 (10 μM) inhibits archenteron formation, indicating that production of cAMP by sAC is required for gastrulation. Similarly, exposing embryos to membrane-permeable pCPT-cAMP (20 μM) causes embryos to hyper-invaginate. Finally, in C5a-knockdown embryos, pCPT-cAMP rescues hindgut
invagination defects. Together our data suggest that C5a-mediated efflux of cAMP from pigment cells controls late
invagination of the hindgut. The functions of ABCC5/MRP5 transporters remain unclear in any organism, and our study
elucidates a novel role for C5a-mediated mesoderm-endoderm signaling in embryogenesis.

Program Abstract #122
Connecting the Dots: Linking the Biochemistry and Cell Biology of Wnt Lipid Modifications to Embryonic
Development
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Hannoush²
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The developing spinal cord represents one of the best-characterized gradients of Wnt activity in vertebrate models.
Specifically, gain and loss of function experiments show that a dorsal to ventral gradient of Wnt1 and Wnt3a activity
drive the patterning and outgrowth of the spinal cord. The post-translational modification of Wnt ligands with palmitate is
required for the formation of the Wnt gradient. The addition of palmitoyl groups to a conserved serine residue on WNT
proteins is catalyzed by Porcupine (PORCN). Mutation of the palmitoylated WNT1 Ser residue (Ser224) to Ala reduces
the lipid raft association and secretion of WNT1. The WNT amino acid residues required for recognition and
palmitoylation by PORCN have not been fully characterized. We show that WNT1 residues 209–239 are sufficient for
PORCN-dependent palmitoylation of Ser224. Substitution of Ser224 with Thr, but not Cys, is tolerated in palmitoylation
and spinal cord assays. Interestingly, this fusion protein recapitulates the PORCN-dependent properties of WNT1 with
respect to lipid raft association and secretion. To define the specificity of PORCN for different Wnt family members, we
are now comparing the ability of PORCN to bind and palmitoylate all Wnt family members that are expressed in the
spinal cord. In sum, our data establish PORCN as an O-acyl transferase and highlight the importance of palmitoylation for
WNT activity in cells and in the developing spinal cord. Acknowledgements: Matilde Miranda was supported by an NIH
MARC fellowship while Muryam Gourdet is supported by NIH BRIDGES. Michael Enriquez was supported by an NSF
REU fellowship. This research was made possible by NSF RUI MCB-1244602, NSF RUI IOS-0950892, NIH
1R15HD070206-01A1, CSUPERB grants as well as SFSU IRA and ORSP mini-grants to Dr. Laura Burrus and a NIH-
RIMI (P20MD000262) Grant to San Francisco State University.

Program Abstract #123
Restriction of Sonic Hedgehog Signaling Competency within the Cell Cycle
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Cell-cell signaling is essential for the development of a diverse range of metazoans as these pathways pattern body axis
polarity, determine cell fate, and regulate cell cycle progression. Mechanisms of signal transduction regulation have been
extensively characterized. However, relatively little attention has focused on a potential layer of regulation; whether a
cell’s ability to respond to a signaling cue is restricted to or enhanced within a specific phase of the cell cycle, a
phenomenon termed cell cycle gating. Previous work supports cell cycle gating; the Wnt signaling pathway is gated to
G2/M by Cyclin Y, a G2/M cyclin, in Xenopus laevis and Notch signaling is enhanced by G1 cyclins and repressed by G2
cyclins in C. elegans. We are investigating the regulation of five major vertebrate signaling pathways – Hedgehog, BMP,
Retinoic Acid, FGF, and Wnt – by the cell cycle. Here we present our data on the Hedgehog pathway. For this analysis,
we have exploited a fluorescing cell-cycle reporter system (FUCCI) in both proliferative NIH-3T3 cell populations and
mouse neural tube sections. Our approach provides high temporal resolution by analyzing expression of nascent pre-
mRNAs, which are processed or degraded quickly after their synthesis. Our results show that responses to Hedgehog
appear to be gated to late G1/early S-phase of the cell cycle. This gating does not temporally correlate with changes in
ciliogenesis, although cilia are essential for vertebrate Hedgehog signaling. This shows that there may be a cell-cycle
related component priming cells for Hedgehog signaling competency. Changes in the relative length of each cell cycle
phase over development may drive differential signaling competency as cells mature. Funding Sources: Office of the
Vice President for Research, University of Georgia

Program Abstract #124
FGFR1 regulates development through the combinatorial use of signaling pathways
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FGF signaling governs multiple processes important in development and disease. Many lines of evidence have implicated ERK1/2 signaling induced through FRS2 as the predominant effector pathway downstream of FGFRs, but these receptors can also signal through other mechanisms. To better understand the function of ERK1/2-independent signaling downstream of FGFRs in the mouse, we engineered an allelic series of knock-in point mutations designed to disrupt FGFR1 signaling functions individually and in combination. Analysis of each mutant indicates that FRS2 binding to FGFR1 has the most pleiotropic functions in development, but also that the receptor utilizes multiple pathways additively in vivo. In addition to FRS2, CRK proteins and PLC\(\gamma\) also contribute to ERK1/2 activation, affecting axis elongation, craniofacial and limb development, providing a biochemical mechanism for additive signaling requirements. Disruption of all known signaling functions diminished ERK1/2 and PLC\(\gamma\) activation but did not recapitulate the peri-implantation Fgfr1 null phenotype or affect PI3K/AKT signaling. This indicates that ERK1/2-independent signaling pathways are functionally important for FGF signaling in vivo. Funding: NIH/NIDCR RO1 DE022778; NYSTEM IIRP N11G-131; NIH/NIDCR F31 DE023686

Program Abstract #125
TNF\(\alpha\)-mediated reverse signaling in the developing peripheral nervous system
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TNF\(\alpha\) is a member of the tumour necrosis superfamily. It signals via two receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). In the immune system it has been shown that these receptors can function as ligands for membrane-integrated TNF\(\alpha\) and initiate reverse signaling. Recently, we have discovered that TNFR1 can act as a ligand for membrane bound TNF\(\alpha\) expressed by developing sympathetic neurons of the superior cervical ganglion (SCG). TNFR1-activated TNF\(\alpha\) reverse signaling promotes sympathetic axon growth in vitro and is required for the establishment of sympathetic innervation in developing mice in vivo (Kisiswa et al, Nature Neuroscience, 16, 865-873, 2013). We show by whole mount staining that Tnf\(\alpha\)-/- and Tnfrsf1a-/- mice have reduced sympathetic innervation in target organs of the SCG (submandibular gland, trachea, pineal gland, kidney and stomach) and stellate ganglion (heart). In addition, treating dissociate sensory cultures (nodose and trigeminal) with recombinant soluble TNFR1-Fc chimera significantly increases axonal growth and branching. However, the developmental time-frame of response to soluble TNFR1-Fc is different between sympathetic and sensory neurons. These results suggest that TNF\(\alpha\) mediated reverse signaling is widely involved in regulating axon growth in the developing peripheral nervous system. This work is funded by the Wellcome Trust and the Neuroscience & Mental Health Research Institute (NMHRI, Cardiff).

Program Abstract #126
The Role of Microglia in the Developing Retina
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Recent evidence suggests that microglia, the resident immune cells of the central nervous system, have multifaceted roles regulating neurogenesis. During cortical development, microglia limit the number of newborn neurons embryonically, but promote the generation of neurons during postnatal time periods. Therefore, microglial function is complex and may change over developmental time. To address this, we first investigated molecular changes in microglia over the course of mouse retina development. We used whole retina quantitative RT-PCR to analyze microglia gene expression at multiple stages from e12.5 to P21. We confirmed that microglia are more “activated” at early developmental stages by increased expression of ionized binding adapter molecule 1 (Iba1) and F4/80. Nerve growth factor (Ngf), which is secreted by microglia to promote developmental apoptosis, was highly expressed at the peak of retinal ganglion cell (RGCs) developmental cell death. We then assessed the expression of multiple microglia-specific genes. Some microglial specific genes, such as Trem2, were expressed embryonically, while others, such as purinergic receptor P2Y (P2ry12), were upregulated at specific times postnatally. Pathways important for microglial maturation also increased in expression postnatally. This suggests microglia may “mature” and acquire specific functions during postnatal development. To investigate the relationship between microglial activation and retinal neurogenesis, we administered minocycline to pregnant dams. Reducing embryonic microglial activation (e12.5-e16.5) increased retinal progenitor cell proliferation and the production of Brn3\(^3\) newborn RGCs. These preliminary results suggest that at embryonic stages, activated microglia limit the generation of newborn neurons. Overall, our findings suggest that microglia may be important regulators of mammalian retinal development and may change function over the course of development. Supported by EY025082.
Program Abstract #127
The intellectual disability gene Kirrel3 regulates target specific synapse development
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Synaptic target specificity, whereby neurons make distinct types of synapses with different target cells, is critical for normal brain function, yet mechanisms driving target specificity remain poorly understood. We discovered that the cell adhesion molecule Kirrel3 regulates target specificity at hippocampal mossy fiber synapses, which are compound synapses connecting dentate granule (DG) neurons to both CA3 and GABAergic neurons. During development, Kirrel3 selectively regulates MF filopodia; the structures that give rise to DG-GABA neuron synapses. Alterations in the Kirrel3 gene have been repeatedly associated with intellectual disability, autism, and Jacobsen’s syndrome but the role of Kirrel3 at synapses is unexplored. Our study demonstrates that alterations in target-specific connectivity during development can cause changes in circuit function that may underlie intellectual disabilities. Funding provided by the NIMH and the University of Utah Seed Grant.

Program Abstract #128
Defining the role of BMP signaling during development of the mouse Cortex
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Development of the mammalian cortex is a complex process known to be regulated by several signaling molecules. Among these, bone morphogenetic proteins (Bmps) have been implicated in the regulation of cortical neurogenesis primarily through in vitro studies. We observed that BMP signaling is active in the ventricular zone (VZ) as well as in the cortical plate in the developing mouse cortex, when we carried out immunohistochemical detection of phosphorylated Smad1/5/8, a readout of BMP signaling. We hypothesized that since BMP signaling is active in multiple locations at different time points within the developing mouse cortex, it is likely to be involved in regulating multiple aspects of cortical development. To investigate in depth the possible roles played by BMP signaling in the developing mouse cortex we employed the following two-pronged strategy. We first depleted BMP signaling during the period of cortical neurogenesis using a Bmp2/Bmp4 double conditional knockout mouse. Here we observed that on depleting of Bmp2 and Bmp4 there is an increase in the number of cells expressing Pax6 and Svet1 in the VZ and the sub-ventricular zone (SVZ) respectively, accompanied by an expansion in the overall number of proliferating cells. This indicated that BMP signaling is indeed a key regulator of cortical neurogenesis in vivo. Next, we inhibited BMP signaling in the developing mouse cortex by delivering a construct expressing the dominant negative Bmp receptor 1b (DNBmpr1b) through in-utero electroporation at 15.5 days post coitus (dpc). When we examined the cells expressing DNBmpr1b at postnatal day 6 (P6) we observed a dramatic disorganization of their dendritic processes accompanied by mis-localization of the Golgi complex. This study has thus provided evidence in support of BMP signaling playing multiple temporally distinct role during mouse cortical development particularly in the regulation of neurogenesis and dendritogenesis of cortical pyramidal neurons.

Program Abstract #129
co-Smad independent canonical BMP signaling in early mouse sternum development
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It is well understood that the canonical Bone Morphogenetic Protein (BMP) signaling required both R-Smad and co-Smad as the intracellular mediator for the signal transduction. Therefore, loss of R-Smad (Smad1, Smad5 and Smad8) or co-Smad (Smad4) is predicted to have similar phenotypes. However, our previous finding in embryonic cerebellum development showed that R-Smad is still functional in the absent of co-Smad. In this study, we used sternum as a model of early endochondral bone development to study the functional discrepancy between R-Smad and co-Smad. We generated the En1-Cre driven Smad1/5 double conditional knockout mice which displayed total loss of sternum components except the manubrium at E18.5. At E14.5, Sox9 and N-cadherin expression was highly down-regulated in the sternum progenitors. Besides, expressions of Sox5 and Sox6 were lost. Together, these data suggest that Smad1/5 are required for the early sternum development, especially in the mesenchymal condensation. To investigate the functional discrepancy between R-Smad and co-Smad, En1-Cre driven Smad4 conditional knockout mice were also generated. To
our surprise, all sternum components were still presented in the Smad4 conditional mutants. And the expression of Sox9, N-cadherin, Sox5 and Sox6 could still be maintained in the mutant sternum progenitors. These differences in phenotypes between Smad1/5 and Smad4 mutants, again, challenge our current understanding on the role of Smad4 as the central mediator in the canonical BMP signaling pathway. This research was supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. AoE/M-05/12, CUHK2/CRF/11G and CUHK 466708).

Program Abstract #130
Electrical activity regulates BMP Release in Drosophila wing development
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Disruption of the inwardly rectifying Kir2.1 potassium channel is responsible for cardiac arrhythmia, periodic paralysis, and developmental defects of Andersen–Tawil Syndrome. These defects include cleft palate, small jaw, wide set eyes, low set ears, dental defects and digital defects such as syndactyly. The molecular mechanism underlying how an ion channel affects morphological development is poorly understood. Disruption of a Kir2.1 homolog in Drosophila called Irk2 causes developmental defects in wings and in eyes that are reminiscent of BMP loss of function. Mutant versions of the channel inhibit correct BMP signaling. Here we show that inwardly rectifying K+ channels are needed for regulating pulses of electrical activity in BMP producing cells in dissected Drosophila imaginal wing discs. Inhibition of Irk2 changes the period of the electrical activity and affects BMP release to disrupt development of the wing. (Funding: NSF IOS 1354282)

Program Abstract #131
Dpp signaling antagonism is mediated by a chondroitin-sulfated sink
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Glycosylation has been identified as a mechanism of protein regulation in a growing number of contexts, of which Drosophila embryogenesis is no exception. Our lab and others have shown that Dpp signaling is regulated by glycosylation during development, and our lab has characterized the role of mummy (mmy) in a unique form of Dpp signal antagonism dependent upon chondroitin sulfate (CS). mmy encodes the enzyme required for the last step in the synthesis of UDP-N-acetylglucosamine (UDP-GlcNAc), a sugar used for glycosylation and synthesis of glycosaminoglycans like CS proteoglycans. mmy mutants exhibit hyperactive Dpp signaling cuticle phenotypes, as well as molecular phenotypes that include expanded embryonic epidermal domains of both pMad and dpp consistent with a role for mmy in Dpp signal antagonism. To better understand the mechanism by which glycosylation is used to antagonize Dpp signaling and with the expectation that enzymes required for GlcNAc utilization and Dpp signal antagonism will exhibit shared loss-of-function phenotypes with mmy, we carried out an RNAi screen targeting all transferases involved in GlcNAc utilization. Here we present data showing that one of the transferases identified in this screen, the chondroitin sulfate synthase wanderlust (wand), is required for the creation of a localized Dpp sink. From its position close to the Dpp signaling source, this sink limits signaling to the dorsal-most domains of the epidermis during dorsal closure. Our data suggest a novel role for glycosylation in maintaining the proper Dpp signaling range in the embryonic epidermis and provide the first evidence for a localized Dpp sink.

Program Abstract #132
Activin-Beta/TGF-Beta signaling in skeletal muscle controls insulin/TOR signaling, metabolism and final body size
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Inter-organ communication is essential for regulating development, growth and homeostasis. Mutations in Drosophila Activin-Beta (Act-Beta), a TGF-Beta ligand, cause accelerated pupariation and reduced final body and organ size. To determine how Act-Beta affects size and timing, we first identified cells expressing 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. Skeletal muscle-specific knockdown of the TGF-Beta signaling transducer/transcription factor dSmad2 reduces body size, indicating skeletal 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 are correlate with phospho-dSmad2 levels, suggesting TGF-Beta signaling in muscle positively regulates insulin/TOR 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 when Act-Beta is lost. 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 in skeletal-muscle using RNAseq and will test potential target genes using tissue-specific knock-downs to determine how TGF-Beta signaling influences insulin signaling and how the muscle coordinates body growth and final organ size.

Program Abstract #133
Identifying Transcripts Involved in Neural Tube Closure Using RNA sequencing
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Anencephaly is a fatal human developmental defect in which the anterior neural tube remains open. When Nodal signaling is reduced, zebrafish display a disorder similar to anencephaly. Previous work from our laboratory suggests a model where Nodal signaling acts through induction of the head mesendoderm and mesoderm. The mesendoderm/mesoderm then promotes adhesion between neural tube cells. This cell adhesion is required for the movements that bring the edges of the left and right folds together so that they fuse at the dorsal midline and close the neural tube. Consistent with a central role for mesendoderm/mesoderm, we found that Nodal signaling is required up to dome stage (4.3 hpf) for a closed neural tube, which is within the time frame when Nodal activity is required for mesendoderm/mesoderm induction. However, the mechanism for interaction between the mesendoderm/mesoderm and the developing neural tube is still unknown. We used RNA-sequencing to identify potential candidates. Embryos treated with a Nodal inhibitor at sphere (neural tube will be open) or 30% epiboly (neural tube will be closed) stages were snap frozen at three time points important for neurulation: shield (6 hpf, neurectoderm induction), tailbud (10 hpf, neural plate forms), and 7 somites (12 hpf, forebrain neural rod is closed). Resulting mRNAs were selected based on degree and significance of differential expression. Consistent with our model, we found mRNAs that are known to be regulated by Nodal signaling, including goosecoid, sox17, and bonnie and clyde. The screen also identified many mRNAs involved in Wnt, FGF, and BMP signaling, suggesting these pathways might be involved in the communication between the mesoderm/mesendoderm and the neuroectoderm. This research was supported by NIH R15 grant 1 R15 HD068176-01A1.

Program Abstract #134
Fishing for Factors of Self-Organization: Heparan sulfate proteoglycans regulate FGF signaling in the lateral line
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The posterior lateral line (PLL), a sensory system that zebrafish use to sense water movement, is an extraordinarily tractable system in which to study cell migration and pattern formation. The posterior lateral line primordium (PLLp) spearheads the early development of this system, migrating from the otic vesicle to the tail, depositing rosettes of cells that will develop into mechanosensory neuromasts. Two signaling systems work in concert to achieve this pattern in the PLLp: Wnt signaling in the leading domain and fibroblast growth factor (FGF) signaling in the trailing domain. The balance of Wnt and FGF signaling is a critical determinant of the rate of neuromast formation, so it is essential to identify the factors that can influence this balance. Heparan sulfate proteoglycans (HSPGs) in the extracellular matrix and on the cell surface, known to associate with Wnt and FGF, have the potential to influence the efficacy of PLLp signaling. Heparan sulfate 3-O-sulfotransferase (HS3ST) known to be involved in HSPG synthesis was previously shown to be specifically expressed within the PLLp. Preliminary results show that hs3st expression, initiated by the Wnt signaling system, is required for effective establishment of the FGF signaling system. We also show that the HSPG syndecan4 (SDC4) is expressed within the PLLp and, like HS3ST, facilitates FGF signaling and protoneuromast formation. Additionally, SDC4 appears to play some role in PLLp migration, as knockdown of sdc4 significantly slows the speed of migration. Together, these studies show how HSPGs help to establish and maintain FGF signaling in the trailing domain of the PLLp and contribute to effective migration and neuromast formation. We would like to thank the National Institute of Child Health of Human Development Intramural Research Program for funding this research.

Program Abstract #135
Functional role of Annexin A6 during trigeminal gangliogenesis.
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The cranial sensory ganglia are formed from two migratory embryonic cell populations, neural crest cells (NCCs) and 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. The molecules and pathways that govern NCC-PC interactions to assemble the ganglia, however, are not well characterized. To this end, we are using the cranial trigeminal ganglia (TG) of the developing chick embryo as an in vivo model to elucidate the mechanisms underlying NCC-PC interactions. The Annexin protein superfamily is crucial for the development of the sensory ganglia, a process that likely requires remodeling of the actin cytoskeleton through actin cross-linking in order to generate forces for NCC and PC motility and promote intercellular interactions. Our lab previously identified an in vivo function for chick Annexin A6 in creating migratory NCCs from immotile precursors, but a later role for Annexin A6 in cranial TG assembly has not been envisioned or investigated. We hypothesize that Annexin A6 acts a core cytoskeletal scaffold in PCs to facilitate TG formation. In support of this, our results show that PCs, not NCCs, express Annexin A6 during TG assembly. In addition, Annexin A6 perturbations lead to defects in the assembly of the TG. Finally, Annexin A6 possesses a functional and putative binding site for F-actin and the actin cross-linking protein α-actinin, respectively. Future studies will be aimed at understanding how the association of Annexin A6 with the actin cytoskeleton, and subsequent effects on cytoskeletal remodeling, impact TG formation. Our results will be significant because they will provide insight into the molecular mechanisms that underlie cellular movements essential for cranial gangliogenesis and in other tissues generated from heterotypic cell types. This work is supported by a grant to LAT (NIH R01DE024217).

**Program Abstract #136**

**Increased glutamine catabolism mediates osteoblast activity in response to Wnt signaling**

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Wnts are secreted ligands that are necessary for cellular differentiation and embryonic development. Cellular differentiation is the process by which cells obtain their final specialized form and function. As such differentiation is one of the most fundamental aspects of human development. Perhaps the most important aspect of differentiation is how cells both obtain and sustain specific cellular activities. Bone forming osteoblasts are characterized by sustained high rates of protein synthesis and secretion. Wnt signaling stimulates bone formation by increasing both the number of bone-forming osteoblasts and their protein-synthesis activity. This increased activity is predicted to be very demanding both energetically and in terms of amino acids and other synthetic building blocks. Currently, it is unclear how Wnt augments the cell’s capacity to meet the increased energetic and synthetic needs. Here we discover that Wnt stimulates glutamine catabolism through the TCA cycle, and consequently lowers intracellular glutamine levels. The reduced glutamine concentration triggers the GCN2-mediated integrated stress response (ISR) that stimulates the expression of genes responsible for amino acid supply, tRNA aminoacylation and protein folding. Mechanistically, Wnt-induced glutamine catabolism and ISR is β-catenin independent, but requires mTORC1 activation. In a mouse model for human osteosclerosis caused by hyperactive Wnt signaling, inhibition of glutamine catabolism or GCN2 removal suppresses excessive bone formation. Thus, glutamine is both an energy source and a protein-translation rheostat responsive to Wnt. Manipulation of the glutamine-GCN2 signaling axis may provide a valuable approach for normalizing deranged protein anabolism associated with human diseases.

**Program Abstract #137**

**The ciliary localization of Gli2 is critical for cilium-dependent activation of Hedgehog signaling**

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Hedgehog signaling is crucial for vertebrate development and physiology. Gli2, the primary effector of Hedgehog signaling, localizes to the tip of the primary cilium, but the importance of its ciliary localization remains unclear. We address the roles of Gli2 ciliary localization by replacing endogenous Gli2 with Gli2ΔCLR, a Gli2 variant not localizing to the cilium. The resulting Gli2ΔCLR and Gli2ΔCLR;Gli3 double mutants resemble Gli2 null and Gli2;Gli3 double mutants, respectively, suggesting the lack of Gli2ΔCLR activation in development. Significantly, Gli2ΔCLR cannot be activated by either pharmacological activation of Smo in vitro or loss of Ptch1 in vivo. Finally, Gli2ΔCLR exhibits strong transcriptional activator activity in the absence of Sufu, suggesting that the lack of its activation in vivo results from a specific failure in relieving the inhibitory function of Sufu. Our results provide strong evidence that the ciliary localization of Gli2 is critical for cilium-dependent activation of Hedgehog signaling. This work was supported by NSF grants IOS-0949877 and IOS-1257540.
Program Abstract #138
Hedgehog signaling activates autophagy to regulate follicle stem cell lifetime.
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Stem cells maintain tissue homeostasis and are essential for repair and regeneration. Niche adhesion and self-renewal are important for long-term stem cell maintenance; but the mechanisms essential for maintaining stem cell functional lifetime are not well understood. In the Drosophila melanogaster ovary, the follicle stem cell (FSC), an adult epithelial stem cell population, proliferates actively in the presence of food. We have previously shown that during low-nutrient conditions, a transmembrane protein called Boi sequesters Hh ligand and limits FSC proliferation. Flies bearing a loss-of-function boi mutation (boi mutants) have increased Hh release and subsequent increased FSC proliferation. Despite increased FSC proliferation, boi mutants become infertile rapidly relative to the wild-type (WT) flies, suggesting that excessive Hh diminishes the functional lifetime of FSCs. This also correlates with a decrease in the pool of FSCs in hh gain-of-function (gof) mutants as compared to WT flies. To identify the mechanisms that mediate the Hh signal to control FSC lifetime, we conducted a genetic suppressor screen to isolate genes that are important in this process. Reduced expression of genes that encode components of the autophagy regulatory pathway restored the fertility of boi mutants to WT levels. Moreover, boi and the hh gof mutants have elevated levels of autophagy within FSCs. The effects of increased Hh activity on autophagy induction are separable from the Hh-mediated proliferation response in FSCs. Together, these results support a model in which the Hh signaling pathway activates two responses, autophagy and proliferation, that contribute independently to regulation of FSC lifetime. This work was supported by grant from NIH (HD065380) awarded to AOR.

Program Abstract #139
Folate Receptor Alpha (FRα) signaling in cranial neural crest stem cells
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Folic acid (FA) has been associated with prevention of neural tube defects. Although folate uptake is mediated by a proton-coupled folic acid transporter and reduced folic acid carrier, a high affinity folate receptor, FRα, is critical for embryonic development. For instance, FRα transcriptionally regulates Pax3 downstream targets, Fgfr4, and Hes1. Several genes involved in the development of neural crest (NC) are responsive to maternal FA intake. FA through interaction with FRα appears to regulate stem cell characteristics of cranial neural crest stem cells (NCSCs) as they migrate. The mechanism for this regulation is not known. We hypothesized that FRα regulates coding genes responsible for pluripotency and down-regulates non-coding miRNAs which target these coding genes in cranial NCSCs. Using Q-RT-PCR and ChIP-q-PCR we show that FRα transcriptionally regulates Oct4, Klf4, and Sox2, by binding to the cis-regulator elements on their respective promoters and recruiting H3K27Ac. Additionally, FRα down-regulates miRNAs: miR-138 which targets Oct4 and miR-let-7, which targets two members of the tripartite motif (TRIM) family, TRIM71, an E3 ubiquitin protein ligase, and TRIM6. The mechanism by which FRα down-regulates miR-138 and miR-let-7 may involve Drosha, as in this work we show that FRα binds to Drosha. Thus the results presented here on FRα signaling in NCSCs propose that (i) FRα directly regulates Oct4, Klf4 and Sox2, genes responsible for pluripotency; and (ii) FRα inhibits miR-138 and miR-let-7 biogenesis by binding to Drosha.

Program Abstract #140
Timing and range of temperature effects on neural tube development of Zoep mutants
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Neural tube defects (NTD) occur when the flat neural plate epithelium fails to fold into the neural tube, the precursor to the brain and spinal cord. In zebrafish, Squint (Sqt/Ndr1), a Nodal ligand, and One eyed-pinhead (Oep), a component of the Nodal receptor, are required for anterior neural tube closure. The penetrance of several defects, including NTD, in sqt mutants increases upon heat- or cold-shock. Although other defects in zygotic oep (Zoep) mutants are the same in all embryos, the neural tube in these mutants can either be open or closed. The variable penetrance of the open neural tube phenotype in Zoep mutants is unexpected because other aspects of their phenotype are present in all embryos. In this project, undergraduate students tested whether temperature also influences the Zoep open neural tube phenotype. Embryos
from single pairs of adults were spawned at 28.5 °C, the normal temperature for zebrafish, and one half were moved to 34 °C at different developmental time points. ANOVA indicated temperature and clutch/genetic background significantly contribute to the penetrance of the open neural tube phenotype. Heat-shock affected the embryos only at or before mid-blustula stage. This sensitive period corresponds to the time when Nodal signaling is inducing endoderm, mesoderm, and mesendoderm, suggesting that the higher temperature is affecting the formation or function of one of these germ layers. Many factors, including temperature changes in the mother, nutrition, and genetic background contribute to NTD in humans. Thus, sqt and Zoep mutants may serve as valuable models for studying the interactions between genetic environment and external environment during neurulation. This research was supported in part through NIH grant 1 R15 HD068176-01A1 to J.O.L. and University of Minnesota Undergraduate Research Opportunity Program Fellowships to P.M. and A.K.

Program Abstract #141
Lin28 Promotes the Proliferative Capacity of Neural Progenitor Cells in Brain Development
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Neural progenitor cells (NPCs) have distinct proliferation capacities at different stages of brain development. Lin28 is an RNA-binding protein with two homologs in mice: Lin28a and Lin28b. Here we show that Lin28a/b are enriched in early NPCs and their expression declines during neural differentiation. Lin28a single-knockout mice show reduced NPC proliferation, enhanced cell cycle exit, and a smaller brain, whereas mice lacking both Lin28a alleles and one Lin28b allele display similar but more severe phenotypes. Ectopic expression of Lin28a in mice results in increased NPC proliferation, NPC numbers, and brain size. Mechanistically, Lin28a physically and functionally interacts with Imp1 (Igf2bp1) and regulates Igf2-mTOR signaling. The function of Lin28a/b in NPCs could be attributed, at least in part, to the regulation of their mRNA targets that encode Igf1r and Hmga2. Thus, Lin28a and Lin28b have overlapping functions in temporally regulating NPC proliferation during early brain development.

Program Abstract #142
Biochemical and Functional Characterization of the Semaphorin6A-PlexinA2 Signaling Pathway in Zebrafish Eye Development
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During embryonic development, environmental cues help direct migrating axons towards their ultimate synaptic target by initiating intracellular signaling pathways in the growth cone of the neuron. The repulsive guidance molecule Semaphorin6A induces growth cone collapse via interaction with the PlexinA2 receptor. PlexinA2 is hypothesized to interact with the src-family tyrosine kinase Fyn to activate downstream effector molecules. Collapsin Response Mediator Proteins (CRMPs) are a family of microtubule associated proteins that are regulated by phosphorylation through Semaphorin-induced signaling events. CRMP2 is known to be regulated by Semaphorin6A-PlexinA2 signaling and has important roles in the proper positioning and lamination of cortical neurons. However, the function of CRMP2 is not yet understood in laminated, non-cortical areas of the brain, such as the retina. Furthermore, the upstream mechanisms underlying Semaphorin6A-induced CRMP2 regulation are not fully understood. The objectives of this study are to functionally characterize the role of CRMP2 in the zebrafish visual system as well as to biochemically characterize the functionally-important interactions between PlexinA2 and Fyn. We show that decreasing CRMP2 protein levels results in impaired optic tract formation and retinal lamination. These results suggest a novel role for CRMP2 in the appropriate lamination of the retina and the development of the optic tract. Furthermore, we show data testing the proposed model that Fyn phosphorylates PlexinA2 at specific sites. This project is funded by the Vermont Genetics Network and the National Science Foundation.

Program Abstract #143
Disparate Regulatory Mechanisms Control Fat3 and P75 Protein Trafficking Through a Conserved Kif5B/Kinesin1 Interacting Domain
Haixia Cheng1, Jacqueline Birkness2, Michael Deans1
Polarized protein transport is one mechanism by which cells spatially restrict protein function to establish cellular polarity, thereby regulating tissue patterning, morphogenesis and function. The cellular requirements for polarized protein transport can be quite dynamic and context dependent. Although Kinesin1 plays a critical role in the intracellular transport of diverse proteins and organelles along polarized microtubule bundles, the regulatory mechanisms underlying cargo selection are not well known. We have identified a unique Kinesin1/Kif5B interaction (KI) domain in the atypical cadherin Fat3 that mediates Fat3 distribution in MDCK cells and neurons. Fat3 is a single pass transmembrane protein containing 34 extracellular cadherin repeats that is necessary for neuronal lamination and synaptic stratification in the developing retina. In the present study we identified two splice isoforms of Fat3 mRNA during eye development that differ in KI domain function. Through GST-pulldown and co-immunoprecipitation we demonstrate that the Fat3 KI domain and a conserved tyrosine within this domain are required for binding to Kif5B by mass spectrometry analysis and western blot. This interaction mediates delivery of Fat3 to the apical surface of polarized MDCK cells and to the tips of neurites in cultured neurons. Alternative splicing of the Fat3 mRNA can prevent Fat3 binding to Kif5B and Fat3 distribution in MDCK cells by disrupting the Fat3 KI domain. Furthermore, essential residues of the KI domain are present in P75NTR, which similarly contribute to Kif5B-dependent trafficking of P75NTR in MDCK cells. Despite these similarities, distinct mechanisms regulate kif5B-mediated protein transport, with the association between Fat3 and kif5B regulated by alternative splicing while P75NTR binding to kif5B is not, suggesting that the regulation of Kif5B-mediated traffic is cargo mediated.

Program Abstract #144
ESDN/DCBLD2 serves as a scaffold for the signaling adaptor CrkL and is essential for proper development of the zebrafish eye
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Src family kinases (SFKs) and Crk family adaptors are known to collaborate in the proper development of neuronal systems in metazoans. A quantitative proteomics screen to identify substrates of SFKs whose phosphorylation promotes CrkL-SH2 binding identified the orphan receptor ESDN/DCBLD2 whose features suggest it plays roles in cell migration. Mutagenesis analysis of ESDN’s seven highly conserved intracellular tyrosines in CrkL-binding YxxP motifs found that several contribute to the binding of ESDN to the SH2 domains of both CrkL and a representative SFK Fyn. In zebrafish ESDN shows strong expression in the developing eye and ESDN morphant zebrafish showed dramatically impaired retinal lamination and optic tract formation. Together these data uncover a novel signaling pathway essential for proper development of the vertebrate eye and represent an interactive merger between biochemical and organismal studies of development. This work was funded by the Vermont Genetics Network and the National Science Foundation.

Program Abstract #145
The Drosophila Lactate Dehydrogenase gene promotes 2-hydroxyglutarate synthesis during larval development
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The metabolism of proliferating cancer cells not only generates energy, but also synthesizes the biomolecules required for growth. In response to these metabolic demands, cancer cells rely on aerobic glycolysis, a metabolic program that synthesizes biomolecules from carbohydrates. Drosophila also uses aerobic glycolysis to promote the nearly 200-fold increase in biomass that occurs during larval development, and the fly has emerged as an ideal model to study this conserved metabolic program. We are using larval development as a model to understand how the inhibition of aerobic glycolysis affects rapid growth and animal physiology. Our initial studies are focused on inhibiting key enzymes involved in aerobic glycolysis, many of which are also potential therapeutic targets. Toward this goal, we have determined that mutations in Lactate Dehydrogenase (Ldh; also known as ImpL3) significantly disrupt aerobic glycolysis and induce an L3 lethal phase. Intriguingly, our GC-MS-based metabolomic analysis of the Ldh mutants revealed an unexpected link between the production of lactate and the oncometabolite 2-hydroxyglutarate (2-HG). This compound acts as a competitive inhibitor of 2-oxoglutarate-dependent dioxygenases, suggesting that LDH-dependent 2-HG production could link glycolytic flux with genome-wide changes in histone methylation and gene expression. Intriguingly, although 2-HG synthesis occurs in cancer cells that harbor neomorphic IDH1 mutations, it has never been linked with normal
developmental growth. Our findings suggest that rather than an aberrant metabolite produced in cancer cells, 2-HG is part of a conserved metabolic program that contributes to organismal growth and aerobic glycolysis.

Program Abstract #146
Role of Ecdysone in the Migration of Border Cells in Drosophila Melanogaster Egg Chambers
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Cell migration is a very important mechanism during animal development. Understanding how cells migrate can help build information that future generations can use in the fight against birth defects and diseases like cancer. As in humans, steroid hormones in Drosophila melanogaster (fruit flies) control the timing of key developmental events, including cell movements, so it is important to investigate how these hormones signal. The goal of this project is to study the role of the steroid hormone Ecdysone in the border cells of fruit fly egg chambers. Border cells are a cluster of motile cells that must migrate during egg development to fulfill their functions. Steroid hormone signaling controls the timing of when border cells exit from the epithelium at the anterior end of the egg chamber and move to the oocyte. Work from multiple labs has identified several factors that are regulated by steroid hormone signaling, such as the protein Abrupt, and cell adhesion regulators. We have identified many other potential downstream targets of ecdysone signaling through expression analysis. Through genetic experiments, we are determining which of these targets most significantly contribute to cell migration. Mutations that cause an abnormal phenotype are being further characterized. These results will inform us about the important signaling effectors downstream of ecdysone steroid hormone in cell migration.

Program Abstract #147
Functional analysis of B56 δ/γ within the canonical Wnt signaling pathway in Xenopus laevis.
Celso Catumbela
Sam Houston State University, United States
The canonical Wnt pathway is crucial to embryogenesis and tumorigenesis. Components of this pathway are deregulated in several carcinomas, as well as Alzheimer’s and type II diabetes. Protein phosphatase 2A (PP2A), consisting of a structural A, catalytic C, and regulatory B subunits, both positively and negatively regulates Wnt signaling. The largest B subunit family, B56, is regulated by five paralogous genes (α, β, γ, δ, ε) that inhibit Wnt tumorigenesis, with the exception of B56ε, an activator. B56 subunits differ in their N- and C-termini, while displaying a high degree of conservation within the core domain. The novel conserved mixed-isoform B56δ/γ, an alternatively spliced variant of B56γ, contains a B56δ-like N-terminal and a B56γ core. Specificity and subcellular localization of the PP2A holoenzyme is largely determined by the B56 N- and C-termini. We expect B56δ/γ to confer to the holoenzyme Wnt-inhibitory properties, similarly to B56δ and B56γ. We found that B56δ/γ rescued both Xenopus wnt-8-induced, and mutant β-catenin-induced (non-degradable β-catenin due to lack of CKI and GSK3β phosphorylation sites), secondary axes. The former finding shows that B56δ/γ inhibits exogenous Wnt signaling, whereas the latter finding suggests that B56δ/γ acts downstream of β-catenin within the Wnt signaling pathway. The latter finding indicates that relative to other B56 isoforms, B56δ/γ is currently the furthest downstream regulator of Wnt signaling, making it a valuable candidate for biomedical studies. B56δ/γ also inhibits formation of a primary body axis, therefore, we hypothesize that B56δ/γ acts as a ventralizing factor. Our findings lead us to hypothesize that B56δ/γ dephosphorylates key components of the canonical Wnt pathway whose phosphorylation lead to Wnt signaling. Future analyses will aid in determining whether B56δ/γ is specific to the Wnt pathway, elucidate on its interaction with Lef/Tcf, and its potential as a biomedical treatment candidate.

Program Abstract #148
Mitochondrial dynamic is affected by UVB radiation during organogenesis of Macrobrachium olfersi embryos
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High levels of ultraviolet-B (UVB) radiation can induce DNA damage and also impairment in proteins involved with mitochondrial dynamic. Then, we investigate whether UVB radiation affects embryos of Macrobrachium olfersi, a prawn that lives in clear shallow water, whose yolky eggs are exposed to environmental radiation during development. Ovigerous females with embryos in early post-nauplius stage (E5- E6) were irradiated using a UVB lamp 6W (310 mW/cm²) for 30 min, and kept in darkness until 48 h after irradiation. Non-irradiated embryos were used as controls. DNA damage, specifically formation of pyrimidine dimmers (CPD) was observed after 1 h of irradiation (4.83% ± 0.21) and 48 h (4.13% ± 0.68) in comparison to controls (1.78% ± 0.16). After 48 h to UVB exposure, embryos showed no significant upregulation of p53 (5.66% ± 0.68), accompanied by an upregulation of Bak (3.44% ± 0.68), a mitochondrial
pro-apoptotic protein. However, no changes were observed in Bcl2, a mitochondrial anti-apoptotic protein. On the other hand, mitochondrial dynamics were altered by UVB radiation. We observed a significant decrease of Mfn-1, involved in mitochondrial fusion and a significant increase of Drp-1, in mitochondrial fission. Our results showed that UVB radiation causes DNA damage and negatively impacts the permeability and function of mitochondria in embryos of *M. olfersi*.

**Program Abstract #149**

**Bis-GMA, a Dental Composite, Affects Embryonic Development and Survival in Zebrafish (*Danio rerio*)**

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Bisphenol-A (BPA) is an endocrine disrupting compound found in plastic and epoxy products and used to make a variety of common items including cell phones, food containers, adhesive labels, and dental sealants. Bisphenol-A Glycidyl Methacrylate (Bis-GMA), a major component in dental sealants, is synthesized from BPA. Zebrafish Tg(sox10:gfp) embryos were exposed to 10⁻⁶, 10⁻⁷, or 10⁻⁸ M Bis-GMA starting at 12 hours post fertilization (hpf). Solutions were refreshed every 24 hours after initial treatment. With increasing concentrations of Bis-GMA, we observed a higher mortality rate, increased edema, jaw malformation, decreased eye size, and curvature of the spine. Some of these phenotypes are mimicked by estrogen treatment. Effects were concentration dependent with lower concentrations resulting in more subtle defects.

**Program Abstract #150**

**Developmental Assay for Cadmium Toxicity: Utilizing Zebrafish as a Model for Fish and Humans**

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Chronic exposure to cadmium, long recognized as an environmental hazard, is increasingly cited as a threat to public health. Current ways to assess cadmium exposure in humans (urinary Cd or markers of kidney tubule damage) fail to detect low levels of exposure that lead to cancer, bone damage and cardiovascular disease. Climate change and chemically-contaminated water increasingly impact coastal aquatic ecosystems and the effects of small temperature change fluctuations on developmental metal sensitivity are not well understood. This project utilized a cadmium toxicity bioassay in zebrafish embryo-larvae to 1) test the validity, relevance and applicability of biomarkers of Cadmium exposure and 2) to examine the combined effect of low-level cadmium exposure with otherwise-tolerable temperature shifts. General morphology and developmental milestones were assessed as were specifics of cardiovascular development and gene expression changes. Results suggest this assay is effective at signaling low levels of cadmium exposure and that very low levels of metal contamination of coastal waters pose a significant future environmental threat.

**Program Abstract #151**

**Mechanisms Regulating Embryonic Gonad Morphogenesis**

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In the embryo, the gonad is composed of two well-characterized cell types, the primordial germ cells (PGCs) and the somatic gonadal precursor cells (SGPs). These two cell types are specified at distal locations, and they must migrate, converge, and interact to form a functional gonad. This work focuses on the characterization of the molecular mechanisms that regulate the migration of the SGPs, their interactions with each other, and their interactions with the PGCs during *Drosophila* gonadogenesis. A genetic screen resulted in the identification of a number of genes linked to the cytoskeleton genetically or molecularly, including two transcription factors, *ribbon* (*rib*) and *longitudinals lacking* (*lola*), which encode members of the BTB (Broad complex, Tramtrack, Bric-à-Brac) family of proteins, as well as RhoGEF2, an activator of the Rho family GTPases. These genes are required for the coalescence and compaction of SGPs and PGCs into a cohesive, spherical gonad. Given that BTB proteins form homo- and heteromeric-complexes and frequently contain DNA binding domains by which they regulate target gene transcription, we hypothesize that Rib and Lola interact to control gene expression to promote gonad morphogenesis. Both genes are expressed in the embryonic gonad, and genetic studies reveal that these genes may function in a common pathway to promote gonad morphogenesis. We are currently testing if these proteins physically interact to regulate a common set of target genes. Previous studies have identified a number of cytoskeletal regulators and adhesion proteins that may function downstream of Lola. Therefore, we are evaluating these genes as potential Rib and Lola targets in the gonad. The findings in this study will provide insight into how defects in these proteins can lead to a failure of normal developmental programming.
Directed migration and actomyosin contractility drive germline stem cell niche formation
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Adult stem cells are maintained by a local microenvironment termed the niche. Cells of the niche direct self-renewal of resident stem cells and are therefore crucial for both normal homeostasis and tissue regeneration. In most mammalian tissues, niche cells cannot be unambiguously identified and are thus difficult to observe in vivo. Fortunately, the Drosophila germline stem cell (GSC) niche is well-defined, allowing unambiguous identification of both niche cells and resident stem cells. The testis niche is composed of a few cells that are selectively activated for Notch among a larger pool of somatic gonadal precursors. Upon specification, these so-called hub cells assemble at a defined position within the gonad, become enriched for homotypic adhesions and secrete factors for self-renewal and attachment that serve to organize a rosette of stem cells around the hub. We are investigating the mechanisms of niche assembly by genetic analysis and live imaging of embryonic gonads. We identified a branched pathway for hub formation downstream of Notch. Along one arm of the pathway, the large Maf factor Traffic jam is downregulated to allow for hub adhesion and signaling. In a parallel arm, Bowl is activated for hub assembly. Our data indicate preferential adhesion is not sufficient for hub assembly. In fact, our in vivo imaging of gonadogenesis revealed that hub cells migrate individually along a basement membrane. Furthermore, we have identified two tissues as candidates for directing this migration as each tissue adjoins the gonad precisely where the hub assembles. We are currently testing their role in niche formation. Finally, imaging hub compaction revealed that Myosin II becomes enriched along hub cell membranes while f-Actin becomes polarized to the GSC-hub cell interface. Inhibition of Rho Kinase, which activates Myosin II, disrupted hub cell compaction and germ cell recruitment, demonstrating that actomyosin contractility is required for niche formation.

Osterix is a Novel Regulator of Male Reproductive Tract Differentiation in Mouse
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Early in mammalian development the reproductive tract of both sexes consists of the Wolffian duct and the Müllerian duct (MD), two epithelial tube pairs surrounded by mesenchyme. During male development, mesenchyme-epithelia interactions mediate MD regression to prevent its development into a uterus, oviduct and upper vagina. Previous studies in humans and mice have demonstrated that 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. At E14.5 in mouse, the MD of both males and females are structurally equivalent. However in males, AMH has been produced for two days and AMH-signaling is well underway. This made it possible to isolate RNA from the same tissue type at the same developmental time point in males (where AMH-signaling is on) and in females (where AMH-signaling is off). RNA-Seq analysis of YFP positive MD mesenchymal cells sorted from E14.5 Amhr2Cre/+;R26Ryfp/+ reproductive tracts was used to globally identify transcriptome differences in the MD mesenchyme in males versus females during MD regression. This analysis identified Osterix (Osx), a C2H2-type zinc finger transcription factor required for osteoblast differentiation, as a possible downstream effector of AMH-signaling during MD regression. Osx expression was found in a male-specific pattern in the MD mesenchyme. Additionally, transgenic mice ectopically expressing AMH in females resulted in a male pattern of Osx expression. Further, MD regression was delayed in functional null Osx lacZ/lacZ males. Together this suggests Osx is downstream of AMH-signaling and contributes to MD regression. The BMP signaling effectors, Msx2, Dlx5 and Sp6 were also identified as potential mediators of AMH-signaling during regression. We are currently examining the in vivo role of these candidate factors during MD regression using available mouse models.

Epithelial Development of the Mouse and Human Urethra
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Hypospadias is a congenital defect characterized by incomplete closure of the urethral tube and affects ~1 in 250 births. To better understand how hypospadias develops and to establish better treatment options, a basic knowledge of urethral epithelial development is needed. The bladder and the urethra develop from a single structure called the urogenital sinus.
Once the structures are differentiated, the bladder urothelium develops a stratified epithelium with distinct cell layers: basal, intermediate and superficial. Although the lining of the lower urethra has been described as having an urothelial character, the distribution of cell type-specific molecular markers of bladder urothelium have not been mapped in the human lower urethra. The goal of this study is to map the distribution of epithelial markers from the bladder neck to the glandular urethra in mouse and human embryos and to identify embryonic origins of those cell types. To test the hypothesis that the human urethra is derived from both endodermal and ectodermal origins, the classic textbook description of urethral cell origins, we examined markers for derivatives of each germ layer in the developing urethral epithelium. We found that Foxa2, an endodermal marker, is expressed throughout the developing human urethral epithelium all the way to the distal tip of the glans, suggesting the human urethra is derived entirely from endoderm (similar to that of the mouse). To test whether the urethral tube is lined with urothelial cells, we looked at the distribution of known bladder urothelial markers that distinguish distinct cell layers: Islet1, P63, Keratin 5 and Uroplakin III. We found that like the bladder these markers are found throughout urethra development in both the mouse and human; however, their spatial and temporal distributions differ significantly. Our data suggest that once the urogenital sinus differentiates, epithelium of the urethra develops independently from bladder urothelium. Funded by NIDDK.

Program Abstract #155

The role of Sox4 in urogenital development
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Defects in nephrogenesis and ureteric branching result in Congenital Anomalies of the Kidney and Urinary Tract (CAKUT). CAKUT constitutes one of the most frequent birth defects in humans, and the major cause of childhood renal failure. Nephron deficiency is a hallmark feature of CAKUT. Low nephron endowment - although asymptomatic early in life - is associated with adult-onset hypertension, a leading cause of coronary heart disease, stroke, and renal failure in North America. We previously identified the Sry-related HMG box (Sox)-C subfamily (composed of Sox4, Sox11 and Sox12) of transcription factors as gene targets bound by the well-known master regulator of kidney development WT1. We have also previously shown that conditional ablation of the transcription factor Sox4 in nephron progenitor cells and their derivatives results in reduced glomerular number and early-onset glomerular injury, which progresses to end-stage renal failure in mice. Examination of renal development in Sox4 conditional-knockout embryos demonstrated a major reduction in nephrogenesis both ex vivo and in vivo. Current experiments are underway to investigate the primary cause of reduced nephron endowment in Sox4-deficient kidneys. Preliminary data indicate that increased apoptosis, rather than decreased proliferation or a change in cell fate of nephron progenitor cells is at fault. Our preliminary data also demonstrate abnormal testicular development in Sox4 knock-out mice and experiments are in progress to further substantiate these findings. These data indicate that, like another well studied member of the Sox family of transcription factors, Sox9, Sox4 may also play a critical dual role in male sex determination as well as normal kidney development and function in vivo. Funding for this project provided by the Kidney Foundation of Canada, and the Kidney Research Education and National Training Program (KRESCENT).

Program Abstract #156

GUDMAP – GenitoUrinary Development Molecular Project, an open resource.
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The GenitoUrinary Development Molecular Anatomy Project (GUDMAP) is an open access online resource developed by an international consortium of laboratories working to provide the scientific and medical community with gene expression data, transgenic mice and tools to facilitate research and teaching in genitourinary (GU) development. The goal is to identify novel cell types and expression domains in the kidney, ureter, lower urinary tract and reproductive tract. The GUDMAP database includes data from large-scale in-situ hybridisation screens, 3D Optical Projection Tomography (OPT) data, microarray gene expression data and sequencing data of the developing mouse GU system. Expression data are annotated using a high-resolution ontology specific to the developing murine GU system. More specifically, gene expression is annotated by describing both the presence and strength of expression in different sub-compartments. The database is searchable based on gene name, function or by anatomical structure. An advanced Boolean query allows users to further refine their search according to the stage, organ, or expression pattern. Initially, GUDMAP focused on the murine GU system. More recently, GUDMAP has extended its focus to two new projects: Nociceptive GUDMAP (nGUDMAP) and Human GUDMAP (hGUDMAP). nGUDMAP focuses on nociceptors and associated cell types in pain processing of the murine urinary tract and pelvic region. hGUDMAP extends the gene expression data to include Human
Program Abstract #157

Concurrent BMP7 and FGF9 signaling governs AP-1 function to promote nephron progenitor self-renewal
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Defining growth factor circuits controlling self-renewal and differentiation of nephron progenitor cells (NPCs) is essential for our understanding of renal organogenesis and for developing ex vivo renal tissue from stem cells. Three major growth factor pathways are essential for NPC self-renewal during normal kidney development: BMP7, WNT9b and FGFs 9/20. Previous work has reported cross-talk between these growth factors in synergistic maintenance of NPCs in organ culture in vitro. However, the molecular mechanism(s) by which these signaling factors synergistically control NPC self-renewal is largely unknown. In this study, we demonstrate that BMP7 activates the TAK1-JNK-JUN signaling cascade to promote NPC proliferation. More specifically, we show that BMP7 signaling directly governs G1-S phase cell cycle transition through transcriptional activation of key G1 phase regulators such as Ccnd1 and Myc. We find that while BMP7 activates JUN, its partner FOS is regulated by FGF9 in NPCs. Concurrent BMP7 and FGF9 signaling coordinately regulate AP-1 transcriptional activity to promote G1-S cell cycle progression and NPC proliferation. Together, our findings uncover a molecular mechanism explaining the synergistic effect of BMP7 and FGF9 in NPC self-renewal. Ongoing experiments are aimed at elucidating the cross-talk mechanism by which BMP7 and FGF9 govern AP-1 function in NPCs.

Program Abstract #158

Specification of nephron and stromal progenitor populations during kidney development
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Multipotent self-renewing progenitor cells ensure formation functional organs containing various cell types. A human kidney contains about a million cells of various cell types. A mouse kidney contains approximately 13,000 cells of different cell types for specialized physiological functions. Currently, it is not well-studied how multipotent self-renewing progenitor cells are regulated during kidney development. By using genetic fate mapping in the mouse, we have previously discovered that Six2+ cap mesenchyme and Foxd1+ cortical stroma are multi-potent, self-renewing progenitor populations during kidney development. However, at the very onset of kidney development, small fractions of Six2+ cells differentiate into FOXD1+ cortical stromal cells, while a small fraction of Foxd1+ cells give rise to SIX2+ cap mesenchyme cells. Subsequently, contributions of Six2+ and Foxd1+ cells become restricted to the nephron epithelia and renal stroma, respectively. We further found that cap mesenchyme cells lacking Pax2 activity are ectopically converted into Foxd1+ cortical stromal cells through transient SIX2+ FOXD1+ double positive cells, and subsequently, can contribute to terminally differentiated PDGFRB+ interstitial cells of the kidney. Taken together, our observations suggest that cellular specification of nephron and stromal progenitor cells has not been strictly determined at the very onset of kidney development. Our data also suggest that, once the specification for nephron and stroma progenitor cells is established, Pax2 activity maintains the lineage boundary between the nephron and stroma compartments by repressing stromal progenitor cell fates in nephron progenitor cells. Funding: NIH-NIDDK, March of Dimes, Harvard Stem Cell Institute, American Society of Nephrology, National Kidney Foundation

Program Abstract #159

Cell motility in the cap mesenchyme drives niche swapping, cell dispersal, and maintains nephron progenitor position at the tips of the ureteric tree
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The collecting duct system of the developing kidney grows through a dynamic program of branching morphogenesis driven by reciprocal interactions between the epithelial tips of the ureteric tree and surrounding ‘cap’ domains of mesenchymal nephron progenitors. The dynamics of ureteric tree branching are broadly appreciated. However, questions concerning how the cap mesenchyme is maintained at the tips of the tree during branching, and whether differentiation and interaction with stromal and tip-produced signals involves cell movement are unclear. Using live imaging of fluorescent reporters that label the cap mesenchyme and underlying tip compartment, we observed extensive cell motility in the cap mesenchyme. Cap cells migrate within their niche, and can cross between adjacent niches. Cap cells in the top

studies in bladder, urethra and kidney. GUDMAP data are curated and freely accessible at www.gudmap.org. Funded by the National Institutes of Health (NIH) via DK092983 (JAD) and DK094523 (RAB).
of the niche migrate more freely than those that are adjacent to the terminal regions of the tips. Cells in the latter region have an elongated morphology and appear to be physically attached to the tip epithelium. Across an 18 hour time window the majority of cap cells maintain their position relative to the underlying ureteric tip as it grows and branches. Occasionally, individual cells are seen to actively migrate from what is thought to be the uninduced region of the cap to the site of nephron formation. These data support two modes of migratory behaviour for nephron progenitors: collective cell migration that is likely coordinated by tip-produced factors to maintain cap position during branching; and individual motility that can drive niche swapping, dispersal of progenitors through the cap, and has implications for the way we consider cellular interactions and differentiation within the nephrogenic niche.

Program Abstract #160
Role of Etv4 and Etv5 in kidney development: from branching morphogenesis to nephron differentiation
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Etv4 and Etv5 are two transcription factors expressed in the developing mouse kidney, specifically in the ureteric bud (UB) tips, the metanephric mesenchyme (MM) and the renal vesicle. In the UB, the expression of these two transcription factors is downstream of the signaling pathway mediated by GDNF, its receptor tyrosine kinase Ret and the co-receptor Gfra1. We have shown that Etv4 and Etv5 act redundantly during kidney development; in mouse models that lack both transcription factors, the UB fails to branch resulting in kidney agenesis. Our studies using Mosaic Analysis with Double Markers (MADM) further demonstrate that Etv4 and Etv5 regulate UB branching by promoting directed cell movements that allow UB tip cells to remain at or migrate towards the tip. Using a mouse model of Etv4 and Etv5 deletion in the MM and its progeny we also demonstrate a critical role for these transcription factors during nephrogenesis; absence of Etv4 and Etv5 cause premature interruptions in the nephrogenic zone and a severe cystic phenotype at birth. Because Etv4 and Etv5 are not expressed in the mature nephron epithelia, the cystic phenotype of these mutant mice is likely a consequence of a defect that arises during early nephrogenesis. Altogether, we propose a global role for Etv4 and Etv5 in kidney development mediated, at least in part, by modulating cell migration.

Program Abstract #161
An integrated cell, tissue and whole organ profile of kidney morphogenesis
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While cell, tissue and even organism level analyses of morphogenesis are feasible in invertebrates, the size, opacity and complexity of mammalian organs has impeded systematic analyses of developmental processes critical to organ function. Here, we integrate optical projection tomography, single-cell resolution confocal and quantitative image analysis to comprehensively document mouse kidney organogenesis across time. This reveals a previously unappreciated structurally stereotypic organ architecture undergoing a temporally non-uniform process of development with respect to rates of cellular proliferation, dominant morphogenetic processes and spatial relationships between key cellular compartments. The existence of such distinct phases predicts temporal sensitivity to genetic / environmental insults, potentially enhancing our understanding of the mechanism of developmental anomalies. This approach facilitates quantitative analysis of even subtle perturbations to kidney development and is also applicable to other organ systems.

Program Abstract #162
Planar Cell Polarity Signaling in Kidney Development and Ciliogenesis
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Cystic diseases of the kidney result from ciliopathies and prevent normal kidney function. Kidney tubules consist of a ciliated epithelium, and disruption of cilia polarity leads to aberrant nephrogenesis and cyst development. Recent work suggests that planar cell polarity (PCP) genes influence the development of cilia, but the mechanism behind this involvement is unknown. Daam1 and WGEF, two components of the PCP pathway, are expressed during nephrogenesis. Our previous work established that knockdown of Daam1 and WGEF or inhibition of Daam1 or Rho lead to decreased kidney tubulogenesis in *Xenopus laevis* (frog) embryos. Dishevelled, a component upstream of Daam1 and WGEF in the PCP pathway, is known to influence ciliogenesis. We hypothesize that inhibition of Daam1/WGEF modulates kidney tubule formation through formation of cilia. Our preliminary data suggest that knockdown of Daam1 leads to reduction of cilia in *X. laevis* skin and kidneys, as well as in polarized mammalian kidney cells, supporting a working hypothesis that a reduction in kidney cilia may contribute to nephrogenesis defects. We are beginning to investigate the mechanisms by which Daam1 is involved in ciliogenesis, which may lead to its functional role in nephrogenesis. A yeast 2-hybrid screen indicates that Daam1 interacts with Tuba, a component of the exocyst vesicle trafficking complex. Our preliminary work suggest that overexpression of Tuba in *X. laevis* embryos leads to kidney defects, including enlargement of the kidney tubules, as well as a decrease in the number of cilia on the skin, suggesting that PCP signaling regulates ciliogenesis and nephrogenesis through interaction with exocyst trafficking components. Our recent data also suggest that knockdown of Tuba in the kidney results in reduced tubulogenesis. Together, these results indicate that PCP signaling is necessary for tubulogenesis within the developing kidney, in part due to effects upon ciliogenesis.

**Program Abstract #163**

**Foxp4 is critical for acinar cell homeostasis in mouse pancreas**

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Pancreas is critical for the nutrients digestion and glucose homeostasis via its exocrine and endocrine functions respectively and thus it is a vital organ for investigation. We recently found that Foxp4, a newly identified Foxp superfamily member, expressed in both embryonic and adult mouse pancreas and it is important in exocrine acinar cell physiology. In embryonic stage (E)10.5 mouse pancreas, Foxp4 co-express with Pdx1+ cells, the progenitor cells of developing pancreas. It then expresses in the tip cells (future acinar cells) and most of the trunk cells (the future endocrine and ductal cells) in later pancreas development. In postnatal stages, Foxp4 is detected in both acinar cells and most endocrine cells except ductal cells. The persisting Foxp4 expression in embryonic, neonatal and mature acinar cells prompts us to hypothesize that Foxp4 may have important roles in the differentiation, functioning, maintenance and/or homeostasis of the acinar cells. Thus we adopted genetic approach to inactivate Foxp4 in mouse pancreas to elucidate the function(s) of Foxp4 in pancreas. Inactivation of Foxp4 in mice resulted in acinar cell abnormality including dispersed zymogen granule, high level of calreticulin located to cell membrane and abundant appearance of autophagosome-like vesicles. Thus it indicated that the mutant acinar cells were under cellular stress. Interestingly, the level of Mist1 protein, a critical factor in acinar cell maturation and maintenance, was much reduced in Foxp4 mutant pancreas. In summary, our data suggests that Foxp4 has a novel and critical role in acinar cell homeostasis in exocrine pancreas. This research was supported by grants from Research Grant Council of the Hong Kong Special Administrative Region, China (Project No. CUHK 466412)

**Program Abstract #164**

**The anterior and posterior liver bud contributes to different portions of the embryonic liver.**

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Although it is clear that the murine liver bud is produced from two discreet endodermal progenitors, it has been assumed that the liver bud is a homogenous tissue population. We now show that the liver bud is comprised of an anterior and posterior population that can be distinguished using molecular markers, histology or by the surrounding mesenchyme. *Ex vivo* fate mapping demonstrates that the anterior liver bud produces the caudal liver lobes while the posterior liver bud produces the single prominent rostral lobe. Endoderm-specific loss of Ying-Yang1 (YY1) highlights the distinct genetic requirements and lobe contributions of these two liver bud populations. In the absence of YY1, Vascular Endothelial Growth Factor and Hepatocyte Nuclear Factor 4-α are reduced in the posterior liver bud resulting in rostral liver lobe hypoplasia E10.0. Conversely, the anterior liver bud and the E10.0 caudal lobes are unaffected by YY1 loss. Together
these results demonstrate that the liver bud contains two populations of hepatoblast with unique molecular requirements that each contributes to different regions of the embryonic liver. This work is funded by R01DK87753 to KT.

Program Abstract #165
Canonical Wnt signaling regulates the pituitary organizer and pituitary gland formation
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The pituitary gland is often called the master gland because it plays a central role within the endocrine system, regulating vertebrate physiology through the secretion of hormones. The mouse pituitary gland originates from an interaction between the neural ectoderm and the oral ectoderm, where signals emanating from the ventral diencephalon cause the adjacent oral ectoderm to thicken and invaginate, forming Rathke’s pouch, the precursor of the pituitary anterior and intermediate lobes. The pituitary posterior lobe forms from an evagination of the ventral diencephalon, which forms an infundibulum. Within the infundibular region of the ventral diencephalon the morphogenetic proteins BMP4, FGF8, and FGF10 comprise a pituitary organizer that is necessary for the induction of Rathke’s pouch. Previous research demonstrated that the WNT signaling pathway regulates the pituitary organizer such that loss of either Wnt5a or Tcf7l2 leads to an expansion of the pituitary organizer and an enlargement of Rathke’s pouch. WNT5A is typically classified as a non-canonical WNT; however, TCF7L2 can partner with β-CATENIN to mediate the transcriptional responses of canonical WNT signaling. We sought to determine if canonical WNT signaling regulates the pituitary organizer by deleting β-catenin within the ventral diencephalon. Surprisingly, we found that loss of β-catenin in the ventral diencephalon leads to a loss of Rathke’s pouch. This result suggests that canonical WNT signaling is necessary for promoting pituitary organizer function. To test this hypothesis we stimulated canonical WNT signaling in the ventral diencephalon using an inducible gain-of-function allele of β-catenin and found that stimulating canonical Wnt signaling causes an increase in pituitary organizer function and a hypermorphic pituitary. We believe that a balance of both canonical and non-canonical WNT signaling is necessary to ensure proper regulation of pituitary organizer activity.

Program Abstract #166
Foxn1 levels in thymus organogenesis and maintenance - too much is not enough
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The transcription factor Foxn1 is essential for fetal thymus organogenesis, including thymic epithelial cell (TEC) differentiation and proliferation, and in its absence the thymic rudiment is poorly differentiated and extremely hypoplastic. In the postnatal thymus, Foxn1 levels vary widely between different TEC subsets, and its expression is required for maintenance of the postnatal microenvironment, as premature down-regulation of Foxn1 causes a rapid involution-like phenotype. Transgenic overexpression of Foxn1 in TECs has also been shown to cause thymic hyperplasia and delayed postnatal involution. In the current study, we investigated the effects of Foxn1 over expression using a K5.Foxn1 transgene. To our surprise, although this transgene drives Foxn1 over-expression in TECs, it does not cause thymic hyperplasia, nor does it delay or reduce the reduction in thymus size associated with aging-related involution. However, it does maintain TEC differentiation longer than in control mice. Similarly, this transgene cannot rescue thymus size in mice which undergo premature involution due to reduced Foxn1 levels, but TEC differentiation is significantly improved including expression of beta5t, AIRE, and UEA-1, and improved corticomedullary organization. Expression of this transgene in Foxn1 null "nude" mice also improves thymus size and differentiation, although the thymus is smaller and there is a bias towards medullary differentiation. These results demonstrate that the functions of Foxn1 in promoting TEC proliferation and differentiation are separable, and suggest that differential modulation of Foxn1 levels in different TEC subsets at different stages is critical for normal organogenesis and maintenance of the thymus.

Program Abstract #167
Bmp2 regulates epithelial-mesenchymal interactions at the initiation stage of tooth mineralization
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Tooth organogenesis relies on reciprocal epithelial mesenchymal interactions. Various conserved signaling pathways orchestrate these interactions, one of which is BMP signaling. The importance of Bmp signaling for early stages of tooth development is well established. In comparison little is known about its involvement during later mineralization stages. Expression of Bmp2 shifts from epithelial and mesenchymal cells just prior to onset of mineralization. In this study we aim to understand how Bmp2 is involved in progression of tooth formation. We used a conditional KO approach to delete
Bmp2 in mesenchyme-derived cranial neural crest cells (NCC) including dental mesenchyme. Though this deletion was perinatal lethal no changes were observed during tooth development. At birth (P0), when tooth mineralization was at its beginning, the mesenchyme derived dentin producing odontoblast layer appeared disorganized. Markers associated with odontoblast differentiation, such as nestin and dentin sialoprotein (Dsp) were reduced. To assess the consequences of Bmp2-deletion in fully developed teeth, E14.5 Bmp2-deficient or control tooth germs were implanted under kidney capsule and the resulting teeth were analyzed 4-6 weeks later. Loss of Bmp2 led to dentin dysplasia and associated pulp obliteration (mineralization). Expression of Nestin and Dsp was altered and ectopic Bmp signaling, as assessed by pSmad1/5/8 staining, was observed. In order to understand the molecular basis of these changes during development/initiation of mineralization we performed RNA seq analysis at P0 – the initial stages of cytodifferentiation when molecular changes were first observed. We found expression changes both in epithelium-derived genes, alongside changes in mesenchyme-derived genes involved in bone and cartilage development. This indicates that Bmp2 regulates epithelial-mesenchymal interactions at the initiation stage of tooth mineralization.

Program Abstract #168

**Atrazine Affects Craniofacial Chondrogenesis in Zebrafish (Danio rerio)**

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Atrazine is a commonly used herbicide that has been implicated as an endocrine disrupting compound. Atrazine has been shown to have effects on developing organisms similar to the steroid hormone estrogen, but its role in cartilage formation has yet to be established. Craniofacial cartilage and bone deformities account for a large number of birth defects in the United States. Previous studies have shown estrogen affects the development of cartilage formation in the craniofacial region of zebrafish (Danio rerio). In order to determine if atrazine has similar effects, zebrafish embryos were treated with differing concentrations of atrazine. Zebrafish treated with 1 µM atrazine had gross morphological defects at 5 days post fertilization, while embryos treated below this threshold had cartilage elements with increasingly wide angles, resulting in a shorter, fatter face with increasing atrazine concentration. Further investigation into the effects of atrazine on heart and skull suture formation could lead to a better understanding of the overall effects of this drug.

Program Abstract #169

**An Fgf-Shh signaling hierarchy regulates early specification of the zebrafish skull**

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The neurocranium generates most of the craniofacial skeleton and consists of prechordal and postchordal regions. Although development of the prechordal region is well studied, we know virtually nothing regarding postchordal neurocranial development. We have characterized a signaling hierarchy necessary for postchordal neurocranial development. The postchordal neurocranium requires Fibroblast growth factor (Fgf) signaling for early specification of mesodermally-derived progenitor cells. The expression of hyaluron synthetase 2 (has2) in the cephalic mesoderm requires Fgf signaling and Has2 function, in turn, drives postchordal neurocranial development. While Hedgehog (Hh)-deficient embryos also lack a postchordal neurocranium, this is due to a later defect in chondrocyte differentiation. Inhibitor studies demonstrate that postchordal neurocranial development requires early Fgf and later Hh signaling. Collectively, our results provide the first mechanistic understanding of early postchordal neurocranial development and demonstrate a hierarchy of signaling between Fgf and Hh in the development of this structure.

Program Abstract #170

**Adrenergic signaling modulates bone growth and chondrocyte function in vivo**

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Regulation of organ and body size is one of the least understood processes in developmental biology. Vertebrate growth occurs in the growth plates (GP) located at both ends of the long bones. Chondrocytes within the GP undergo successive stages of differentiation from the ends towards the bone center: resting, proliferative, and hypertrophic chondrocytes (HTC). Regulation of HTC number and size determines bone length, as HTCs provide the scaffold that will be replaced by bone. While most growth regulation occurs intrinsically within the GP, animals also adapt their growth rate in response to external cues, such as varying nutrient levels and disease outbreaks, but the mechanisms by which they do so are mostly unknown. In addition to their role in the regulation of bone mass, it has been shown that the catecholamines epinephrine and norepinephrine (produced by the adrenal gland and sympathetic neurons) affect chondrocyte
Differentiation in vitro, signaling through adrenergic receptors (AdRs). Since G-protein-coupled receptors such as AdRs can crosstalk with growth-related signaling pathways, we hypothesized that adrenergic signaling can modulate long bone growth in vivo. Immunodetection of AdRs in murine GPs revealed notable changes in expression during development, especially for alpha1B-AdR in HTCs. To test their role, we blocked alpha-adrenergic signaling pharmacologically during the first two weeks of postnatal mouse growth, and found that bone growth was reduced at endpoint, associated with decreased size of the hypertrophic region of the GP, and decreased expression of the chondrocyte differentiation markers Ihh and Col2a1. We next tested by chemical denervation whether the in vivo source of the ligand is the sympathetic nerve fibers, but found that their removal did not recapitulate the results from the pharmacological experiments. Our preliminary conclusion is that adrenal gland-derived catecholamines play a so-far overlooked role in bone growth modulation.

**Program Abstract #171**

The ciliary basal body protein Wdpcp mediates hedgehog pathway responsiveness necessary for normal skeletogenesis

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The Cys40 mouse bearing a loss of function mutation in Wdpcp and displaying severe skeletal defects was recovered from a mutagenesis screen for cardiac defects. Wdpcp has been shown to localize to the basal body of primary cilia and play a role in ciliogenesis1. Initial characterization of the Cys40 mutant described polydactyly and associated hedgehog pathway defects2. Alizarin red/alcian blue staining of mice with limb bud mesenchyme specific deletion of Wdpcp (Prx1-Cre;WdpcpCys40/c) showed this mouse recapitulates the appendicular skeletal phenotype of the Cys40 mutant. Consistent with observed polydactyly, we observed delayed onset of chondrogenesis as assayed by decreased expression of chondrogenic marker genes Col2a1 and Col9a1 in E11.5 Cys40 forelimb buds. Diminished chondrogenesis coincided with increased Gli3 activator to repressor ratio and increased expression of Gli3 regulated BMP inhibitor Grem1 in E10.5 Cys40 forelimb buds. Accordingly, we observed decreased activation of the BMP signaling pathway as assayed by decreased expression of BMP pathway target gene Msx2 and decreased phospho-Smad1/5/9 in E10.5 Cys40 forelimb buds. Alcian blue staining of in vitro limb bud micromass cultures demonstrated decreased Cys40 chondrogenesis could be rescued with Gli inhibitor GANT61, but not Smo inhibitor cyclopamine consistent with disruption of hedgehog pathway at primary cilia. Von kossa staining of E14.5 humerus of mice with chondrocyte specific deletion of Wdpcp (Col2a1-CreERT;WdpcpCys40/c injected E11.5) revealed decreased trabecular bone. Cys40 mouse embryonic fibroblasts had decreased osteogenic differentiation in response to Smo agonist as assayed by alizarin red staining and expression of osteogenic marker genes Osx, Col1a1, and Ocn. Our results suggest a role for Wdpcp and the primary cilium in chondrogenesis in the limb bud and osteogenesis in chondrocytes.


**Program Abstract #172**

Goosecoid regulates a Spemann organizer-like function for neurogenesis in the inner ear.

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Neurons of the Statoacoustic Ganglion (SAG), which innervate the inner ear, originate as neuroblasts in the floor of the otic vesicle and subsequently delaminate and migrate towards the hindbrain before completing differentiation. In all vertebrates, locally expressed Fgf initiates SAG development by inducing expression of Neurogenin1 (Ngn1) in the floor of the otic vesicle. However, not all Ngn1-positive cells undergo delamination, nor has the mechanism controlling SAG delamination been elucidated. Here we report that Goosecoid (Gsc), best known for regulating cellular dynamics in the Spemann organizer, regulates delamination of a subset of Ngn1-positive neuroblasts in the otic vesicle. In zebrafish, Fgf co-regulates expression of Gsc and Ngn1 in partially overlapping domains. Loss of Gsc function severely inhibits delamination of SAG neuroblasts whereas overexpression of Gsc greatly increases delamination. Importantly, overexpression of Gsc promotes delamination of otic cells even when neurogenesis is blocked by disrupting Ngn1. Thus, Gsc and Ngn1 control delamination and neural specification as distinct, experimentally separable outputs of Fgf signaling. The role of Gsc in otic delamination, together with its regulation of cell movement in the Spemann organizer and promotion of tumor metastasis indicates that Gsc might have a more widespread role in epithelial-mesenchymal dynamics than previously thought.
The role of carbonic anhydrase for otolith formation in the zebrafish embryo.
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The otoliths in the inner ear are the essential organ to maintain balance equilibrium and auditory function in vertebrate. They are formed by covering the core matrix consisted of glycogen and proteins with calcium carbonate. In order to investigate the process of the otolith calcification in zebrafish Danio rerio, the normal development of otoliths through its organogenesis was pursued. Localization and mRNA expression of isoforms in Carbonic anhydrase that would be involved in the calcification were also studied in the course of the development. At one day post fertilization (dpf), two immature otoliths of utricle and saccule were found in each otocyst in embryos. They were floating in the endolymph in the inner ear and located just above the basal layer of sialialy hair cells without their calcification. This was confirmed on 3 dpf by Calcein staining with isolated otoliths. The otolith growth continued beyond 3dpf that was the date of zebrafish hatching. The growth rate in the saccule was rapid than that in the utricle especially in the period between 3 to 5 dpf. Prior to the initiation of calcium deposition, immunohistochemical reactivity to anti Carbonic anhydorase II (CA II) antibody was detected in the basal cell layer on 2 dpf, and the immunoreactivity was maintained by 5 dpf. The precedent and sustained expression of CA II activity suggests that calcium carbonate depositing on the immature otoliths would be supplied from the basal cell layer through CA II function. The different growth rate between two otoliths would depend on the regional difference in gene expression of CA II in the cell layer. Present study describes the normal development of otoliths during zebrafish ontogenesis and possible involvement of CA II in otolith calcification. These results would supply the basal knowledge of zebrafish as a model for studies on development of equilibrium and auditory organs.

Deciphering the role of Foxi3 in early mouse development
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Foxi3 is a Forkhead transcription factor that is expressed in the preplacodal region of mice. This region gives rise to craniofacial placodes, such as the neurogenic trigeminal and epibranchial placodes in addition to otic placode, the precursor to the inner ear. The division of embryonic ectoderm into neural plate, neural crest, pre-placodal region and epidermis is achieved in part by the establishment of different domains of transcription factors. As the pre-placodal region is induced, Gata3 and Dlx5 genes become restricted to this region, together with definitive markers of the pre-placodal region such as, Six1, Six4 and their co-factors Eya1 and Eya2. A posterior group of pre-placodal cells respond to FGF signaling from the adjacent structures and differentiate into the otic placode. Our lab has knocked out Foxi3 in mice and Foxi3 mutants completely lack all ear structures. Ear induction appears to fail at the very earliest stages, as we see no expression of markers of the otic placode, such as Pax2, Sox9 or Dlx5 induced in Foxi3 mutants. We have shown that transcription factors downstream of Foxi3 (Gata3, Eya1 and Six1) are absent in mutants, and that FGF signaling is received by the pre-placodal region in Foxi3 mutant embryos but it fails to execute a program of otic placode differentiation. Moreover, the neurogenic epibranchial and trigeminal placodes are also disrupted, although not absent, in Foxi3 mutant mice. Together, our data suggest that Foxi3 acts at multiple stages of otic placode induction and is necessary for pre-placodal ectoderm to execute an inner ear program in response to FGF signaling, as well as being necessary for proper cranial ganglia development. Supported by RO1 DC013072 (A.K.G.), RO3 DC007349 (T.O.)

The chemokine CXCL14 regulates neurovascular patterning during ocular development
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Spatiotemporal signaling events in the developing anterior eye guide the intricate process of neurovascular patterning, which results in the formation of a highly innervated but avascular cornea. Although there is increased interest in chemokines due to their involvement in cell proliferation, migration, and differentiation during embryogenesis, very little is known about the function of CXCL14. Examination of the expression of CXCL14 during chick development by section in situ hybridization and immunohistochemistry revealed novel patterns of localization in the corneal stroma, iris, and trigeminal ganglion. To investigate the function of CXCL14 during ocular development, we knocked down its expression by over expressing RCAS-CXCL14-shRNA. Gross analysis of embryos after CXCL14 knockdown revealed relatively smaller eyes compared to RCAS-GFP and untreated controls. Whole-mount analysis of ocular nerves indicated exacerbated projection of sensory nerves into the pericorneal nerve ring, corneal stroma, and iris, which subsequently
elevated nerve thickness in these tissues. Furthermore, Knockdown of CXCL14 in Tg(tie1: H2B:eYFP) transgenic Japanese quail resulted in ectopic migration of fluoroscencently labeled angioblasts into the cornea. Collectively, these results demonstrate that CXCL14 plays a role in the precise patterning of ocular nerves and blood vessels. Therefore, CXCL14 may act indirectly by enhancing the response of sensory axons and angioblasts to the guidance signals that regulate neurovascular patterning.

Program Abstract #176
Mutations of chromosome assembly factor 1b correlate with abortive retinal morphogenesis
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An ethylnitrosourea mutagenesis screen in zebrafish for eye developmental defects uncovered families with recessive, lethal mutations that initially display normal eye development with rapid degeneration during middle phases of retinal morphogenesis. One of these families, the good effort (gef) mutant, is characterized by markedly abnormal morphological defects after two days post fertilization. The most obvious of these defects is a smaller retina, without significant effects on lens morphology. Cell death was elevated in the central neuroretina of the 48 hour gef mutant embryos compared with wild-type controls. Cell proliferation was found to be unaffected by anti-Proliferating cell nuclear antigen antibody labeling. Meiotic mapping of the gef allele localized to an interval of chromosome 9 that included the candidate gene, chromosome assembly 1b (chaf1b), a protein implicated in the survival of proliferating cells. Cloning of gef genomic DNA found a disruption to the splice donor sequence of chaf1b exon 3, a defect predicted to severely truncate the Chaf1b protein. Morpholino knockdown of Chaf1b in wild-type embryos phenocopied the gef morphant phenotype. Overexpression of wild-type chaf1b mRNA rescued the gef mutant phenotype. Taken together, these data point to gef as a loss-of-function allele of the chaf1b gene.

Program Abstract #177
Myc transcription factors coordinate proliferation and differentiation during mouse lens development
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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 and form fiber cells. The differentiation of these fiber cells includes the degradation of their organelles resulting in a fully transparent structure. Thus, cell cycle dynamics must be precisely coordinated with the fiber cell differentiation to form a functional lens, making it a suitable model to investigate Myc function during development in vivo. Targeted deletion of c-myc from mouse lens precursors resulted in severe eye and lens growth impairment. c-myc inactivation led to reduced cell proliferation in the developing lens, associated with ectopic localization of Prox1 and p27Kip1 proteins within epithelial progenitor cells. These proteins drive cell cycle exit of lens epithelial progenitors and their promoters have Myc binding sites. Additionally, we detected a sharp decrease in both mRNA and protein levels of Foxe3, a transcription factor important for epithelial cell proliferation. Interestingly, we observed that N-myc-deficient lenses do not have cell proliferation defects, but instead present disrupted fiber cell differentiation, including defective denucleation and cataracts formation. Furthermore, N-myc/c-myc double-inactivated lenses exhibit cataracts and striking volume reduction. Our findings support the hypothesis that Myc transcription factors regulate distinct cellular events during lens formation in vivo. These data constitute the first description of the physiological roles played by Myc genes during mouse lens development.

Program Abstract #178
EDA and its targets DKK4 and LRP6 regulate Wnt action during Meibomian Gland development
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Meibomian glands (MGs) are large sebaceous glands in the eyelids. They produce lipids that protect tears from evaporation. MG dysfunction is thought to be a leading cause of “dry eye”, a condition that affects over 10 million people in the United States. Previous data have demonstrated that mutations in the EDA gene cause MG ablation both in “EDA” patients and in the Tabby (Eda-negative) mouse. Besides EDA, however, other possible signaling pathways may control MG development and physiology but remain unidentified. To study the development and function of MG, we utilize cDNA microarrays, mutant mouse models and CRISPR technology to clarify signaling crosstalk. First we performed
expression profile of MG tissues from Tabby and wildtype controls. Data strongly suggested that the Wnt pathway may be regulated by EDA, and in particular via DKK4 and LRP6, which were markedly down-regulated in Tabby. Further experiments including CHIP, luciferase assays and cell-based functional studies confirmed that both DKK4 and LRP6 are direct transcriptional targets of EDA. These findings suggested canonic Wnt signaling as a key player and led us to analyze MG growth phenotypes in KO and TG mice. Interestingly, a MG ablation phenotype was observed in DKK4 transgenic mice, and further histological analysis indicated that the phenotype is due to growth arrest at the early “germ” phase, a phenotype similar to Tabby. We also analyzed the phenotype of b-catenin KO mice and found a more severe defect in which even early germ induction was totally blocked. All lines of evidence confirmed the importance of the Wnt pathway. To study the function of LRP6, a second direct target of EDA, we are using MG tissue culture as an ex vivo assay with CRISPR KO and activation of LRP6 to analyze phenotypes during MG formation. Overall, this study focuses on previously unknown signaling crosstalk during MG development, and may provide potential targets to alleviate related eye conditions.

Program Abstract #179
A retinoic acid mediated segment-coordinating mechanism terminates anterior limb bud outgrowth
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Five and fewer digits are autopod patterns seen in modern tetrapods, indicating highly conserved regulatory programs in constraining digit number. In early limb bud development, autopod mesenchyme is divided into anterior and posterior populations depending on distinct responses to Shh signal. The posterior mesenchyme growth is terminated by increased distance between Grem1-expressing mesenchyme, zone of polarity activity (ZPA) and apical ectodermal ridge (AER), but little is known about how the growth mode of anterior mesenchyme is switched off. Here we show that retinoic acid (RA) derived from the proximal limb bud mesenchyme is a critical signal to terminate the growth of anterior mesenchyme of autopod. In Mef8C193RC193R mutant, a transient downregulation of Aldh1a2 in the proximal mesenchyme causes an absence of Tbx3 expression and consequently an expansion of Grem1 in the anterior mesenchyme of autopod, which inhibits cell apoptosis and increases cell proliferation, thereby resulting in preaxial polysyndactyly. Maternal RA supplementation is sufficient to rescue this digit defect. These data support a segment-coordinating mechanism that Mef8/RA/Tbx3/Grem1 pathway synchronizes the developmental progresses of proximal and distal limb bud segments. It exemplifies the notion of coordinating development that may be used in various developmental settings to explain coherent growth among different cell populations.

Program Abstract #180
Gata6 interacts with Gli3 and enhances its repressor activities for limb anterior-posterior patterning
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Gli3 is a major regulator and mediator of Hedgehog signaling. GLI3 is a bifunctional transcription factor, regulated by proteolytic processing that converts a full-length transcriptional activator form (GLI3A) into a C-terminal truncated repressor form (GLI3R). Hedgehog signaling inhibits the processing of GLI3, and thus, GLI3 acts as a repressor in the absence of Hedgehog signaling and represses Hedgehog target gene expression. While it has been known that Shh and Gli3 counteract for anterior-posterior patterning in the limb, mechanisms that regulate GLI3R repressor activities are still to be understood. We report that a zinc finger factor, Gata6, genetically and physically interacts with Gli3 to enhance its repressor activity, and that the interaction is essential for normal autopod development in mice. Conditional knockout (CKO) of Gata6 in the mesoderm resulted in hindlimb-specific preaxial polydactyly, while forelimbs developed normally. We found that Tcre; Gata6CKO embryos exhibited ectopic activation of Hedgehog signaling in the anterior of hindlimb buds, which was rescued in Gata6 CKO; Shh+/− mutants. However, ectopic digit primordia still developed in Gata6 CKO; Shh+/− the hindlimbs. Given that Shh and Gli3 counteract with each other in limb patterning, we tested the hypothesis that Gata6 interacts with Gli3 and found that Tcre;Gata6-Cre;Gli3+/− mutants developed preaxial polydactyly. Unexpectedly, the preaxial polydactyly was developed both in forelimbs and hindlimbs. Analysis of marker gene expression suggests that Gli3-Gata6 interaction enhances GLI3R activity. In vitro experiments demonstrated that GATA6 physically interacts with GLI3R and enhances its repressor activities. Our results identified that Gata6 is necessary for Gli3R to elicit sufficient repressor activities and that the interaction between Gli3 and Gata6 maintains proper levels of repressor activities for normal autopod development in fore- and hind-limbs. Supported by NIAMS (R01AR064195)
**Program Abstract #181**

**How homocysteine affects the proteins involved in vasculogenesis during limb development?**

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Homocysteine (Hcy) has been implicated in the development of limb defects. However, vasculotoxic properties of Hcy have been poorly described. Thus, we investigated whether Hcy can induce impairments in proteins involved in vasculogenesis during limb development, in a chicken embryo model. Embryos were treated with 20 µmol D-L Hcy/50 µL saline at E2 and analyzed at E6. Control embryos received exclusively 50 µL saline solution (Ethics Committee for Animal Research: 254/2009). We observed a decrease in expression of all investigated proteins involved in vasculogenesis in embryos treated with Hcy. The number of cells labeled with anti-VCAM-1, vascular cell adhesion molecule, was small in Hcy-treated (38.20 ± 13.85) in comparison to control embryos (2,944.20 ± 266.50, p ≤ 0.0001). Also, a small number of cells labeled with anti-VEGF, growth factor, was verified in Hcy-treated embryos (1,247.40 ± 233.27), which differed from control embryos (2,346.20 ± 349.02, p ≤ 0.05). Finally, Hcy-treated, showed few cells labeled with anti-NOS3, an endothelial cell marker, in Hcy-treated embryos (68.00 ± 13.18) that differed from control embryos (149.40 ± 13.11, p ≤ 0.01). Our data indicates that the treatment with Hcy changes the expression of proteins involved in vasculogenesis during limb development. These findings provide information to better understand the cellular basis of the toxicity of Hcy on vasculogenesis during limb development.

**Program Abstract #183**

**Understanding the roles of the proepicardium and Fgf10 in heart development**

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The heart is formed by the coordinated accretion of different progenitor cell populations during embryogenesis. One such population, the proepicardial organ (PEO), is a mesothelial cluster of cells that forms at the caudal end of the heart and contributes cells that traverse the pericardial space, adhere to the myocardium and migrate over the surface to form the epicardium. This thin epithelium sheathes the mouse heart by E11.5. Some epicardial cells undergo EMT and enter the myocardium, predominantly generating coronary smooth muscle cells and interstitial fibroblasts. In addition to its critical role in the genesis of coronary vasculature, partial mechanical or genetic perturbations of the epicardium suggest that it also provides trophic signals supporting expansion of the ventricular compact zone during late gestation. To determine the earliest role of PEO-derived cells in murine cardiac development, we employed diphtheria toxin-mediated genetic ablation of Tbx18+ proepicardial cells and found that loss of this lineage causes heart failure and death at E11.5-E12.5 consequent to failure of ventricular myocardial expansion, and hemorrhage in the absence of subepicardial vascular plexus formation. We previously reported a spectrum of heart defects in Fgf3/Fgf10 double mutants, including reduced epicardial ensheathment and adhesion, and thinned myocardium, with double mutants dying of heart failure by E11.0. In an Fgf3 mutant background, conditional mutagenesis of an Fgf10 floxed allele in the PEO (Tbx18Cre driver) causes embryonic lethality between E11.5-E12.5 with cardiac phenotypes similar to those of Tbx18 lineage-ablated embryos. Together, these data suggest the hypothesis that the PEO and its derivatives play a critical and much earlier role in heart development than previously appreciated and that FGF signaling is required. Genetic profiling in Tbx18 lineage-ablated hearts and examination of Fgf target genes in Fgf3−/−;Fgf10−/−;Tbx18Cre+ hearts are underway.

**Program Abstract #184**

**Carbamyl-treated zebrafish embryos exhibit neuronal and cardiac defects as well as increased expression of a subset of cytochrome P450 genes**

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Carbamyl (1-napthyl-N-methylcarbamate) is one of the most commonly used insecticides in the United States. It functions as a neurotoxin by inhibiting the acetylcholinesterase (AChE) enzyme by blocking the vital breakdown of the acetylcholine (ACh) neurotransmitter in the nervous system of both insects and vertebrates. While the inhibitory effect that carbaryl has on AChE is well documented, some studies have also shown that carbaryl can bind to the aryl hydrocarbon receptor (AhR) to ultimately activate the expression of the cytochrome P450 genes. This potential novel role for carbaryl is not well studied and no research has addressed this question using animal model systems. Upon exposure to carbaryl, zebrafish (*Danio rerio*) embryos have cardiac defects characterized by a decrease in heart rate, edema, and a developmental delay/defect in cardiac looping. In addition to this cardiac defect, there is also a neuronal defect. Carbaryl-treated embryos have a significant decrease in the number of spinal cord neurons as well as cell death in the spinal cord.
Since these defects are very similar to those neuronal and cardiac defects observed when zebrafish embryos are treated with TCDD- a known ligand of AhR, we tested whether carbaryl would also induce expression of known effector genes of the AhR pathway- the cytochrome P450 genes. Using RT-PCR analysis, carbaryl-treated zebrafish embryos were shown to have induced expression of the cyp1a and cyp1b genes, but not the cyp1c1 and cyp1c2 genes suggesting that carbaryl may not only be inhibiting the AChE enzyme, but also may have a novel mechanism by which it activates the AhR pathway.

Program Abstract #185
A Novel Role for Hedgehog Signaling in Cardiac Patterning
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The mature heart is comprised of multiple types of specialized cardiomyocytes, each with distinct functional attributes. However, the mechanisms that specify discrete populations of cardiac progenitors are not well understood. For example, it is clear that cardiac pacemaking activity is confined to a specialized population of cells in the cardiac inflow tract (IFT), but the signals that create the appropriate number of IFT cardiomyocytes remain unknown. For this reason, we were intrigued to discover that Hedgehog (Hh) signaling has a particularly potent effect on formation of the zebrafish IFT. Given our prior finding that Hh signaling is required to promote cardiomyocyte production, we were surprised to find that Hh signaling seems to delimit the number of IFT cardiomyocytes. Using both genetic and pharmacological manipulations of Hh signaling, we have shown that loss of Hh signaling results in dramatically expanded expression of IFT markers. Timed manipulations have demonstrated that Hh signaling acts during early steps of cardiac patterning to limit IFT size. Furthermore, we have determined that Bmp signaling drives IFT formation, as embryos with reduced Bmp signaling have a diminished IFT. Intriguingly, reducing both Hh and Bmp signaling restores a nearly normal number of IFT cells, suggesting that Hh and Bmp signaling act in opposition during IFT development. We therefore propose a model in which high levels of Bmp signaling in emerging cardiac progenitors biases cells toward IFT fate, whereas high levels of Hh signaling biases cardiac progenitors toward other cardiac lineages. These findings reveal novel mechanisms of cardiac patterning and broaden our understanding of congenital heart disease and arrhythmia.

Program Abstract #186
FGF signaling maintains cardiac chamber identity in zebrafish
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The atrial and ventricular chambers of the heart behave as distinct subunits with unique morphological, electrophysiological, and contractile properties. Proper maintenance of atrial and ventricular features is therefore essential for a functional heart. Several transcription factors, including Nkx2.5, COUP-TFI, and Irx4, are known to be key regulators of chamber-specific programs. However, the signaling pathways that function upstream of these factors to control the retention of chamber-specific characteristics are less well understood. Here, we show that the FGF signaling pathway, which facilitates ventricular specification, is required to preserve ventricular identity. We find that inhibition of FGF signaling with the FGFR antagonist SU5402 can generate ectopic atrial cardiomyocytes within the already differentiated ventricle. Similar phenotypes are evident in fgf8 mutants and in embryos overexpressing a dominant-negative FGFR receptor. Additionally, temporally restricted manipulation of the FGF pathway reveals a specific time window during which ventricular chamber identity is vulnerable to the loss of FGF signaling. Analysis using photoconvertible reporter transgenes indicates that the ectopic cells in SU5402-treated embryos are not derived from the atrium. Instead, examination of the dynamic localization of atrial and ventricular proteins suggests that these ectopic cells are produced through transformation of ventricular cardiomyocytes. Furthermore, we find that FGF signaling is required for normal maintenance of nkh2.5 and nkh2.7 expression. Overexpression of nkr2.5 can improve the preservation of ventricular character in SU5402-treated embryos, suggesting that FGF signaling may function downstream of Nkh factors to promote retention of ventricular identity. Together, our data suggest a model in which differentiated ventricular cardiomyocytes retain some plasticity and require continuous FGF signaling to preserve their chamber-specific identity.

Program Abstract #187
Pitx2 drives organ-specific arterial and lymphatic vascularization of the midgut
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A key feature of organogenesis is the tightly regulated patterning of organ-specific vascular networks. These channels are critical for facilitating exchange of gas and nutrients and have to be structurally and molecularly suited to meet the unique requirements of specific organs. The blood and lymphatic vascular networks of the intestine have important functions of (i) efficient absorption of nutrients through specialized blood and lymphatic capillaries in the intestinal villi and (ii) transport of gas and nutrients via the mesenteric vessels. The dorsal mesentery (DM), a mesodermal tissue suspending the intestines from the dorsal body wall, is the sole conduit joining the dense intestinal vascular network to the systemic circulatory system. However, the molecular and cellular mechanisms underlying the patterning of this critical vascular network are undefined. We show that Pitx2, a master regulator of left-right organ asymmetry, initiates arteriogenesis and subsequent lymphangiogenesis exclusively on the left side of the DM via regulation of the Cxcl12-Cxcr4 axis. Cxcl12 expression is necessary but not sufficient to drive vascular patterning in the absence of Pitx2, indicating a critical dependence on Pitx2 patterned microenvironment. Genetic or pharmaceutical inhibition of arterial branches to the looping gut arrests local lymphangiogenesis in the left DM. Surprisingly, we show that Pitx2-dependent lymphatic precursors in the left DM are independent of the venous derived mesenteric lymph sac at the root of the DM, suggesting a dual origin of intestinal lymphatics. We further show that in Cxcr4 mutant neonates, disrupted mesenteric arterial branching is accompanied by disorganized collecting mesenteric lymphatic vessels and absent intestinal villi vasculature. Our data reveal developmental heterogeneity among intestinal lymphatic population and hold therapeutic promise to selectively affect intestinal lymphatics during disease.

**Program Abstract #188**

**A Novel Non-Neuronal Role of Acetylcholinesterase in Intestinal Development**

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Acetylcholinesterase (AChE) is a highly conserved protein well studied for its role in terminating nervous signaling through degradation of the neurotransmitter, acetylcholine. However, a growing body of evidence suggests AChE also has non-neuronal activities associated with cell adhesion and polarized cell migration, cell behaviors that underlie much of embryogenesis. Consistent with a non-neuronal role in embryonic development, we detected AChE within the non-inervated endoderm cells of the *Xenopus* embryo that rearrange to lengthen the embryonic gut and form the epithelial lining of the intestine. Exposing embryos to chemical AChE inhibitors, or knocking down endoderm AChE via antisense agents (morpholino), resulted in shortened guts with disrupted cell polarity, decreased expression of adhesion molecules, and failure of the endoderm to differentiate and rearrange into a single epithelial layer. To better define the mechanism(s) by which AChE promotes adhesion and migration during development, we isolated gut endoderm cells and performed ex vivo cell-cell and cell-substrate adhesion assays. Cell-cell and cell-substrate adhesion were unaffected by loss of AChE activity. However, cell protrusive activity and movement were reduced when AChE was inhibited. This suggests that AChE may be required for cell polarization, but not adhesion during gut development. This work reveals a previously unrecognized role for a neurotransmitter hydrolase in coordinating polarized cell rearrangement during organ morphogenesis.

**Program Abstract #189**

**Fish Guts and Genome Cuts: Investigating FIP5 roles during epithelia polarization and apical lumen formation in vivo**

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Epithelial cells are structurally and functionally polarized to transport specific molecules while maintaining a trans-epithelial barrier. This cellular asymmetry is essential for the proper functioning of epithelial tissues and depends on polarized endocytic transport routes. Epithelial cells coordinate their polarization with neighboring cells to form an apical lumen, a key step in the establishment of renal and gut architecture and tissue function. Recent work from our laboratory implicated endocytic Rab11 and its binding protein FIP5 as major components that regulate apical endosome transport during apical lumen formation in vivo. To assess whether FIP5 mediates epithelia morphogenesis and lumen formation in vivo we generated mutant FIP5 zebrafish lines using CRISPR/Cas targeted mutagenesis and analyzed zebrafish intestine and pronephron development. Our work indicates that the zebrafish FIP5 homolog, Fip5a, is predominantly localized in the zebrafish intestinal bulb and is required for intestinal lumen formation and expansion during embryonic intestinal development. Interestingly, we also show that Fip5a function is not limited to intestinal lumen formation. We found that mutation of Fip5 resulted in severe defects in morphogenesis of additional “hollow” organs, including the otic vesicle. Further, our results suggest Fip5 may be required for neural tube closure and subsequent neural development. Finally, we
found that depletion of Fip5a severely disrupts the morphology of microvilli and affects the formation of terminal web in gut enterocytes. Taken together, our data demonstrates that FIP5a is a key regulator of apical protein targeting and thereby the establishment of epithelial polarity and formation of apical lumen during morphogenesis of multiple zebrafish tissues.

Program Abstract #190
Bilayered mammary epithelia form through radial intercalation with dynamic enrichment of PIP3 and actin in sub-cellular protrusions
Neil M. Neumann, Robert J. Huebner, Andrew J. Ewald
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Morphogenesis of the mammary gland requires dynamic, collective cellular movements to reorganize and form the mature tissue. The final stage of mammary gland development is polarization from a stratified to a bilayered epithelial architecture. However, both the cellular rearrangements and the molecular pathways that form a bilayer are not fully understood. Here, we demonstrate that transition to a bilayer results from highly migratory epithelial cells that radially intercalate with sub-cellular protrusions. Direct observation of in vivo mammalian organ development is difficult; therefore, we use three-dimensional organotypic culture coupled with time-lapse confocal microscopy. Individual cells are observed using mosaic fluorescent cytoplasmic labeling, and fluorescent biosensors for actin and PIP3 allow molecular dynamics visualization. Interestingly, upon treatment with 11 different inhibitors of caspase-dependent apoptosis, we observed transition to a polarized, bilayered epithelium. This demonstrates that there are additional mechanisms leading to bilayer formation, apart from cavitation. Additionally, throughout the stratified epithelium, both actin and PIP3 are specifically and dynamically enriched in the sub-cellular protrusions, demonstrating that these are a truly migratory epithelial cell population. We conclude that polarization results from radial intercalation by using directed actin and PIP3 enriched sub-cellular protrusions. Ultimately, we would like to understand how individual cells exchange cellular contacts and transition to an apicobasally-polarized cell, thus providing insights into how cancer cells migrate and metastasize.

Program Abstract #191
Kdf1 is a critical regulator of the periderm, an essential but understudied component of the epithelium in development.
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Keratinocyte Differentiation Factor 1 (Kdf1; 1810019J16RIK) is a novel, relatively unstudied protein. From a forward genetics screen, we identified a Kdf1 mouse mutant and our previous work showed that Kdf1 regulates the balance of proliferation and differentiation in keratinocytes that compose the epidermal layer of the skin. Our recent studies show that Kdf1 is expressed not just in the epidermis, but also in the vast majority of epithelial cell types in the developing mouse embryo including parts of the esophagus, teeth, salivary glands, lungs, kidneys, bladder and the oral cavity. We identified morphological defects in many of these tissues and in this presentation we focus on cleft palate, fused esophagus, and shortened, fused digits. We found that the common feature of these defects in Kdf1 mutants is abnormal formation and function of the periderm. The periderm is an epidermal-derived layer of cells that covers the embryo and lines the oral cavity and esophagus. The periderm is a transient cell layer that is normally shed before birth, but whose function during development was largely unknown. Recent studies suggest that the periderm forms a barrier to prevent adhesions between epithelial tissues. We found that Kdf1 is critical for periderm function and in Kdf1 mutant periderm, adhesion proteins are no longer basally restricted within the periderm. This results in fusion between the walls of the esophagus, within the epithelial layers of the limb, and between the palatal shelves and tongue, causing cleft palate. We further found that Kdf1 physically interacts with Ikkα, and 14-3-3σ, two factors essential for periderm formation. Our studies reveal a critical role for Kdf1 in the development of multiple epithelial tissues and provide novel insight into the genetic pathway regulating the periderm, an essential but poorly understood developmental tissue. FUNDING: NIH-NIAMS-R01AR059687

Program Abstract #192
Expression profile of Claudin family members in the developing lung
Joshua Lewis, Felix Jimenez, Michael Chavaria, Tyson Jergensen, Paul Baker, Jason Gassman, Ryan Morris, Dallin Millner, Jared Bodine, Scott Albright, Tessa Schrader, Conner Christiansen, Jaun Arroyo, Brigham Merrill, Paul Reynolds
Claudins are tight junctional proteins that are implicated in cell polarity and in establishing and maintaining epithelial barrier function. Our published research has also revealed that claudin misregulation adversely impacts cell differentiation and proliferation. Impairment of such critical functions related to various claudins has been linked to anomalous barriers in abnormal lung development and diseases such as acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and cancer. The current research evaluated expression of claudins 1-8 in order to elucidate expression patterns within the developing murine lung. Claudins 1-8 were selected based on their phylogenetic similarity and published reports of potentially diverse pulmonary expression patterns. Wild type mice from embryonic days 12.5, 14.5, 16.5, 18.5 and post-natal day 2 were sacrificed and assessed to demonstrate cell-specific expression via immunohistochemical analysis. Protein and RNA expression was quantitatively confirmed using immunoblotting and qPCR techniques. The results suggest increasing and decreasing patterns of expression that included redundancies and temporal transition between family members. Furthermore, cell-specific expression of individual claudins implicated a subset as orchestrators of proximal vs. distal lung barrier establishment. These data support the need for further studies using claudin-specific transgenic mice that knock-in/out specific claudins so that precise functions in the normal and diseased lung can be determined. This work was supported by a grant from the Flight Attendant's Medical Research Institute (FAMRI, PRR) and a BYU Mentoring Environment Grant (PRR).

Program Abstract #194
Role of miR-126 in placental development
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The placenta is a highly vascularized, transient organ that is indispensable for the developing mammalian embryo. It acts as a maternal-fetal interface for the exchange of gases, nutrients and waste. Placental insufficiencies can lead to embryonic growth retardation and death. MiR-126, an endothelial cell specific micro-RNA is embedded in intron7 of Egfl7 and is expressed along with Egfl7 in various tissues. Deletion of miR-126 in mice of a mixed background strain results in partial embryonic lethality due to leaky vessels. Based on our hypothesis that miR-126 plays a role in placental development, miR-126-/- mice (C57Bl6 congenic strain) were intercrossed. None of the miR-126-/- offspring survived at birth, and miR126-/- embryos displayed widespread edema and hemorrhaging, consistent with previous reports. H&E stained sections from E12.5 miR-126-/- placentas displayed large lesions in the junctional zone. Staining with antibodies against CD31 (EC marker) and cytokeratin (trophoblast marker) suggest that the lesions are confined to the trophoblast layer of the junctional zone in the placentas. Interestingly, preliminary observations indicate no major abnormalities in the placental vasculature. Potential miR-126 targets were identified by collating genes from multiple target prediction software programs, and their expression levels were measured by qRT-PCR. Insulin receptor substrate 1 (IRS1) was one of the target genes predicted by multiple programs. IRS1 transcripts were increased 3-fold in miR-126-/- placentas, and western blot analysis showed increased protein levels of p-IRS1 (Tyr 632) and its downstream effector p-Akt. Ongoing studies investigate the implications of dysregulation of IRS1 due to loss of miR-126 on glucose metabolism during pregnancy and gestational diabetes.

Program Abstract #195
Evolution of the genetic network regulating bract suppression in maize
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Plant architecture is highly modular, with repeating units known as phytomers that consist of a leaf attached to a stem with a meristem in the axil of each leaf. The transition from vegetative to reproductive growth in plants involves dramatic changes to the phytomer. In many, but not all, flowering plants this transition includes the inhibition of leaf growth subtending flowers and inflorescence branches. Leaves produced after the inflorescence transition are known as bracts, and bract suppression has convergently evolved in many flowering plant lineages. We are interested in understanding the genetic network regulating bract suppression in maize because it provides an excellent model to investigate the evolution of convergent morphologies. Genetic analysis has revealed that bract suppression in maize is regulated by numerous genes that interact in complex ways. We have positionally cloned three maize bract suppression genes including two recessive mutants (tasselsheath1 and tasselsheath2) and the dominant Few branched1 gene. Characterization of these mutants demonstrates that bract suppression is tightly correlated with inflorescence branching, and this developmental constraint may help explain the complex diversification of grass inflorescence morphology that began after the origin of bract suppression. Comparison of the bract suppression network in maize and Arabidopsis, which evolved bract
suppression independently, shows that unique genes are involved in each and further suggests that a novel, integrated developmental network has arisen to control bract suppression in the grass lineage.

**Program Abstract #196**  
**Maternal Deposition versus Zygotic Transcription during Early Development in Nematodes**  
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A long-standing view of development is that transcription is silenced during the oocyte-to-embryo transition and early embryogenesis. Maternally deposited RNAs and proteins are differentially and coordinately used to drive these developmental processes. We compared maternal deposition and zygotic transcription between two nematodes, *Caenorhabditis elegans* and *Ascaris suum*, that exhibit highly similar early developmental patterns but differ dramatically in their cell cycle lengths (~50x difference). In the fast developing nematode *C. elegans*, transcription does not occur until the 4-cell stage, and development continues under the control of maternal genes until ~120-cell stage. We found that in the slow developing nematode *A. suum*, ~4,000 genes are transcribed prior to pronuclear fusion and in 1-4 cell embryos. Intriguingly, we did not detect the maternal contribution of many orthologs of maternally contributed *C. elegans* mRNAs, but instead found these genes are newly transcribed in the *A. suum* zygotes. Early transcription is detected using RNA polymerase II antibody staining in both the maternal and paternal *A. suum* nuclei in zygotes prior to pronuclear fusion. We identified ~4,000 active promoter regions/transcription start sites in 1-cell *A. suum* embryos using H3K4me3 ChIP-seq as well as other histone marks. Our data suggest that major transcription can occur immediately after fertilization and prior to pronuclear fusion in some metazoan and that the contribution of maternal vs. zygotically transcribed genes differs dramatically between nematodes with similar development. These data illustrate significant plasticity in the gene regulatory networks that control and play important roles in developmental outcomes in nematodes. We postulate that the balance between transcriptional and post-transcriptional regulation during the oocyte-to-embryo transition may largely be determined by cell cycle length and thus the time available for the genome to be transcribed.

**Program Abstract #197**  
**Characterization of the Body Pigment in the Planarian Flatworm Schmidtea mediterranea**  
John Dustin, Brad Stubenhaus, Emily Neverett, Megan Beaudry, Leanna Nadeau, Jason Pellettieri  
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The Platyhelminthes, or flatworms, exhibit a striking variety of pigment colors and patterns. Many species also have the ability to regenerate lost body parts by forming new tissue at sites of amputation. Thus, the development of genomic tools for model flatworms such as *Schmidtea mediterranea* and *Dugesia japonica* provides new opportunities to investigate mechanisms of pigment cell differentiation, and also how those mechanisms have evolved across this phylum. Toward this end, we have initiated an analysis of *S. mediterranea* pigment biosynthesis pathways. Results of previous research on related planarian species suggested the body pigment is a melanin or an ommochrome. We identified and cloned 6 *S. mediterranea* homologs of tyrosinase, the rate-limiting enzyme in melanin biosynthesis, and 7 homologs of enzymes that produce ommochromes from the amino acid tryptophan. Whole-mount in situ hybridization and RNAi experiments revealed that the tyrosinase homologs do not exhibit expression patterns or RNAi phenotypes consistent with a role in body pigment synthesis. Conversely, the ommochrome biosynthesis enzyme *kynurenine 3-monooxygenase* exhibited expression in dendritic cells dispersed over the surface of the body, but excluded from the non-pigmented areas of the photoreceptors. Knockdown of this gene reduced bodily pigmentation; we are currently conducting combinatorial RNAi experiments to address the possibility that the lack of complete depigmentation reflects genetic redundancy at this step of the ommochrome biosynthesis pathway. We also found the absorbance spectrum of purified body pigment resembles that of previously characterized ommochromes. Taken together, these results suggest the *S. mediterranea* body pigment is an ommochrome synthesized from tryptophan via evolutionarily conserved mechanisms. Our work sets the stage for future evo-devo analyses of flatworm pigmentation, as well as mechanistic studies of pigment cell differentiation during regeneration.

**Program Abstract #198**  
**LEP-2/Mkrn regulates LIN-28 to promote the juvenile-to-adult transition**  
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*C. elegans* and mammals share a developmental milestone: the juvenile-to-adult (J/A) transition, or puberty. In mammals, the timely onset of puberty requires LIN28b and Makorin-3 (Mkrn); altering their levels results in early or delayed
puberty. The *C. elegans* J/A transition is scheduled by heterochronic genes and *lin-28* is a key regulator. Here we show that in *C. elegans* LEP-2/Mkrn also controls the timing of the J/A transition by promoting LIN-28 protein degradation. *lep-2* was found in a screen for defects in L4 male tail tip morphogenesis (TTM), a process where the tail tip changes shape from long and pointed to short and rounded. *lep-2* mutants display retarded phenotypes: 1) TTM is delayed until adulthood and young adult *lep-2* males have long larval-like tail tips that will undergo TTM as the male ages. 2) The adult cuticle of both sexes does not have the adult-specific collagen ROL-1. 3) Supernumerary molts also occur. 4) *lep-2(-)* males cannot perform wild-type mating behavior, although male tail morphology is unaffected. Also, production of adult alae is normal in *lep-2* mutants. This suggests that *lep-2* acts redundantly or is not involved in regulating the timing of seam cell development. It also explains why screens for seam cell phenotypes failed to find *lep-2*. Epistasis analysis shows that *lep-2* represses *lin-28*: *lin-28* RNAi is sufficient to suppress the *lep-2* mutant phenotype. In wild type, LIN-28 is down-regulated during the L2 stage. In *lep-2* mutants this down-regulation does not occur. *lin-28* mRNA levels are unaffected, thus LEP-2 regulates LIN-28 post-transcriptionally. We use a LIN-28::Dendra2 fusion protein to see how LEP-2 represses LIN-28: by halting translation or aiding degradation. Together, our findings suggest conservation of genes involved in the mechanisms of pubertal timing in animals. Due to this conservation, we suggest that *C. elegans* is a model to study basic mechanisms of pubertal timing in animals. Funded by the NIH.

**Program Abstract #199**

**How We’re Made: The Process (CELLULAR DARWINISM) and Mechanism (BINARY BIOLOGY) of Multicellular Organization**

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We begin life as a fertilized cell and become a multicellular organism of trillions of cells, each doing the right thing, at the right time and place. How does this happen? 3 decades of research have taught us that we are made by CELLULAR DARWINISM, where cellular diversity arises by stochastic gene activation and developmental order arises by cellular selection (differential cellular proliferation and death). This can be seen by immunofluorescence of plasma protein synthesis by the liver, revealing each protein to be produced by separate cells, whose abundance is determined by differential cellular proliferation, while cellular heterogeneity arises by stochastic gene expression, revealed by allelic exclusion of immunologically distinguishable alleles of albumin. Underlying this Darwinian process lies the molecular mechanism of BINARY BIOLOGY, the either/or switching of cells and molecules, made possible by their fundamentally discrete nature. A vivid example can be seen when cells interact with small numbers of molecules, like IGF-II, for which there is \(~1\) bound molecule for every 6 cells. Such discreteness provides a way for cell division to occur in a fraction of cells, in this case, in one-in-every-six cells. We have come to conclude that such binary biological switching occurs in many aspects of multicellular organization, including the creation of cellular diversity by stochastic gene expression. The practical utility of such a binary biological approach has been borne out by mathematical methods for capturing many multicellular processes, including the prediction of cancer survival, the generation of morphogenesis, and the identification of a universal growth equation, which accurately captures the relation of size to age, from fertilization to adulthood, for many types of animals. This binary biological mathematics of growth also allows one to relate the biochemical features of mitotic signaling to the macroscopic features of normal and cancerous growth.

**Program Abstract #200**

**Tracing the Role of bab Gene Duplication and Divergence Events in the Evolution of a Fruit Fly Pigmentation Trait**

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Diversity is largely shaped by genetic differences in orthologous genes. The functional expression of these genes are controlled by *cis*-regulatory elements (CREs) which typically are located in introns, upstream, or downstream of the exon protein coding sequences whose transcription they control. It remains unresolved whether gene functional evolution more frequently follows paths of CRE evolution, protein-coding evolution, or a blended evolutionary path of the two. Moreover, mutation events can duplicate a gene, creating a pair of paralogous genes which can then undergo protein-coding and/or CRE evolution. The fruit fly species *D. melanogaster* possesses the paralogous *bab1* and *bab2* genes from an ancestral duplication event. These paralogs are thought to encode transcription factor proteins that similarly repress abdominal pigmentation in females, whereas pigmentation is unimpeded in males who largely lack abdominal *bab* expression. It has been shown that this dimorphic trait and *bab* gene expression pattern were derived from a monomorphic ancestor through CRE evolution. However, the possibility and the historical time point for protein-coding sequence
evolution have not been explored. We are using the \textit{bab} genes as a model to investigate whether and when \textit{bab} protein coding sequence evolution contributed to this trait’s origin. This involves testing whether the \textit{D. melanogaster bab} paralogs are functionally equivalent through loss-of-function and gain-of-function methods. Furthermore, the protein coding sequences of orthologous \textit{bab} genes will be tested for functional equivalence and DNA-binding necessity in pigmentation suppression. Collectively, this work will provide a comprehensive overview of how historical changes to an animal gene made possible the origin of a novel trait. Funding sources: American Heart Association (11BGIA7280000); National Science Foundation (IOS-1146373); and the University of Dayton Honors Program and Berry Summer Thesis Institute.

**Program Abstract #201**

\textbf{The role of Polycomb and Trithorax genes in the development and evolution of an animal trait.}

Claire Konys, Thomas Williams

\textit{University of Dayton, United States}

Animal traits result from intricate patterns of gene expression that are regulated during development. Differences in gene expression between individuals and species are a prominent cause for variation. In eukaryotes, gene expression regulation occurs at two levels. One is via interactions of transcription factor proteins with \textit{cis}-regulatory DNA sequences. A second level is via the compaction of DNA sequence into chromatin through interactions between DNA and histone proteins. Gene expression by default is shut “OFF” through a repressive compacted chromatin state, but this state can be turned “ON” through histone modifications and remodeling (repositioning or removals). Histone modifications and remodeling actions are performed by evolutionarily conserved genes. In the fruit fly \textit{Drosophila melanogaster}, the Polycomb Group of genes are needed for the formation of repressive chromatin and the Trithorax Group of genes are needed for the formation of permissive chromatin. How Polycomb and Trithorax genes collaborate to regulate the development of individual traits remains poorly understood, as is the extent to which these genes contribute to trait evolution. The objectives for my research project are to investigate three questions. One, how many Polycomb and Trithorax group genes contribute to a fruit fly pigmentation trait? Two, which pigmentation network genes are regulated by Polycomb and Trithorax genes? Three, has the expression patterns for these Polycomb and Trithorax genes changed to shape evolutionary changes in pigmentation? Completing these objectives will advance an understanding of chromatin and how its management shapes the development and evolution of an animal trait. Funding sources: American Heart Association (11BGIA7280000); National Science Foundation (IOS-1146373); and the University of Dayton Honors Program and Berry Summer Thesis Institute.

**Program Abstract #202**

\textbf{Understanding gene expression regulation and its evolution through genome editing and transgenesis approaches}

Jessica Grilliot, Alexandra Hallagan, Thomas Williams

\textit{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 \textit{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. Funding sources: American Heart Association (11BGIA7280000); National Science Foundation (IOS-1146373); and the University of Dayton Honors Program and Berry Summer Thesis Institute.
Program Abstract #203
The evolution of eggshell morphology is guided by spatiotemporal change in TGFα-like ligand distribution.
Nir Yakoby, Matthew Niepielko
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Eggshells of the genus Drosophila display a remarkable morphological diversity among species, providing an excellent opportunity to study mechanisms underlying the evolution of morphologies. One such morphology is the dorsal ridge, a lumen-like structure along the dorsal side of eggshells from numerous Drosophila species. The dorsal ridge varies in shape and length among species and is absent from D. melanogaster eggshells. We associated distinct dynamic patterns of epidermal growth factor receptor (EGFR) activation with the final shape of the dorsal ridge. Among species with and without a dorsal ridge, we showed the corresponding changes in the distribution of the TGFα-like ligand Gurken (GRK), a crucial ligand for axes formation. Remarkably, we demonstrated that GRK is necessary for dorsal ridge formation in D. willistoni. Furthermore, we established that not only does GRK from D. willistoni rescue a grk-null D. melanogaster fly, but it is also sufficient to generate a dorsal ridge-like structure on its eggshell without changing the fly’s axes formation. We concluded that spatiotemporal changes in GRK distribution underlie the formation of a new eggshell morphology, the dorsal ridge. Funding acknowledgment: The research was supported by the Rutgers Faculty Research Grant (281715), and by the National Institute of General Medical Sciences of the National Institutes of Health Award (R15GM101597) granted to NY.

Program Abstract #204
Recapitulating the evolution of fly extraembryonic tissue: the role of mmp1
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Evolution is characterized by changes of gene expression that lead to the generation of new morphological features between species. Here, we aim to understand such a step in change of shape by studying the formation of the fly extraembryonic tissue topology as an example. During gastrulation, flies form a specialized extraembryonic tissue that eventually seals the embryo dorsally. In most flies and insects, this extraembryonic tissue consists of two distinct epithelia, the amnion and the serosa, which develop by extension of an epithelial fold (so-called amnioserosal fold). In Drosophila melanogaster, the extension of this amnioserosal fold is strongly reduced and only a single epithelium is formed, the amnioserosa. In spite of this morphological difference, extraembryonic tissue in all flies is specified by the homeobox gene zerknüllt (zen). We are interested in the cellular and genetic mechanisms that changed during evolution downstream of zen and gave rise to the single epithelium observed in D.melanogaster. To address this question we have focused our attention on the scuttle fly Megaselia abdita, which has been previously shown to develop a distinct amnion and serosa. Based on a combination of functional analyses and high resolution light-sheet in vivomicroscopy, we found that the crawling of the serosa can be blocked by manipulating the expression of two factors: Mab-dorsocross (doc) and Mab-matrix metalloproteinasel (mmp1). The knock-down of either of these two genes in M.abdita appears to recapitulate evolution, as it gives rise to a single dorsal extraembryonic tissue similar to the amnioserosa in D.melanogaster. Our results suggest that changes in the regulation of mmp1 are sufficient to explain the evolutionary transition from two to a single extraembryonic epithelium.

Program Abstract #205
The small peptides Polished rice/mille-pattes play an evolutionarily conserved role in insect segmentation.
Miriam Rosenberg1,3, Hélène Chanut2, Claude Desplan1, François Payre2
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Despite similar developmental gene toolkits, insects deploy strikingly different segmentation modes to control axial patterning in embryos. Two extreme modes, referred to as long- and short- germ band, are exemplified by Drosophila and Tribolium embryogenesis, respectively. In the “long germ” Drosophila mode, all segments form almost synchronously and embryos are largely patterned by cellularization. In “short germ” Tribolium embryogenesis, only head and thorax are patterned before cellularization, and posterior segments are sequentially added and patterned thereafter. One outstanding question is whether any “new” genes beyond those identified in the classical screens in Drosophila are required to achieve sequential segment formation in a cellular environment, and how these may connect with known networks. A locus called mille-pattes (mlpt) encoding four similar small peptides (smORFs) was initially identified as a novel gap gene in Tribolium. Although the expression of mlpt (aka polished rice or tarsal-less) resembles that of a typical pair rule gene in early Drosophila embryos, no segmentation phenotype was detected in flies. We have studied mlpt in the parasitic wasp
Nasonia, which exhibits aspects both of long and short germ patterning. We present expression and RNAi data from Nasonia, as well as more basal insects, and revisit the apparent absence of segmentation function in Drosophila. Our results show that, in contrast to initial models, mlpt plays an ancient and widely conserved role in the axial patterning of insects, providing new insights into the evolution of long and short germ embryogenesis.

Program Abstract #206
Functional Characterization of the Role of the Pole Plasm Component Oskar in the Adult Brain of the Cricket Gryllus bimaculatus
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The gene oskar was first identified as having a role in holometabolous insects (“higher insects”) in specifying the germ line by forming specialized maternally supplied cytoplasm called germ plasm. However, insect germ plasm is thought to be a derived trait within insects and unique to Holometabola. The fact that germ plasm is a derived trait in holometabolous insects, and that oskar and other well known genes with germ line functions are expressed in the Drosophila adult brain and function in long term memory, suggests that these genes might have had an ancestral association with the nervous system. Previously in our lab, an orthologue of oskar was recently identified in a basally branching insect, the cricket Gryllus bimaculatus. Unlike Drosophila oskar, Gb-oskar does not play a role in germ line formation. Instead, cricket oskar mRNA and oskar protein are enriched in embryonic and adult neuroblasts. However, very little is known about the function of oskar in nervous system of insects. The goal of this work is identify the functional role of oskar in the adult brain of Gryllus bimaculatus. Gb-oskar mRNA is expressed in the adult brain in neuroblasts that are responsible for adult neurogenesis in the mushroom body, which is the anatomical substrate of olfactory memory in insects. Olfactory learning experiments revealed that RNAi against Gb-oskar impairs long-term olfactory memory. Thus far, we have observed no changes in gross brain anatomy, division rate of adult neuroblasts, or expression of prospero between oskar RNAi and controls. However, we found that mushroom body neuroblasts also express orthologues of Vasa and Piwi. Here we present progress towards elucidating how the function of Oskar might have been co-opted from its neuronal role to a new function in germ cell specification in insects, including preliminary analyses of what sort of cellular changes in the adult brain are caused by oskar RNAi that might account for the loss of long-term memory.

Program Abstract #207
Evolution and Development of a Novel Larval Organ
Amy McElhinney
University of Mount Union, United States
The Phylum Echinodermata is ancestrally indirect developing via a feeding larva lacking a skeleton, and has evolved to include both direct and indirect developing species. Larvae are bilaterally symmetrical with a ciliary band dividing oral and aboral ectoderm territories and serving a purpose in locomotion and feeding. The classes Echinoidea and Ophiuroidea develop via feeding pluteus larvae with underlying calcified skeletons. These pluteus larvae likely arose independently and had to evolve the skeleton and overlying arm ectoderm from pre-existing adults. This evolution involved reorganization and co-option of numerous genes in the production of the novel feature, pluteus arms. The research pursued here aims to understand the evolution and development of pluteus arms by beginning to dissect the regulatory controls involved in their development. The expression patterns of the arm-associated genes carbonic anhydrase, msp130, and tetraspanin were analyzed using whole mount in situ hybridization. The expression patterns for carbonic anhydrase, msp 130, and tetraspanin were compared between direct and indirect developing species of sea urchins. Additionally, expression patterns were determined in indirect developing species with perturbation of arm development using teratogenic compounds interfering with signaling pathways in early stage embryos. Following developmental perturbation, changes in marker gene expression were assessed and the following questions were answered, how do expression patterns of marker genes change when normal development is disrupted? Do their region(s) of expression expand, contract, disappear, or remain unchanged? What does a change in their expression reveal about the evolution of developmental regulation? This study in conjunction with existing knowledge of ancient gene pathways shed light on the evolution of axial regulation and development in echinoderms.
Funding from NSF IGERT fellowship and the Indiana Molecular Biology Institute.
Development of somites and their derivatives in amphioxus, and implications for the evolution of vertebrate somites
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Vertebrate somites become subdivided into lineage compartments with distinct tissue fates including muscle, cartilage, tendon and dermis. There is some variation in the position, relative sizes, and inductive mechanisms of these compartments across higher vertebrates, which contributes to variation in musculoskeletal morphology. Further, skeletal tissue is an evolutionary novelty of vertebrates, raising the question of how a compartment of skeletal precursors arose within early vertebrate somites. The evolutionary history of higher vertebrate somites can be better understood by comparison to basal groups. Here, we have examined somite organization and fate in the invertebrate chordate amphioxus. Amphioxus somites contain myotome and non-myotome compartments, but the development and derivatives of the latter are not well understood. We used transmission electron microscopy (TEM) and light microscopy on a developmental series to examine the development and fate of amphioxus somites and their derivatives, and to describe the development of the collagenous axial support system. Our series indicates that non-myotome somite cells give rise to mesothelial cell layers directly apposed to the extracellular collagen layers that comprise the axial support system and dermis of amphioxus. Based on their ultrastructure, collagen gene expression, and their position abutting extracellular collagenous layers, these non-myotome somite derivatives may be secretory cells that contribute to the collagenous connective tissues. The non-myotome cells of amphioxus somites include a population similar to vertebrate sclerotome in their initial position, apparent movement to surround midline structures and into the myosepta, and likely contribution to collagenous extracellular matrix of the axial support system. Therefore, many aspects of the sclerotome developmental program may have evolved prior to the origin of the vertebrate mineralized skeleton.

How to stop embryo development? Using African killifish Notobranchius furzeri as a model system to study embryonic diapause in vertebrates
Chi-Kuo Hu
Stanford University, United States

Organisms experience seasonal changes in the wild, and extreme environmental conditions give rise to extreme adoptions. Diapause is a unique surviving approach by which embryos put all developmental processes in temporary arrest to live through an adverse environment. Similar dormancies are widely adopted from plants, insects, to mammals. While some knowledge has been acquired from studying diapause in invertebrates such as insects, still little is understood about the underlying mechanical principles that govern a more complex vertebrate system. African killifish Notobranchius furzeri (N. furzeri) embryos possess a rare ability among vertebrates to enter diapause at the mid-stage of embryogenesis, with well-developed neural, muscle, and circulation systems. N. furzeri naturally lives in emerald ponds, with newly laid embryos preparing the coming drought by entering diapause. This diapause might last months or even years until another rain season refills the ponds with water again. During diapause, embryos show higher tolerance to stresses, and this additional period of time spent in diapause does not affect the adult lifespans. N. furzeri embryos enter diapause by obligation, but maternal effect can reverse this fate on selected embryos to skip diapause. A fine window, rather than a specific developmental point, is identified that diapause can only be initiated around the end of somitogenesis, with circulation system potentially involved. At molecular level, diapause embryos are epigenomically reprogrammed, to selectively up-express functional genes while global gene expression is in quiescence. Together, my diapause research provides a unique opportunity to understand that how a robust embryogenesis process can be stopped embryo-wide in different organs and tissues simultaneously, and importantly, for an extensive length of time without suffering the harmful consequence from various stresses and aging. [Funding sources: LSRF Fellowship & Glenn Foundation]

Divergence of the Pharyngeal Apparatus in Bluegill and Pumpkinseed Sunfish
Kelly Grant, Corrie Olson, Sofiya Bychkova, Sourabh Goyal, Brad Nowieslski, Greg Andraso
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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. 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 hoping to address the developmental mechanisms underlying these differences. In young fish, the PA bones are similar in size, shape, and tooth-type. In wild-caught pumpkinseeds, bone is rapidly added to cb5 along both the medial and lateral aspects, widening the tooth plate area. The rate of widening is greater in fish, 20 mm to 40 mm, than larger fish. We are trying to determine exactly how the early growth trajectories differ by analyzing in vitro fertilized, lab-reared larvae. In addition, we are beginning to explore gene expression in each species that might contribute to the morphological differences in the bone. 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 A (eda) and Beta-catenin (ctnnb1) and are continuing to clone other candidates. We plan to evaluate the expression of these genes in both species, as well as in their hybrids.

Funded by the Cooney-Jackman endowed professorship to GA and Gannon University Faculty Research Grant to KG.

Program Abstract #211
The Slit-Robo Pathway: Potential Regulator of Convergence and Extension in Zebrafish Axial Elongation
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Convergence and extension (C&E) is the process by which animal embryos elongate in the anterior-posterior direction. During gastrulation, mesoderm cells converge towards the midline thereby extending and elongating the anterior-posterior axis. During C&E, mesoderm cells “choose sides” establishing a boundary between axial (future notochord) and paraxial cells (future somites). Axial mesoderm cells align perpendicular to the boundary cells (becoming mediolaterally polarized), which is essential for C&E. The Zebrafish mutant ugly duckling (udu) has a C&E defect, which presents a short axis phenotype. It was found that this mutant overexpresses slit3. The Slit ligands and their Robo receptor are known to provide repulsive cues and slit3 expressed in axial mesoderm of Zebrafish embryos. For these reasons, we decided to test if we can mimic aspects of the udu phenotype by overexpressing slit3 by RNA injection. After the overexpression, the notochord length and width was found to be shorter and wider than that of the WT embryos. The injected embryos were also shorter in total length. Interestingly, embryos injected with robo3 and dominate negative robo3 RNA, produced similar phenotypes. Through live imaging, we observed the disrupted formation of notochord boundaries. Polarity of axial mesoderm cells is specifically disrupted adjacent to the notochord boundary. This shows that through improper Slit-Robo signaling, notochord boundary cells lose polarity, and C&E is disrupted. We therefore conclude that intact Slit-Robo signaling is necessary for axial elongation. Future direction of this project would be to investigate the possible mechanisms by which Slit-Robo contribute to positional cues during gastrulation.

Program Abstract #212
FingRs for in vivo Mapping of Synaptic Connectivity
Jong-Hyun Son, Tamara Stevenson, Joshua Barrios, Scott Anjewierden, James Newton, Adam Douglass, Joshua Bonkowsky
University of Utah, USA

Genetically-determined visualization of neural circuits, particularly tools to study synaptic dynamics and interactions in vivo, are necessary for efforts to build functional connectomics of the vertebrate brain. Here we describe the application of FingRs (fibronectin intrabodies generated by mRNA display) technology for use in live, transgenic zebrafish. We demonstrate and validate the use of FingRs in defined neuron subgroups under inducible control, and show their applicability for tracking synapse development.

Program Abstract #213
Spectral reprogramming of photoreceptors by vitamin A dehydrogenation
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A classic example of adaptive plasticity is the spectral reprogramming of the photoreceptors of freshwater organisms in response to changes in their light environment. This reprogramming, known as the rhodopsin-to-porphyrpsin switch, red-shifts the sensitivity of photoreceptors and is mediated by the dehydrogenation of retinol (vitamin A1) to 3,4-didehydroretinal (vitamin A2). The distinction between rhodopsin and porphyropsin was first observed in the late 1800’s,
The laminar structure of the chick optic tectum is formed as a result of proliferation of neuronal precursors in the ventricular layer followed by three temporally overlapping waves of radial migration of post mitotic cells to their destinations. Cells migrating in the first two migratory waves populate the inner and outer layers of the tectum and those migrating in the last wave populate the middle layers, the rostral-lateral-ventral part of the tectum being developmentally advanced compared to the caudal-medial-dorsal region. We found the expression of Raldh2 in a small population of cells in the anterior-lateral part of the tectum as well as in the meninges surrounding the tectum at stages HH30 to HH39. At these same stages there was strong expression of the RA degrading enzymes Cyp26B1 in the ventricular layer, and Cyp26A1 in some cells in the tectal lamina. The presence of both the source and the sink of RA in the developing tectum points towards its possible role in tectal development. I used the avian replication-competent retroviral vector to transduce Cyp26A1 in some cells in the tectal lamina. This study was funded by DST, India.

Program Abstract #214

Cell Differentiation and Morphogenesis of the Squid Eye: Establishing a Lophorochozoan model for Complex Eye Evolution and Development

Peter Sun, Kristen Koenig
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Many organisms rely on photoreception in order to survive, yet how the eye evolved is still not completely understood. As complicated as photoreceptive organs can be, they have independently evolved numerous times using multiple different optical designs. Development of the vertebrate and Drosophila complex eyes are well researched, yet a large group of organisms within the Bilateria, the Lophotrochozoa, remain largely unstudied. We can gain a greater understanding of the evolution of photoreceptive organs and how novelty evolves with a broader sampling of species across the Bilateria. The cephalopods are a member of the Lophotrochozoa and have a single-chambered eye. This is a classic case of convergent evolution as it is similar to the vertebrate eye. Despite the similar structure, the squid eye contains different cell types and has a different organization compared to the eyes of vertebrates. Our goal is to establish Doryteuthis (Loligo) pedesi as a model organism for complex eye development. We have performed histology and transmission electron microscopy on the embryonic cephalopod eye at multiple stages in development. Currently, there is little known about the process of morphogenesis and cell differentiation in the squid eye. This research enables us to better understand differentiation events of specific cells within the eye and how specific tissues are formed. Ultimately, this will enable us to better evaluate phenotypes as our work moves into performing gain and loss-of-function assays as well as understand species specific differences. This research was funded by Undergraduate Research Fellowship from the University of Texas.

Program Abstract #215

The role of retinoic acid in chick tectal laminar formation

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The laminar structure of the chick optic tectum is formed as a result of proliferation of neuronal precursors in the ventricular layer followed by three temporally overlapping waves of radial migration of post mitotic cells to their destinations. Cells migrating in the first two migratory waves populate the inner and outer layers of the tectum and those migrating in the last wave populate the middle layers, the rostral-lateral-ventral part of the tectum being developmentally advanced compared to the caudal-medial-dorsal region. We found the expression of Raldh2 in a small population of cells in the anterior-lateral part of the tectum as well as in the meninges surrounding the tectum at stages HH30 to HH39. At these same stages there was strong expression of the RA degrading enzymes Cyp26B1 in the ventricular layer, and Cyp26A1 in some cells in the tectal lamina. The presence of both the source and the sink of RA in the developing tectum points towards its possible role in tectal development. I used the avian replication-competent retroviral vector to transduce the overexpression of the dominant negative form of retinoic acid receptor, RAR-alpha to disrupt RA signaling in the tectum. We observed that disruption of RA signaling at early stages of tectal development leads to thinning of the tectal wall, with marked expansion of the tectal ventricle. Further analysis of the markers of the cellular and plexiform layers revealed a marked perturbation in the laminar organization of the optic tectum leading to disruption of the lamina ‘g’ of the SGFS and expansion of the laminae formed as a result of the early migratory waves. Analysis of expression of lamina specific markers indicates that the laminar disruption is likely to be a result of abnormal migration of postmitotic cells to their destinations. Thus RA signaling seems to be important for migration of post-mitotic tectal neurons to their destination lamina. This study was funded by DST, India.
**Program Abstract #216**

Characterization of the Meis2A Conserved Non-Coding Region m2de1

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The Meis2 gene encodes a homeodomain containing protein that acts as a Hox cofactor that regulates development in vertebrate embryos. Meis2 is also a member of the TALE superclass, or three amino acid loop extension, which is a subset of homeodomain proteins that is characterized by an extra three amino acids between two of the alpha helices of the homeodomain. We have identified four highly conserved noncoding elements associated with the vertebrate Meis2 gene and named them m2de1-4 (for Meis2 downstream element). While M2de2-4 have to date only been found in land vertebrates, m2de1 is also found in teleosts like zebrafish. The m2de1 sequence is approximately 450bp in length and its sequence and relative position to Meis2 (meis2a in zebrafish) is highly conserved amongst all vertebrates. Using the Tol2 system we have generated transgenic zebrafish in which either the zebrafish element (dr-m2de1) or mouse element (mm-M2de1) have been able to direct reporter transgene expression the mid and hindbrain of developing embryos. The m2de1 sequence was recently described by another group (Parker et al. 2011), however the expression of the reporter transgene being driven by their element was more restricted than the expression that we have observed. Upon closer examination, we have determined that our m2de1 sequence is slightly larger than reported in this publication, suggesting the possibility that their sequence does not represent the full length element. Reference: Parker et al. (2011)  Ancient Pbx-Hox signatures define hundreds of vertebrate developmental enhancers.  BMC Genomics 12:637

**Program Abstract #217**

Analysis of Bmp7 cleavage mutant mice provides strong evidence that BMP4/7 and BMP2/7 heterodimers play essential roles during mammalian embryogenesis

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Heterodimers composed of BMP2 or BMP4 together with BMP7 show a higher specific activity than do homodimers of any single subunit. Biochemical and genetic evidence suggests that these heterodimers are required for maximal activity in vivo, but definitive proof that endogenous BMP7 containing heterodimers form in mammals has not been obtained, nor is it known what roles heterodimers, as opposed to homodimers, play during embryonic patterning. BMPs are synthesized as inactive precursors that dimerize and are cleaved to generate the bioactive ligand. To examine the in vivo role of BMP7-containing heterodimers we generated knock in mice carrying a point mutation at the Bmp7 allele (Bmp7R-G) that prevents cleavage of proBMP7. This point mutation will not only eliminate BMP7 function by preventing its own proteolytic activation, but will also generate dimeric precursors that interfere with the function of any other BMP family members that normally heterodimerize with BMP7 in vivo. Whereas Bmp7 null homozygotes (Bmp7R-/ mice) survive to birth but then die shortly thereafter due to kidney defects, Bmp7R-G/G mice show reduced BMP activity in multiple organs at embryonic day (E)9.5 and die by E11 due to gross heart defects. Furthermore, whereas mice heterozygous for a null allele of Bmp7 together with a null allele of Bmp2 or Bmp4 are adult viable, compound heterozygotes carrying the Bmp7R-G allele together with a null allele of Bmp2 or Bmp4 die during early- to mid-embryogenesis with defects in ventral body wall closure and in the heart, respectively. These defects phenocopy those observed in Bmp2R-/;Bmp4R-/ compound heterozygotes. Co-immunoprecipitation assays confirm the existence of endogenous BMP4/7 heterodimers. Collectively, these studies suggest that BMP7 functions predominantly as a heterodimer with BMP2 and/or BMP4 to generate sufficient BMP activity to ensure normal development of multiple tissues during mammalian embryogenesis.

**Program Abstract #218**

bHLH transcription factors Twist1 and Hand2 are crucial for normal development of the craniofacial vasculature

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Numerous signaling pathways act concomitantly to induce morphogenesis of the lower jaw and tongue. These signals are restricted to well-defined spatio-temporal domains in the mandibular portion of the first pharyngeal arch, establishing proximal-distal organization within the arch by both inducing permissive signaling and inhibiting inappropriate signaling. Two transcription factors involved in this process are TWIST1 and HAND2. TWIST1 is primarily expressed in the intermediate domain of the mandibular arch, while HAND2 is confined to the distal domain. However, both transcription factors have a small region of overlapping expression in the distal domain. To investigate the function and relationship of
these two factors in the overlapping domain, we created mice in which both Hand2 and Twist1 were conditionally deleted in the Hand2 expression domain. In double conditional knockout (dcko) embryos, large blood sacs form in the lower jaw in addition to almost complete disruption of the lower jaw and tongue. Using immunohistochemistry, we determined that these structures were not enlarged blood vessels, nor did they arise via fluid pooling from the first pharyngeal arch artery. In addition, perfusions of a high molecular weight FITC-dextran tracer revealed that the structures were completely sealed off from the embryonic circulation by E18.5. Flow cytometry analysis of blood harvested from the sacs of E18.5 embryos showed the presence of numerous myeloid and lymphoid cell types. These changes were preceded by increased cell death of arch mesenchyme and decreased blood vessel length in E10.5 dcko embryos. In addition, sternal defects were present in dcko embryos, including bifid sternum. Together these findings indicate that TWIST1 and HAND2 have novel functions in multiple aspects of development and could potentially contribute to human birth defect syndromes involving cleft sternum and vascular malformation. This work is supported by NIH/NIDCR (DE018899).

Program Abstract #219
Speckled feathers and bladder eyes: pleiotropic effects of the almond mutation in pigeons
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The domestic rock pigeon (Columba livia) is a compelling model to understand molecular mechanisms of diversity because this species consists of over 300 different breeds with spectacularly variable phenotypes. One derived trait, “Almond,” is characterized by seemingly random sprinkling of pigmented and apigmented regions within and between feathers throughout the body. Classical genetic studies suggest Almond feather pigmentation is caused by a dominant sex-linked mutation. Additionally, these studies found that homozygous Almond males (ZZ sex chromosomes) develop severe eye defects and completely lack pigmentation, whereas hemizygous Almond females (ZW), which lack a wild-type copy of the Almond allele, do not develop these defects. This suggests that dosage of the mutant allele, rather than absence of the wild-type allele, is responsible for these eye phenotypes. We compared the genomes of Almond and non-almond pigeons to identify a candidate region on the Z chromosome. This region is characterized by a substantial increase in coverage in Almond birds, indicative of copy number variation in this region. There are 5 genes in this region, including a melanosome maturation gene. No coding changes have been found in the genes in this region, suggesting that the copy number variant underlies the pigmentation and eye defects of Almond pigeons.

Program Abstract #220
The miR-200 family regulates podocyte differentiation by targeting RSAD2
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The podocyte is a prominent cell type, which encases the capillaries of glomerulus. During kidney development, a series of changes of the morphological characteristics and cellular functions may happen in podocytes. The miR-200 family functions in various biological and pathological processes, and there is gradually accumulating evidence that they can function as potent tumor suppressors. But the underlying molecular mechanisms of miR-200 family that functions in podocyte differentiation remain poorly understood. Herein is shown that miR-200a, miR-200b and miR-429 were significantly upregulated during the differentiation of podocytes, with highest upregulation of miR-200a. In these cells, restraint of miR-200 family by RNA interference assay revealed a prominent inhibition of cell differentiation. Furthermore, microRNA and mRNA integrated analysis(MMIA) revealed radical S-adenosyl methionine domain-containing protein 2(RASD2) as a potential downstream target gene of miR 200 family. Quantitative PCR and Western blotting assay demonstrated a remarkable change of RSAD2 expression during the differentiation of podocytes. Luciferase reporter assay confirmed that miR-200a, miR-200b and miR-429 all downregulated RSAD2 expression during the differentiation of podocytes. What’s more, over-expression of RSAD2 combined with restraint of miR-200 family revealed the inhibition of cell differentiation. Together, these findings indicate that miR-200 family may potentially promotes podocyte differentiation through repression of RSAD2 expression.

Program Abstract #221
Identification of miR206-regulated pathways involved during the formation of skeletal muscle
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MicroRNAs (miRNAs) are post-transcriptional regulators that bind and inhibit translation of target mRNAs. miRNAs are important for tissue growth, differentiation, and maintenance. A subset of miRNAs behaves in a tissue-specific manner. For example, miR-1, miR-133, and miR-206 are expressed in cells destined for muscle formation. miR-206 is particularly interesting as it is exclusively expressed in vertebrate skeletal muscle. Preliminary work in our laboratory demonstrates that knock down and over-expression of miR-206 leads to abnormal muscle formation in the vertebrate embryo, *Xenopus laevis*. To further characterize the role of miR-206 in muscle formation, we are using a reporter assay that measures the relative inhibition of putative targets by miR-206 in *Xenopus* embryos. We have cloned two perfectly complimentary miR-206 recognition elements (MREs) in the 3' UTR of a GFP reporter. The mRNA encoding this reporter construct is injected into fertilized eggs along with mRNA encoding for red fluorescent protein (RFP) in order to allow us to compare the relative expression of GFP to RFP. Injected embryos are allowed to develop to the neurula stage where we then assay for relative differences in GFP and RFP expression levels. In order to find putative targets of miR-206, we utilized a comparative genomic method to query *Xenopus laevis* and *Xenopus tropicalis* cDNA library using Targetscan. We generated a short list of 20 putative miR-206 targets containing at least one miR-206 binding site. This list includes genes involved in cellular metabolism, morphogenesis, and tissue differentiation. We will use our reporter assay to validate these putative miR-206 targets and determine the molecular pathway important for establishing and maintaining the muscle lineage during vertebrate development. NIH MBRS 1SC3GM111118-01

**Program Abstract #222**

**Zygotic Genome Activation Triggers the DNA Replication Checkpoint at the Midblastula Transition**

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A conserved feature of the midblastula transition (MBT) is a requirement for a functional DNA replication checkpoint to coordinate cell cycle remodeling and zygotic genome activation (ZGA). We have investigated what triggers this checkpoint during *Drosophila* embryogenesis. We find that the magnitude of the checkpoint scales with the quantity of transcriptionally engaged DNA. Measuring RNA Polymerase II (Pol II) binding at 20-minute intervals over the course of ZGA reveals that the checkpoint coincides with widespread *de novo* recruitment of Pol II that precedes and does not require a functional checkpoint. This recruitment drives slowing or stalling of DNA replication at transcriptionally engaged loci. Reducing Pol II recruitment in *zelda* mutants both reduces replication stalling and bypasses the requirement for a functional checkpoint. This suggests a model where the checkpoint functions as a feedback mechanism to remodel the cell cycle in response to nascent ZGA. Funding: Ruth Kirschstein NRSA Postdoctoral Fellowship: F32HD072653; NIH: R37HD15587; Howard Hughes Medical Institute

**Program Abstract #223**

**High-throughput sequence stability assay identifies stabilizing and destabilizing RNA motifs involved in maternal mRNA clearance.**

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Development in all animals begins when fertilization triggers cellular reprogramming of oocyte into a zygote during the maternal-to-zygotic transition (MZT). MZT is a universal feature of animal development and constitutes massive remodeling in the transcriptional landscape. The single-cell embryo is endowed with 30-70% of all genes in the form of maternal mRNAs and proteins that drive early cell divisions, zygotic genome activation, and developmental patterning. Zygotic genome activation triggers active maternal mRNA clearance to allow the establishment of the new zygotic program. In zebrafish, miR-430 targets several hundred transcripts for clearance during MZT. Through transcriptional profiling under a variety of chemical and genetic perturbations we have found that several thousand maternal mRNAs are cleared independently of miR-430, implicating the action of an unknown mRNA clearance mechanism. To identify cis-regulatory elements that mediate maternal mRNA turnover we have developed a high-throughput strategy that allows measurements of stabilizing and destabilizing activities of 3'UTR fragments with single-nucleotide resolution. Using this technique we have identified several hundred stabilizing and destabilizing cis-regulatory elements in 550 maternal mRNAs. To determine the exact nucleotides required for mRNA turnover we have developed a novel high throughput mutational analysis and quantified the contribution of each nucleotide to its regulatory element. To assess the developmental function of maternal mRNA clearance, we genetically removed destabilizing 3'UTR regions within individual mRNAs. In this meeting we will present sequence motifs, interacting proteins, and genetic mutants for sequences that drive maternal mRNA clearance. By studying the cis-regulatory architecture mediating dramatic mRNA
turnover during embryonic development we hope to gain insight on the post-transcriptional mechanisms driving in vivo cellular reprogramming.

Program Abstract #224
Defining the Role of Melatonin in the Circadian Rhythms of Developing Zebrafish
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Intracellular circadian clocks generate rhythms with periods of approximately 24 hours. Biochemical, behavioral, and physiological circadian rhythms are present across taxa, from bacteria to humans. In zebrafish, endogenous circadian clocks are found in many different cell types and organs. This leads many to hypothesize that circadian regulation in zebrafish is decentralized. The circadian hormone melatonin is synthesized in the pineal and circulates through the bloodstream. Melatonin receptors are widely expressed throughout developing zebrafish. We hypothesize that melatonin acts to establish and synchronize circadian clocks in zebrafish tissues. To test this hypothesis, we measured the rhythmic expression of *interphotoreceptor retinoid-binding protein (irbp)*, a gene involved in photoreception, and *period 3 (per3)*, a gene involved in the circadian clock. Control embryos raised in a 14:10 hr light:dark cycle exhibited high IRBP mRNA expression during light time points and low expression during dark time points. Embryos raised in light:dark and treated with constant melatonin exhibited increased IRBP mRNA expression and the difference in expression between light and dark was reduced. Embryos raised in light:light conditions had constant high levels of IRBP mRNA, and again constant melatonin treatment caused increased expression. In contrast, *Per3* expression in melatonin treated embryos was indistinguishable from controls. This suggests that melatonin affects the expression of some circadian genes but not others. Supported through a University of Minnesota Grant in Aid of Research, Artistry, and Scholarship.

Program Abstract #225
Functional analysis of DNA methylation and demethylation during zebrafish retina development
Pawat Seritrakul, Jeffrey Gross
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Tet dioxygenases convert 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC). Little is known about the role of Tet proteins and the process of demethylation during the development of complex organs like the eye. The zebrafish retina is composed of neuronal and glial cells derived from a common pool of seemingly indistinguishable retinal progenitor cells (RPCs). Given its unique architecture and development, the zebrafish retina serves as an ideal structure in which to study the epigenetic regulation of cellular differentiation. Gene expression analyses reveal that both *tet2* and *tet3* are expressed in the ganglion cell layer and inner nuclear layer of the developing retina. To functionally test the roles of these enzymes, we generated loss-of-function mutations in the *tet2* and *tet3* genes via TALENs. Homozygous mutant embryos deficient in either *tet2* or *tet3* showed no visible phenotype. However, double mutant (*tet2-/-; tet3-/-*) embryos developed ocular defects; they are microphthalmic, lack an optic nerve, and possess reduced numbers of differentiated neurons. BrdU incorporation assays showed elevated number of proliferative RPCs, and in situ hybridization revealed expanded RPC marker and aberrant cell type-specific marker expression in double mutants. These data support a model in which *tet2* and *tet3* regulate retinal cell differentiation. At the genome-wide level, virtually nothing is known about how the DNA methylation landscape changes during the RPC to differentiated neuron transition. To address this, we performed genome-wide bisulfite sequencing and hydroxymethylation profiling, using pure RPCs isolated from vsx2:GFP embryos, which express GFP in RPCs prior to differentiation. Initial analysis revealed reduced 5mC levels and enrichment of 5hmC at regulatory regions of known RPC genes, while genes expressed in other cell types maintain high 5mC levels. Ongoing work is aimed at elucidating the epigenetic modification requirements for retina formation.

Program Abstract #226
Tissue specific regulation of Igf2r/Airn imprinting during gastrulation.
Jesse Mager, Chelsea Marcho, Ariana Bevilacqua, Kimberly Tremblay
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Appropriate epigenetic regulation of gene expression during lineage allocation and tissue differentiation is required for normal development. Genomic imprinting, defined as parent of origin mono-allelic gene expression is established by epigenetic marks arriving in sperm and egg haploid genomes. Imprinted genes tend to occur in clusters that are coordinately regulated. One such cluster includes the maternally expressed *Igf2r*, *Slc22a2*, *Slc22a3* genes and the paternally expressed long non-coding RNA *Airn*. Although it is known that *Igf2r* and *Airn* are reciprocally imprinted, the timing of imprinted expression and epigenetic modifications have not been in vivo. Here we show lineage- and temporal-
specific regulation of DNA methylation and histone modifications at the \textit{Igf2r/Airn} locus correlating with differential establishment of imprinted expression during gastrulation. Our results show that \textit{Igf2r} is expressed from both alleles in the E6.5 epiblast. After gastrulation commences the locus becomes imprinted in the embryonic lineage with the lncRNA \textit{Airn} expressed from the paternal allele and \textit{Igf2r} restricted to maternal allele expression. We document differentially enriched allele-specific histone modifications in extraembryonic and embryonic tissues. We also document for the first time allele-specific spreading of DNA methylation during gastrulation concurrent with establishment of imprinted expression of \textit{Igf2r}. Importantly, we show that imprinted expression does not change in the extraembryonic lineage even though maternal DMR2 methylation spreading does occur, suggesting distinct mechanisms at play in embryonic and extraembryonic lineages. These results indicate that similar to preimplantation, gastrulation represents a window of dynamic lineage-specific epigenetic regulation \textit{in vivo}. We are now extending these findings to examine tissue specific allele specific expression and DNA methylation throughout the genome during gastrulation.

**Program Abstract #227**

**Investigating the Role of HIF1-a in Anoxia Tolerance in Zebrafish Embryos**

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Anoxia tolerance is a survival mechanism in an environment lacking oxygen. Zebrafish embryos undergo suspended animation, which temporarily halts development and movement. It appears that survival under anoxic conditions decreases with age, possibly due to the inability of embryos to developmentally arrest. Currently, there are two main competing models regarding the molecular basis for of anoxia tolerance: an ATP-sensing and oxygen-sensing mechanism. This project aims to investigate whether an oxygen-sensing mechanism mediated by \textit{hypoxia-inducible factor 1a (hif1-a)} underlies anoxia survival. This transcription factor is stabilized under anoxic/hypoxic conditions and regulates the transcription of genes that promote survival in low-oxygen conditions. However, we hypothesize that \textit{hif1-a} may not be required for anoxia tolerance in zebrafish, as transcription of \textit{hif1-a} target genes is ATP-demanding. To test this, we will analyze the expression of the \textit{hif1-a} downstream targets \textit{insulin-like growth factor binding protein 1a (igfbp1-a)} and \textit{vascular endothelial growth factor (vegf)} that are subjected to anoxia and control embryos that were maintained under normoxic conditions. Whole-mount \textit{in situ} hybridization of control and anoxia-treated embryos will be performed to reveal the distribution and levels of candidate gene expression following 1 or 2 hours of anoxia. In addition, RT-qPCR analysis will be conducted as a quantitative measure of gene expression. Lack of transcriptional up-regulation of \textit{hif} targets would confirm our hypothesis and prompt the investigation of \textit{hif}-independent mechanisms of anoxia survival and ATP-sensing mechanism as well. Findings stemming from this research are relevant to stroke and ischemic injury, which have in common damage associated with anoxia and the sharp onset of re-oxygenation. Acknowledgements: This work was supported by the National Institute of General Medical Sciences grant 5R01-GM085290.

**Program Abstract #228**

**Notch and Hypoxia Inducible Factor Cooperate in the Generation of Hemangioblast and in Sprouting Angiogenesis**

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Adaptive responses to low oxygen tension (hypoxia) are integral to embryogenesis, tumorigenesis, and tissue ischaemia; and during embryonic development, promoting the development of the cardiovascular system. Hypoxic responses mediated by Hypoxia Inducible Factor (HIF) as well as Notch signals mediated by the ligand Delta-4 (Dll-4) stimulate the generation of cardiovascular progenitor cells, hemangioblasts, and their subsequent differentiation into haematopoietic and endothelial cells (ECs). In addition to Notch's involvement in cell-cell signaling, cell-fate determination, and binary cell fate decision, it promotes sprouting angiogenesis. Because of the known physical interaction between cleaved-Notch (NICD) and the HIFα subunit, we propose that noncanonical biochemical crosstalk between these two pathways are critical during these vascular events. We utilize a differentiation system with mouse Embryonic Stem Cells (mESC) to analyze the cooperation of Notch and HIF during the early emergence of hemangioblasts. We also manipulate the activity of HIF and Notch to dissect the effects of hypoxia on Notch-dependent sprouting of ECs. To do this we utilize genetic (DNMM) and biochemical (γ-secretase inhibitor, and hypoxia) approaches to ascertain the biological responses of our systems. Our data thus far shows differential transcriptional regulation of Notch downstream targets, specifically down regulation of Hrt-1 and Hrt-2, upregulation of Notch ligands Jag-1, and Dll-4 and down regulation of Vegf. There are also
differences in sprouting assays concerning the number of sprouts and length of sprouts under various biochemical conditions.

Program Abstract #229
Zebrafish foxk2a/b are multifunctional regulators during neural development
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The forkhead factors are a large group of transcription factors that serve various important biological roles yet many of its members are poorly understood. FOXK2 belongs to the forkhead “K” subfamily and has been associated with cellular functions such as cell cycle regulation, apoptosis and autophagy. Nevertheless the functions of FOXK2 during development have not been characterized. Using zebrafish, we found that morphants deficient in foxk2a/b suffered from slight CNS hypoplasia, owing to neural stem cells failed to exit cell cycle and differentiate. Further molecular analyses indicated that foxk2a/b might regulate the timing of neural stem cells cell cycle exit by repressing cell cycle inhibitor p21. These results suggested that zebrafish foxk2a/b are potential regulators in neural stem cells fate decisions. Taking advantages of the CRISPR technology, we have generated foxk2a/b double knockout zebrafish to further elucidate the roles of foxk2a/b throughout the development. Most of the double mutants developed normally and morphologically indistinguishable to wild type littermates, suggesting maternal foxk2a/b are more essential to early zebrafish development. Surprising, we also observed a small fraction of double mutants suffered from severe apoptosis and growth retardation, implying foxk2a/b may act as regulators but not critical determinants in cell survival. Moreover, spontaneous cell cycle reentry was observed in double mutants retinal ganglion neurons, indicating foxk2a/b functioned as cell cycle barriers for differentiated neurons. To conclude, despite zebrafish foxk2a/b are not critical components in cell cycle progression and cell survival, they act as regulators in maintaining proper timing of neuronal differentiation and help sustaining the neuronal identity by preventing cell cycle reentry. This research was supported by grants from the Research Grants Council of the Hong Kong Special Administrative Region, China (AoE/M-05/12 and CUHK 1/CRI/13G)

Program Abstract #230
Identification of Forkhead box (Fox) family members regulated by Hedgehog signaling during cleft lip pathogenesis
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The Hedgehog (Hh) signaling pathway is a conserved morphogenetic regulator critical for brain and face development. We have previously shown that in utero exposure to the Hh pathway inhibitor cyclopamine from gestational day (GD)8.25 to GD9.5 results in “non-syndromic” cleft lip with or without cleft palate (CL/P) in the mouse. This occurs via a tissue-deficiency of the frontonasal prominence-derived medial nasal process, which forms the medial aspect of the upper lip and primary palate. Here, we aimed to identify Hh target genes involved in the pathogenesis of CL/P. By in situ hybridization for the conserved Hh target gene Gli1, we found that cyclopamine exposure results in robust downregulation of Hh pathway activity in the frontonasal prominence by GD9.25. To identify Hh target genes dysregulated during initial CL/P pathogenesis, we conducted DNA microarray analysis on microdissected and pooled frontonasal prominence tissue from cyclopamine- or vehicle-exposed GD9.25 embryos. Network and clustering analysis of significant differentially expressed genes identified the enrichment of genes involved in ventral-dorsal patterning, neurospecification, and cell proliferation. In addition, the Forkhead box (Fox) family of transcription factors was revealed as a broad, downstream target of Hh signaling in CL/P pathogenesis, with 9 members downregulated in cyclopamine-exposed embryos by microarray and validated by RT-PCR. Fox transcription factors play essential roles in development and disease, partially intersecting with Hh pathway activity, and both signals are considered growth promoting in several contexts. Our study identifies multiple Fox family members as novel targets of Hh signaling during the initial pathogenesis of CL/P. Functional assessment of these genes in normal and abnormal development is a promising avenue to examine the complex etiology of human orofacial clefting. RJL and JLE are supported by R00DE022010-02 and JLE by T32ES007015-37
Program Abstract #231
Identification and characterization of a highly conserved noncoding element associated with the Meis2 gene; M2de2
Hannah Hemingway Freundlich, Kyle Nelson, Ted Zerucha
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The Meis genes are highly conserved across species and play important roles in embryogenesis. There are four known members of the Meis gene family in vertebrates, Meis1-Meis4. Because of the genome duplication event that occurred in the teleost lineage following the divergence from the lineage that would give rise to land vertebrates, zebrafish have 2 copies of the Meis2 gene, meis2a and meis2b, in contrast to the single Meis2 gene in tetrapods. We have identified four highly conserved non coding elements (CNEs) in tetrapods that we hypothesize direct Meis2 expression. We have named these m2de1-4 (for Meis2 downstream element). To date only one of these has been identified in zebrafish. The purpose of this study is to characterize m2de2 using zebrafish as a model organism. Using the Tol2 system, expression constructs containing mouse m2de2 driving expression of EGFP through the cfos minimal promoter have been constructed and microinjected into zebrafish embryos at the single cell stage. Confocal microscopy was used to determine EGFP expression at different time points post-fertilization. Expression was observed in the specific neurons in the brain of the developing zebrafish embryos in a pattern consistent with that observed for the murine Meis2 gene and also in muscle fibers in the trunk of developing zebrafish embryos.

Program Abstract #232
Characterization of a Highly Conserved Novel Meis2 Linked Gene and Protein Product
Tray Neilson, Zachary Williams, Caroline Cochrane, Brandon Carpenter, Brantley Graham, Ted Zerucha
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We have identified a novel gene linked to the Meis2 gene (meis2a in zebrafish) in all vertebrates with publicly available genome data. This gene is always located immediately downstream of Meis2 (meis2a in zebrafish) and is organized in an inverted convergently transcribed manner. Quantitative real time PCR has revealed that transcripts of this gene are maternally expressed at high levels early in zebrafish development and decrease quickly until 8hpf. The zygotic genome produces transcripts of this gene again around 12hpf and low levels of expression are then maintained through development. In situ hybridization shows that these transcripts are expressed ubiquitously early and are later localized to the developing neural tube with further restriction to the retina of the developing zebrafish at 48hpf. Using an antibody raised against a peptide portion of the predicted zebrafish protein product we have shown that the gene is translated into protein within the developing embryo and that it is expressed at various stages throughout development. Western blots show that the protein is expressed as early as 2hpf and is present in significant amounts until 24hpf, at which point its expression is significantly decreased. Immunohistochemistry on 48hpf zebrafish embryo cross-sections show that the protein is present and is highly localized to the retinal area and the optic nerve. Whole-mount in situ IHC hybridization on embryos at the 8-cell and 256 cell stage reveal expression in cell junctions. While later on in development, 24hpf and 48hpf, expression is localized in a specific anterior structure in the developing head along with the developing optic cup and brain.

Program Abstract #233
Zygote arrest (Zar) proteins regulate mRNA translation and disrupt formation of the translation initiation complex.
Jonathan Cook, Amanda Charlesworth
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Zygote arrest (Zar) proteins, Zar1 and Zar2, are found only in the germ cells of vertebrates and are required for successful fertilization and embryogenesis. Synthesis of developmentally important proteins is crucial to embryogenesis and is regulated by translation of maternal mRNAs. Zar proteins bind maternal mRNAs via a specific sequence found in the RNA called the Translational Control Sequence (TCS). It is already known that RNAs containing a TCS are translationally repressed in immature oocytes and activated in mature eggs. It is also known that Zar represses translation when tethered to reporter mRNA. The purpose of this study was to investigate how Zar proteins regulate translation. In a dual luciferase tethered assay, both Zar proteins repressed translation up to 50% in immature Xenopus oocytes and repression was relieved during meiotic maturation, consistent with translational regulation of developmentally important mRNAs. Interestingly, Zar1 required the reporter mRNA have a poly(A) tail to repress translation, whereas Zar2 did not. Proteins recovered from GST-Zar affinity purifications included many known translation factors, such as: CPEB, Rap55, DDX6, Pat1 and Vera. Co-immunoprecipitation and co-purification experiments demonstrated that in immature oocytes,
Zar interacted with eIF4E1b, 4E-T and ePAB, which are translational repression factors, but not with eIF4G, suggesting disruption of the translation initiation complex. After meiosis these interactions decreased, coincident with Zar-mediated translational de-repression. Together, these data suggest Zar proteins regulate translation by disrupting the initiation complex in immature Xenopus oocytes. Funded by American Cancer Society and University of Colorado Denver.

Program Abstract #234
Zygote Arrest Proteins Regulate Embryogenesis via Translational Control
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A Xenopus laevis oocyte embarks on its journey to adulthood when a fertilized egg begins rapidly dividing, and its incredible growth rate is maintained by alternating between mitosis and DNA synthesis, with little time and resources to dedicate to mRNA transcription. Rather, essential proteins are quickly and efficiently translated from previously placed maternal mRNAs, and the regulation of such mRNA is vital to the embryo’s viability. During embryogenesis, maternal mRNA is regulated by translational control, and zygote arrest proteins (Zar1 and Zar2) have been shown to play an essential role in this regulation. Despite Zar's crucial operations prior to the activation of the zygotic genome, many of its details have remained elusive. At present, the translation of two mRNAs that Zar2 regulates are Mos and Wee1. Yet neither Mos nor Wee1 offer adequate explanation for the observed phenotype of Zar knockout mice, indicating Zars regulate additional mRNAs. The identification of the remaining maternal mRNA transcripts Zars bind would clarify their essential role in embryogenesis. Here we use ultra-violet crosslinking and affinity tag purification to show that Zar2 proteins regulate early development and bind maternal mRNAs via a well conserved and novel cis-element, the translational control sequence (TCS). The TCS lies in the 3’ untranslated region of mRNA and is bound by Zar2 in both Mos and Wee1. We used the TCS to identify additional TCS-containing mRNAs that are key to early development. Here, we demonstrate for the first time that Zar2 binds to both the transcription factor POU5f1 and the cell cycle regulator FZR1 mRNAs in Xenopus oocytes. Funded by American Society of Cancer Research and UCD Research Award (AC), and UCD Undergraduate Research Opportunity Award (KV).

Program Abstract #235
Sulforaphane prevents ethanol-induced apoptosis in neural crest cells by increasing histone acetylation at the Bcl2 promoter
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Sulforaphane (SFN) is an isothiocyanate derived from cruciferous vegetables. SFN’s cytoprotective properties have been demonstrated in several models associated with a variety of disorders. Our recent studies have shown that SFN protects against ethanol-induced oxidative stress and apoptosis in neural crest cells (NCCs), an ethanol-sensitive cell population implicated in Fetal Alcohol Spectrum Disorders (FASD). Recent studies have demonstrated that SFN can act as an epigenetic regulator by inhibiting histone deacetylase (HDAC) activities. This study is designed to test the hypothesis that SFN can prevent ethanol-induced apoptosis in NCCs by inhibiting HDAC and increasing histone acetylation at the Bcl2 promoter. We found that exposure to 50 mM ethanol resulted in a significant increase in HDAC activities in NCCs. Treatment with SFN dramatically decreased the activities of HDAC in ethanol-exposed NCCs. We also found that SFN treatment significantly increased the expression of acetyl-histone H3 in NCCs in a time- and dose-dependent manner. ChIP-qPCR assay revealed that ethanol exposure significantly decreased acetyl-histone H3 binding to the Bcl-2 promoter, while supplement with SFN greatly reduced ethanol-induced reduction in acetyl-histone H3 binding to the Bcl2 promoter. In addition, SFN treatment restored the expression of Bcl2 and diminished ethanol-induced apoptosis in NCCs. These results demonstrate that SFN can epigenetically restore the expression of Bcl2 and attenuate ethanol-induced apoptosis by increasing histone acetylation at the Bcl2 promoter and suggest that SFN may prevent FASD through epigenetic regulation of the expression of anti-apoptotic genes. Supported by NIH grants AA021434 and AA020265 (S-Y.C)

Program Abstract #236
The Road to Reprogramming Leads to Protection
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In the auditory field, ectopic expression of transcription factors such as Atoh1 have been used to convert mammalian supporting cells into cells that express many endogenous hair cell markers. However, the reprogramming process of...
transforming supporting cells into hair cells may not be solely about genetic transformation, but also epigenetic transformation. Since HDAC inhibitors are commonly used to increase reprogramming efficiency of iPSCs, we hypothesized that an HDAC inhibitor may also increase cellular reprogramming in the organ of Corti. Adult Fgfr3^CreER^; Atoh1-HA; tdTomato mice were used to test whether systemic treatment of an HDAC inhibitor (SAHA) could improve hair cell regeneration in vivo. Systemic SAHA treatment had no detrimental impact on hearing function compared to littermate vehicle treated controls. However, when kanamycin was administered to SAHA treated mice to induce damage, SAHA treated mice had little to no hair cell loss even 6 weeks post-kanamycin treatment, whereas vehicle treated controls lost >90% of their outer hair cells. Further analysis using wild type mice revealed that SAHA protection is dependent upon both SAHA dosage and kanamycin dosage. Interestingly, dosage dependent SAHA treatment has been reported to protect against cell death in mouse models of inflammation and neurodegeneration. Similarly, the protective effect of SAHA in the organ of Corti appears to be due to the activation of Nf-κB leading to an increase in the expression of the pro-survival genes, whereas littermate vehicle treated controls do not activate Nf-κB and up-regulate pro-apoptosis genes. Our data suggests that SAHA protects against aminoglycoside antibiotic ototoxicity by regulating multiple canonical and non-canonical HDAC targets.

Program Abstract #237

A CRISPR/Cas9 approach to study the mechanism of RA-Fgf8 repression
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Retinoic acid (RA) generated by Raldh2 (also known as Aldh1a2) in the mesoderm of developing embryos controls body axis extension by repressing Fgf8 expression in cells exiting the caudal progenitor zone. RA activates transcription by recruiting coactivators to RXR-RAR heterodimers bound at RAREs, but it is unknown whether RA can directly repress transcription of target genes. In previous studies, we demonstrated that RA directly represses Fgf8 through a RARE-mediated mechanism that promotes repressive chromatin by recruiting H3K27me3, Polycomb, and histone deacetylase to the vicinity of the Fgf8 RARE in an RA-dependent manner using embryo chromatin immunoprecipitation (eChIP). Moreover, deletion of the Fgf8 RARE in transgenic embryos demonstrated that this RARE is required for RA repression of caudal Fgf8 transcription. To further understand how the Fgf8 RARE functions repressively whereas other RAREs such as the Rarb RARE function as activators, we explored additional layers of the RA-FGF repression mechanism. Our eChIP studies on tissues from E8.25 wild-type embryos reveal higher enrichment of NCoR1 and SMRT (NCoR2) near the Fgf8 RARE in trunk (where RA is present) compared to caudal progenitor zone (which is devoid of RA activity), whereas the opposite is seen for the Rarb RARE which recruits NCoR1 and SMRT in the caudal progenitor zone but not the trunk. These findings suggest an important role of these corepressors in ligand-dependent RA repression of Fgf8 during progenitor cell differentiation. We also utilized CRISPR/Cas9 genome editing to quickly create a large pool of Raldh2^-/- mutant mouse embryos for eChIP; in our first attempt at CRISPR, 26 mutants were obtained from 36 embryos generated. These embryos will be used to further study the role of corepressors NCoR1 and SMRT in RA repression of Fgf8. This work is supported by NIH grant GM062848.

Program Abstract #238

RNA-seq time course data reveals gene regulatory interactions during heart looping
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The embryonic heart initially forms as a linear tube and then undergoes extensive morphogenetic remodeling where it loops on itself to form its final shape. Concurrently, the border between the developing ventricles and atria constricts at the atrio-ventricular canal (AVC), and the endocardial cushions (future heart valves) form. To identify the Gene Regulatory Networks (GRNs) driving these complex morphogenetic events, we performed RNA-seq on embryonic zebrafish hearts collected every six hours from early heart looping (30 hpf) through complete looping and AVC formation (72 hpf). We then generated a self-organized map (SOM) to identify groups of co-regulated genes. This analysis revealed several gene expression patterns involving genes known to be involved in multiple processes, including morphogenesis, cell proliferation, transcriptional regulation of differentiation and heart chamber maturation. Individual SOM clusters are also enriched for specific transcription factor binding motifs, revealing the GRNs driving these expression patterns. These data will greatly advance our efforts to construct GRNs driving cardiac cell-type specification, morphogenesis and differentiation. This research is supported by NHLBI F32 HL115881 to JH, NHLBI Bench-to-Bassinet Consortium grant to HJY (U01 HL0981) and a core facilities support grant to NERI (U01 HL098188).
Program Abstract #239
BioTapestry: Version 7 Release and Upcoming New Features
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BioTapestry is a well-established tool for building, visualizing, and sharing models of gene regulatory networks (GRNs), with particular emphasis on the GRNs underlying development. The most recent public release of BioTapestry, Version 7, supports a web application which allows interactive, graphical GRN models to be viewed directly in a web browser. Both the sea urchin endomesoderm and recently released ectoderm interactive GRN models from the Davidson Lab at Caltech, available at http://sugp.caltech.edu/endomes/index.html, are now being hosted using this new platform. Additional published models of developmental GRNs are also available at http://grns.BioTapestry.org/. Researchers who wish to locally host an interactive GRN model can download and install the BioTapestry Web Application on a supported web server. We are now working on new features for the upcoming Version 8, which will support multiple, parallel network hierarchies. Version 8 will also include a new plugin framework, which will enable the user to visualize and compare the results of simple computational modeling of GRN behaviors in a network context. BioTapestry development is supported by NICHD through award number R01HD073113.

Program Abstract #240
Deciphering the gene regulatory network downstream of Bmp signaling during long bone development
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BMP signaling is necessary and sufficient for bone formation. However, Bmp signaling is also operational in many other tissues such as skin, kidney and intestine, which are very distinct from bone. A plausible explanation for this could be that the Gene Regulatory Network (GRN) downstream of BMP signaling is specific to a particular tissue type. Therefore, to better understand the biology of bone development and also the pathophysiology of bone related disorders, it is important to decipher the GRN downstream of BMP signaling during bone development. Towards this goal, we have established a mouse bone marrow derived mesenchymal stem cell line (mBMSC) which can be differentiated into several lineages, such as chondrogenic, osteogenic, and adipogenic. Further, this cell line also has following two unique features: first, it expresses an avian receptor (TVA) for retroviral infection, enabling an easy and stable way of gene delivery; second, this cell line can be depleted of Bmp2 and Bmp4 ligands upon treatment with 4-hydroxy tamoxifen (4-OHT), facilitating loss of function study of BMP signaling during differentiation. We have used this cell line to compare the transcriptome, using RNAseq, of the cells grown under osteogenic condition in presence or absence of BMP signaling. Using a variety of in silico analyses we have shortlisted a number of genes for investigating BMP signaling dependent transcription in developing long bones. For this purpose, we are using RNA in situ hybridization on embryonic wild type and Bmp2/Bmp4 double knockout limb skeletal sections. Using computational cluster analysis we have identified different types of expression patterns. Our list is enriched of different classes of genes such as signaling pathways related genes, transcription factors and metabolic enzymes. Based on our experimental data and in silico analysis we are developing a GRN downstream of BMP signaling during bone development.

Program Abstract #241
Mitosis-Associated Transcriptional Repression in the Drosophila Embryo
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Transcriptional repression is a pervasive feature of development. In Drosophila, sequence-specific repressors delineate stripes and bands of gene expression underlying segmentation and the specification of different cell types. Here, we employ recently developed live-imaging methods to visualize the activity of the Snail repressor, which has been implicated in a variety of developmental and disease processes including gastrulation in Drosophila and tumorigenesis in mammals. Four different Snail target enhancers were attached to an MS2 reporter gene, permitting detection of nascent transcripts via binding of a GFP::MCP fusion protein. All four transgenes exhibit broad ventro-lateral patterns of transcription in syncytial embryos. Prior to gastrulation, these patterns become restricted to the neurogenic ectoderm, due to repression by snail. Interestingly, these restricted expression profiles appear immediately after mitosis, thus providing a previously unknown link between mitosis and repression. We present evidence that the Snail repressor exploits the general transcriptional silencing at mitosis by remaining associated with nucleoplasm during chromosome segregation.
Program Abstract #242
Targeted degradation of Gadd45 mRNA by the nonsense-mediated decay pathway is essential for development and viability in Drosophila and mammalian cells
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Nonsense-mediated mRNA decay (NMD) is a cellular quality control mechanism that selectively degrades nonsense-mutation containing transcripts. NMD also has a significant role regulating normal gene expression as it degrades many native, non-mutant, mRNAs. In complex organisms such as Drosophila, zebrafish, and mammals, loss of NMD is lethal, most likely due to excess expression of specific NMD targets influencing development and viability. However, it is unknown which target(s) are responsible for NMD mutant lethality. To identify NMD targets influencing viability, we performed a genetic screen in Drosophila by using heterozygous deficiencies to suppress the lethality of a semi-viable partial loss-of-function allele of a core NMD factor. From over 400 deficiencies screened, we identified four regions where a deficiency significantly increased the percentage of viable animals. Only one known direct NMD target resides within these suppressing regions: the stress response factor Gadd45. We find that null Gadd45 mutants, and mutants for the obligate downstream signaling partner of Gadd45, Mekk1, suppress lethality resulting from loss of multiple NMD genes, including null alleles of the key NMD factor Upf1. Finally, NMD inhibition in mammalian cells has previously been shown to reduce cell viability, and we find that inhibition of a mammalian Gadd45 orthologue, Gadd45β, suppresses NMD-mediated cell lethality in mouse embryonic fibroblasts. Our findings reveal that Gadd45 is a critical NMD target, whose excess expression and downstream pathway activation is a major factor responsible for the lethality of NMD-deficient cells across species. Importantly, these results also indicate that the NMD quality control function is in itself dispensable for viability. This is the first identification of a direct NMD target that is accountable for mediating NMD defects in any animal.

Program Abstract #243
Embryonic Zebrafish (Danio rerio) as a Bioindicator of Estrogenic Activity in the Roanoke River, VA
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Estrogen pollution poses a serious threat to aquatic ecosystems and human health. Given the prevalence of endocrine disruptors in waterways around the world, we decided to examine the Roanoke River, VA for estrogenic activity. To assess estrogen pollution in environmental samples, we used embryonic zebrafish as bioindicators in two independent techniques for analysis of estrogenic activity. One technique used the Tg(5xERE:gfp) line of zebrafish, which produces the fluorescent molecule GFP upon activation of the estrogen response element, to quantify estrogenic levels. A second technique used RT-qPCR to compare gene induction levels of an estrogen sensitive gene, vitellogenin1 (vtg1), relative to that of a housekeeping gene. We collected samples along the Roanoke River downstream of the Roanoke Wastewater Treatment Plant (RWTP), downstream of the city of Salem VA, downstream of the city of Roanoke VA, and from Tinker Creek. We also acquired a direct effluent sample from the RWTP. We exposed zebrafish embryos to each water sample and measured pixel intensity due to fluorescence at 3 days post fertilization. Using the fluorescent technique, we found no estrogenic activity downstream of the RWTP, downstream of the city of Salem VA, downstream of the city of Roanoke VA, in Tinker Creek, or in the direct effluent from the RWTP. Conversely, our results using RT-qPCR suggest the presence of estrogenic activity downstream of the RWTP as well as in the direct effluent of the RWTP. Given the discrepancies between the findings of our two independent techniques for quantifying estrogenic activity, a definitive conclusion about estrogen pollution in the Roanoke River has yet to be reached. A possible explanation is that the fluorescent technique is less sensitive to environmental estrogens than is the RT-qPCR technique. Nonetheless, further studies on the estrogenic activity of the Roanoke River are needed before a valid conclusion on the subject can be reached.

Program Abstract #244
Gene transcription regulation of anterior hypothalamic development in mouse
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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 #245
Identification of target genes downstream of Semaphorin 6a/Plexin A2 signaling in zebrafish
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During organogenesis, signaling molecules guide precursor cells through coalescence and morphogenesis to maintain a cohesive group of cells throughout. Our lab has shown that the receptor PlexinA2 (PlxnA2) and its ligand Semaphorin 6a (Sema6a) are required together to maintain the correct cohesion and proliferation of precursor cells within eye vesicles during development in zebrafish. PlxnA2 and Sema6a knockdowns phenotypically show a loss of vesicle integrity and decreases in proliferation leading to smaller eyes (Ebert et al., 2014). Microarray analyses of PlxnA2 and Sema6a knockdowns were performed, with several genes showing significant up regulation. This paper aims to validate these microarray results, specifically focusing on a set of up regulated genes common to both knockdowns. Firstly, microarray gene expression changes have been validated via in situ hybridization. Secondly, we have begun and continue to investigate the functions of select genes with a focus on roles in cell migration and proliferation in the developing zebrafish eye.

Program Abstract #246
A high-throughput forward genetic screen to uncover mechanisms of myelination
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Myelin is the multi-membrane structure formed by the wrapping of glial cells around vertebrate axons. Myelin is essential for the efficient conduction of action potentials along axons; additionally, glial cells provide trophic support to neurons. Schwann cells are the myelinating glia of the peripheral nervous system (PNS), and oligodendrocytes are the myelinating glia of the central nervous system (CNS). Diseases affecting myelin can cause devastating symptoms in numerous disorders, including multiple sclerosis in CNS. Despite the importance of myelin, the genetic and molecular mechanisms that govern glial cell development and myelination are still not well understood. To identify novel regulators of myelin development and maintenance, we conducted a high-throughput three-generation forward genetic screen in zebrafish. Zebrafish develop externally and are transparent as larvae, making an ideal model in which to study myelination. Using a fluorescent reporter driving expression of myelin basic protein (mbp) to mark myelinating glia and in situ hybridization, we screened 4770 clutches, 1126 F2 families, and 680 genomes resulting in identification of 31 heritable mutations that affect myelination. These mutations exemplify the high-throughput nature of our screen as we uncovered mutations that affect both the PNS and CNS myelination, resulting in either increased or decreased mbp expression. Currently, we are employing whole genome sequencing to identify the causative mutations, and to date, we have determined 10 confirmed or potential mutations. Here, I will present two mutants identified in the screen: stl72, which exhibit decreased PNS myelination and stl287, which exhibit decreased CNS myelination. Current work to determine causative lesions and phenotypic analyses will be discussed. Unearthing mechanisms of myelination will aid in better understanding of myelin development and treatments for myelin diseases. Funding: NIH HD080601 to KRM.
Identification of a role for splicing of a specific intron in the post-transcriptional control of reporter gene expression in vivo using a novel plasmid-injection system in Xenopus laevis

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Gene regulation during axon outgrowth is mediated largely through the activity of cis-regulatory elements within the transcribed mRNA to coordinate the supply of critical cytoskeletal proteins in response to extracellular cues. Characterizing the activity of these cis-regulatory elements requires an in vivo model system in which the effects of mutating elements on expression can be rapidly screened within the intact, developing nervous system. We have established such a system for evaluating the activity of cis elements in vivo through injection of modified plasmid DNA into early stage Xenopus embryos. Injecting 2-cell stage embryos with a plasmid bearing a φC31 integrase-targeted attB element and two dual β-globin HS4 insulators flanking a reporter transgene in opposite orientations yielded persistent expression with high penetrance without having to inject the integrase. Using this method, we discovered that splicing of a specific neurofilament intron, the nefm Intron II, was essential for robust protein expression with the nefm 3′-UTR, independently of promoter or tissue type. Splicing per se of the intron was required but not sufficient to restore protein expression, neither was substituting Intron II with Intron I of the same gene, indicating preference of elements within a specific intron for promoting reporter expression in vivo. Co-immunoprecipitation with hnRNP K, an RNA binding protein required for nefm translation, indicated that splicing of the nefm Intron II promoted this protein's association with its mature target mRNA. In summary, using a simple and reliable method to evaluate the activity of cis-regulatory gene elements in an intact, developing vertebrate nervous system, we demonstrated that splicing of a specific intron was required for translational regulation by an RNA binding protein associated with the final spliced message. Supported by NSF IOS 1257449.

Regulation of lipid metabolism by the nuclear receptor DHR78
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DHR78 encodes the single Drosophila ortholog of the mammalian orphan nuclear receptors, TR2 and TR4. Recent studies of TR4 mutant mice indicate that it directs a metabolic transcriptional program, including many genes involved in lipid metabolism. These animals are lean and protected against obesity and insulin resistance when fed a high-fat diet. The mechanisms by which this nuclear receptor family regulates metabolism, however, remain unclear. Previous work on DHR78 mutants showed a severe developmental defect where larvae failed to molt their tracheal cuticle correctly and display stunted growth and roving behavior before dying during second and third instar stages. Given the importance of lipids for cuticle formation, we are testing the hypothesis that DHR78 regulates lipid metabolism, both during development and to maintain adult homeostasis. To test this model, we genetically rescued DHR78 expression in the trachea of DHR78 mutants to overcome their lethality and study the effects of this transcription factor on metabolism. Northern blot analysis of RNA samples from mutant larvae revealed reduced expression of key lipid transport genes such as lipophorin (LPP) and microsomal triacylglycerol transport protein (MTP), suggesting that DHR78 is critical for proper lipid transport. Tracheal-rescued DHR78 mutants develop into viable adults but display reduced motility and shortened lifespans. We observed reduced triglyceride (TAG) stores and reduced fertility in DHR78 mutant females, consistent with the phenotypes reported in TR4 knockout mice. Staining with Oil Red O in the ovaries reveals a potential block in oogenesis near the onset of vitellogenesis, suggesting a defect in either lipid transport or uptake into the oocytes. Together, these observed phenotypes implicate a role for DHR78 in the regulation of lipid metabolism during development and in adults. This research is supported by NIH R01 DK075607.

Deciphering the molecular forces driving collective cell migration of the nephric duct
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During embryogenesis, development of the urogenital system (UGS) begins with induction of the nephric duct (ND; or Wolffian duct) by epithelialization of an initial population of intermediate mesoderm cells. The ND is induced at a precise space and time in the developing embryo and rapidly elongate in a caudal direction along a stereotypical pathway to reach the cloaca (primordium of the bladder and urethra). A failure or inappropriate timing in ND formation, migration, guidance or insertion to the cloaca leads to several developmental defects collectively known as congenital anomalies of
the kidney and urinary tract (CAKUT). In spite of this critical importance, the molecular players that control the epithelial-mesenchymal status of ND cells and their collective migration are largely unknown. Our preliminary work evidenced a pivotal, cell-autonomous role for the transcription factor Gata3 in the regulation of the transcriptional program that controls ND morphogenesis, yet the molecular players downstream of Gata3 remain unknown. In this work, we have analyzed the expression of important regulators of epithelial-mesenchymal transition, actin cytoskeleton, as well as cell polarity and uncover the molecular signature of the elongating ND along the anterior-posterior axis. We also present high resolution live imaging of the elongation process in the mouse embryo as well as a functional screening of Gata3 target genes by the CRISPR/Cas9 genome editing technology. This work is supported by a currently operating Canadian Institutes of Health Research grant MOP-130431.

Program Abstract #251
Mical2 mutation alters actin polymerization and is associated with human ectodermal dysplasia
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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. Funding from the University of Colorado Denver AMC Pediatrics Department

Program Abstract #253
Filopodia are Largely Dispensable for Neural Crest Migration
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Neural crest (NC) cells migrate extensively along stereotypical migration routes in vertebrate embryos to form diverse derivatives, including the segmented craniofacial skeleton and peripheral nervous system. Defects in NC migration underlie many craniofacial birth defects and are co-opted during cancer metastasis, however the genetic mechanisms controlling NC migration remain incompletely understood. Filopodia protrusions concentrated at the leading edge of migrating NC cells are thought to act as “cellular antennae” that explore the environment for directional cues to ensure NC reach their correct location. To test this idea, we treated zebrafish fascin1a null mutant embryos with an F-actin polymerization inhibitor Latrunculin B, which have complete loss of robust filopodia. Surprisingly, we found that most NC cells migrate to their correct locations without filopodia. Instead, we found that filopodia are required for directional migration of a subset of cranial NC cells, resulting in asymmetric loss of individual cartilage elements derived from the first cranial NC stream. These defects were significantly enhanced by partial loss of cxcr4a or mis-expression of its ligand Cxcl12b, showing that filopodia function with chemokine signaling to promote directional migration in a subset of NC cells. Overall, our data shows that filopodia are largely dispensable for NC migration and instead are stochastically required in a subset of NC cells to enhance the fidelity of directional migration and maintain craniofacial symmetry. New preliminary data testing alternative models of filopodia-independent NC migration will also be discussed.

Program Abstract #254
miRNA Regulation of Midline Axon Guidance Genes
Tiffanie M. Dahl, Brett Milash, Tamara J. Stevenson, Jong-Hyun Son, Joshua L. Bonkowsky
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Individual molecular pathways functioning in axon guidance and synapse formation have been well described. Little is known, however, about how these different pathways can be coordinately regulated during the establishment of neuronal connectivity. During development, microRNAs (miRNAs) act in the post-transcriptional regulation of target mRNAs. We hypothesize that miRNAs play an important role in the coordinate regulation of axon guidance during development. We
have recently observed a disruption in commissural axon pathfinding in both foxp2 and isl3bx positive neurons following morpholino-induced knockdown of dicer1 in embryonic zebrafish. Morpholino-induced knockdown of dicer1 results in the global knockdown of miRNA processing from pre-miRNAs into mature miRNAs. The presence of pathfinding defects in the dicer1 morpholino injected fish supports our hypothesis of a role for miRNAs during midline axon guidance. In order to determine which specific miRNAs have important roles in midline axon guidance, we have used RNAseq analysis of mRNAs and miRNAs from embryonic zebrafish at different developmental stages, and under normoxic and hypoxic conditions. By the comparison of the expression of mRNAs; miRNAs; and of mRNAs to miRNAs; we have identified groups of axon pathfinding and synaptogenesis genes exhibiting coordinate regulation with other pathfinding/synaptogenesis genes, as well as anti-correlation with one or more miRNAs. One such group includes EphA5; plxna4; itga1; itgav; itgb3b; and miR-196a. Strong anti-correlation between mRNA and miRNA expression (a decrease in mRNA expression corresponds to an increase in miRNA expression) is expected between pathfinding/synaptogenesis genes and the miRNA(s) that regulate those genes. These anti-correlative relationships will provide the basis for our exploration of the role of specific miRNAs in the coordinate regulation of midline guidance genes during the establishment of neuronal connectivity.

Program Abstract #255
Using 4D confocal imaging to investigate ethanol induced axon pathfinding defects in zebrafish embryos
Jennifer Bonner, Aaron Beck, Andrew Ross, Jiajun Fu, Cecilia Culp, Roland Watt, Robert Lemieux, Camille Yongue, Alexander Krazinski, Flip Phillips
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In humans, in utero exposure to ethanol can lead to Fetal Alcohol Spectrum Disorder (FASD). As a disorder that comprises disparate outcomes, the mechanisms that underlie nervous system dysfunction in FASD are incompletely understood. In this study, low doses of ethanol were exposed to zebrafish embryos to elucidate specific interactions between ethanol and axon pathfinding. Embryonic zebrafish were exposed to 50-80 mM or (0.3%-0.48 %) ethanol at 2-4 hours post fertilization (hpf), until the termination of the experiment, between 24 and 36 hpf. To determine what, if any, CNS neurons are misrouted as a result of ethanol exposure, spinal neuron pathfinding was analyzed using znp-1 and anti-acetylated tubulin immunofluorescence. Embryos exposed to ethanol continuously during development exhibit shortened motoneuron axons as well as excess branching. When embryos are exposed to ethanol later in development (during axon outgrowth), motoneurons develop normally, suggesting that early developmental exposure to ethanol is critical for observed defects. To directly investigate whether exposure to ethanol can result in disruptions in axonal dynamics, we have developed methodologies to collect time-lapse confocal laser scanning microscopic data on developing axons in vivo. Future directions will focus on deconvolution of time-lapse data sets to determine axonal velocities and volumes in wild-type and ethanol treated embryos, to better understand immediate effects of ethanol exposure at the cellular level.

Program Abstract #256
The Zebrafish Diencephalic Glial Bridge is Made Up of a Heterogeneous Population of Astroglial Cells During Forebrain Commissure Formation
Caitlin Schneider1, Carla Velez1, Sarah Bashiruddin1, Kristin Alligood1, Rachael Stein1, Kimberly Johnson1, Risha Shina1, Stephen Devoto2, Chi-Bin Chien3, Michael Parsons3, Jeffery Mumm3, Michael Barresi1
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During embryonic development of the central nervous system, neurons send axonal projections across the sagittal midline to form commissures. These serve to connect the two sides of the CNS and are an integral part of its foundation. Little is known about the role of axon-glial interactions during commissure formation, and by using a simple vertebrate model, the cell types and behaviors important for wiring a brain can be parsed out. We use zebrafish to evaluate the developmental underpinnings of forebrain commissure formation. In this study, we are interested in a subpopulation of cells called astroglia and the role they play in commissure formation. Members of the Barresi lab and others have implicated astroglia, which express glial fibrillary acidic protein (gfap), an intermediate filament protein, in assisting commissure formation. Thus far, we have determined 3 distinct morphologies of gfap+ cells that are present during forebrain commissure formation. We have recreated these cell types using 3D imaging software and 3D printing. With mosaic analysis, we can evaluate what cellular behaviors these forebrain astroglia exhibit, which is accomplished through gastrula staged cell transplantation with new gfap reporter lines and live imaging with confocal microscopy. Moreover, these cell morphologies are molecularly distinct, as the Zebrafish radial fiber (zrf-1, 2, 3, and 4) antibodies differentially label gfap+ cell morphologies. While Zrf-1 recognizes gfap, it is unknown what antigens Zrf-2-4 recognize. We are currently using a combination of biochemical analyses, including Western Blot, immunoprecipitation, and LCMS to
identify the antigens of Zrf 2, 3 and 4. These approaches taken together will shed light on which cell types are important for influencing commissure formation and open new doors for the characterization of astroglial development in the forebrain. This work is funded by the NSF #1054168 and #0615594 and by the NIH HD060023 to MJ Barresi.

Program Abstract #257
An agent based model to understand the pattern of neuromast deposition in the zebrafish posterior lateral line primordium
Ajay Chitnis, Damian Dalle Nogare, Jeffery Head, Katherine Somers
Eunice Kennedy Shriver National Institute of Child Health and Human Development, USA
The posterior Lateral Line primordium (pLLp) migrates from the ear to the tip of the tail in the zebrafish embryo periodically depositing neuromasts to pioneer establishment of the posterior lateral line system. We have developed several agent-based models of the pLLp to visualize how interactions between cells coordinate morphogenesis, migration and the fate of cells in the pLLp system. In addition, we have used high-resolution time-lapse imaging to track movement, fate and lineage of every cell in the pLLp. These observations have been used to develop a quantitative agent-based model that illustrates how proliferation and a progressively shrinking Wnt system determines the deposition pattern of neuromasts from the migrating pLLp. Our studies illustrate how relatively simple agent-based models provide an effective platform to integrate what has been learnt from a wide range of experimental studies about pLLp morphogenesis and to evaluate if current hypotheses are adequate to account for the self-organization of pLLp system.

Program Abstract #258
Protocadherin10a is required for migration of neural crest-derived melanocyte precursors
Jason Williams, Christy Rossi, Laura Hernandez-Lagunas, Kristin Artinger
Cell Biology, Stem Cells and Development Graduate Program, and Department of Craniofacial Biology, University of Colorado Anschutz Medical Campus, United States of America
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 functions in 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 required for zebrafish neural crest and melanocyte precursor migration. pcdh10a is expressed in a subset of migrating neural crest cells and in dct+ melanocyte precursors during 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.

Program Abstract #259
Counterclockwise rotation movement of melanophore is generated by rotation of actin fibers
Hiroaki Yamanaka
Osaka University, Japan
In vertebrates, the left-right body axis is determined by the leftward flow, which is generated by unidirectional rotation of the cilia of the primitive node cells. It suggested that the cellular left-right asymmetry generates the left-right asymmetry of animal body. The left-right asymmetric cell behaviors have been reported in several types of cells including neutrophil-like cells, platelets, and neurons. However, the molecular mechanism and the physiological role of the asymmetric cell behaviors have not been elucidated well. In the previous study, we established an in vitro system of pigment cells to analyze the mechanism of the surface pattern of zebrafish. The pigment cells showed characteristic interaction, in which yellow pigment cells (xanthophores) chase running black pigment cells (melanophores). During this interactions, pigment cells showed obvious bias in the direction of cell movement. We observed isolated pigment cells in detail, and found that
the melanophores rotated in counterclockwise direction, and the rotation caused the biased direction of the cell movement. The direction of the rotation was not affected by the extracellular matrix, which suggested that the unidirectional cellular rotation reflected an inherent cellular chirality. Treatment with cytoskeletal inhibitors showed that the cellular rotation was generated by the actin cytoskeleton and was inhibited by the presence of microtubules. We observed the clockwise rotation of actin fibers by expressing Lifeact-GFP. The results suggested that the counter-clockwise cellular rotation was due to the counter-action to the rotation of the actin fibers.

Program Abstract #260
Mrc1 suppresses excess sprouting in the development of venous and lymphatic endothelial vessels
Tim Mulligan, Brant Weinstein
National Institute of Child Health and Human Development, National Institutes of Health, USA
The lymphatic system is critical for the circulation of immune cells, the removal of waste from interstitial spaces, and in pathology as a disseminating route for metastatic cancer cells. Recently it was discovered that the zebrafish has a functional lymphatic system, which can be visualized with transgenic fish harboring fluorescent endothelial cells. This discovery has led to interest in using the enhanced visibility of organogenesis, inherent to the early development of zebrafish, to study genes important for the genesis and maintenance of blood and lymphatic vessels. Through the use of TALEN and CRISPR mutagenesis, we discovered that one such gene, macrophage mannose receptor 1 (mrc1), is essential to limit the sprouting of endothelial cells during the formation of venous and lymphatic vasculature in early development. Mrc1 is a receptor that has been studied in macrophages for its roles in pathogen recognition, MHC presentation, and the clearance of serum glycoproteins. Its role in venous and lymphatic endothelial cells is however relatively unknown. Whole mount in situ hybridization of mrc1a and its paralog, mrc1b, revealed that these genes are robustly expressed in the venous endothelial cells of developing zebrafish prior to formation of the lymphatic vasculature and in lymphatic endothelial cells upon their specification. Determining the role of these genes in venous and lymphatic development and whether their function primarily involves pathways governing directed cell migration or more general cell motility will assist our understanding of how this critical vasculature is established and how it could be altered for therapeutic purposes in pathological situations such as cancer metastasis.

Program Abstract #261
Role of Tmem2 in organization of the extracellular matrix during cardiac fusion
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Cell movement must be precisely coordinated to facilitate proper cardiogenesis. For instance, during early steps of heart formation, bilateral groups of cardiomyocytes must move toward the midline and merge through a process called cardiac fusion in order to assemble the initial heart tube. The critical process of cardiac fusion is governed not only by the intrinsic mobility of the cardiomyocytes, but also by the composition of the extracellular environment within which they organize. However, it is not yet clear which factors act to modulate the extracellular matrix (ECM) during cardiac fusion. Excitingly, our recent findings have uncovered the novel protein Transmembrane protein 2 (Tmem2) as a crucial regulator of cardiac fusion in zebrafish. In maternal-zygotic tmem2 mutant (MZtmem2) embryos, cardiomyocytes fail to move toward the midline. Furthermore, we find irregular ECM deposition surrounding the MZtmem2 mutant cardiomyocytes, which could account for their inability to move properly. In addition to their cardiac fusion defects, MZtmem2 mutants display detachment of skeletal muscle fibers from the myotendinous junction (MTJ), in conjunction with aberrant ECM deposition at the MTJ. Together, these observations prompt us to investigate the molecular mechanism through which Tmem2 could regulate the ECM. We are currently examining whether altered forms of Tmem2 can rescue aspects of the MZtmem2 mutant phenotype. Our data indicate that the cytoplasmic domain of Tmem2 is not required for promoting cardiac fusion or stabilizing fiber attachments at the MTJ. In contrast, it appears that the Pander-like domain of Tmem2 is required for appropriate cardiomyocyte movement and has the potential to be an ECM-interacting motif. Together, our findings implicate Tmem2 as a crucial regulator of ECM organization during both cardiac and skeletal muscle morphogenesis.

Program Abstract #262
Optimizing automated cell tracking protocols to study the collective cell migration of muscle founders in Drosophila
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Caudal Visceral Mesoderm (CVM) cells, which specify the longitudinal visceral muscles that line the gut, are an excellent model system for studying cell signaling and migration in Drosophila. CVM cells make the longest migration of any Drosophila cell type in a coordinate manner as two bilateral groups. Manual tracking of CVM cells over time was instrumental in providing statistics on cell speed and migration paths that contributed to the general understanding of CVM migration mechanisms (Kadam et al., Dev 2012). Automated cell tracking would save considerable time and enable the study of more complex migratory behaviors, but it would require a greater spatial resolution than our current setup can acquire. We addressed this problem by using a high-speed Perkin-Elmer spinning disk confocal microscope to increase the axial sampling while maintaining temporal resolution. This is combined with a sensitive detector ideal for CVM cells deep in the tissue where signal output is reduced. We compared the accuracy of automated vs. manual tracking under these new imaging conditions. Optimized resolution will additionally aid in the visualization of CVM cell membranes so that we may monitor cell shape changes simultaneously with automated tracking. These developments will be important for quantitative analysis of aberrant migratory phenotypes in future experiments.

Program Abstract #263
The Role Of Wnt5 Ligand and the Ryk Family Wnt Receptors in Positioning Neurites along the Antero-posterior Axis of the Developing Drosophila Ventral Nerve Cord
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A distinctive feature of neural development is the positioning of synaptic terminals through targeted growth, termination, and branching to appropriate domains. We use the Drosophila melanogaster nervous system, and a powerful genetic toolbox that allows for the manipulation and characterisation of identifiable embryonic and larval afferents in the ventral nerve cord to study the molecular mechanisms that direct terminals to specific locations in the neuropile. The involvement of Slit/Robo and Sema/Plexin signaling in sensory termination along the medio-lateral and dorso-ventral axes, respectively, were previously reported (Zlatic et al., 2003 and 2009). However, the positional cue system that positions afferent terminals in the antero-posterior axis has been elusive. Using proprioceptive and mechanosensory afferents as in vivo cellular assays for antero-posterior axon growth decisions, we show that Wnt5 and its non-canonical tyrosine pseudokinase receptors, the Ryks, are involved in antero-posterior termination decisions. We will discuss a working model of axon guidance in the antero-posterior axis of the developing Drosophila ventral nerve cord.

Program Abstract #264
Signaling through Rickets, a G-protein-coupled receptor, is crucial for polarity and migration of the border cells in Drosophila
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Cell migration plays crucial roles during development, for instance during gastrulation and organ formation. Research clarifying mechanisms that regulate cell motility in normal developing systems has clinical relevance to understanding the nature of tumor metastasis. Migration of border cell clusters within a developing Drosophila egg chamber provides an excellent model to study coordinated cell movements. A Drosophila egg chamber is comprised of a single layer of somatic follicular epithelial cells surrounding 16 germline cells. During oogenesis, a cluster of cells within the anterior epithelium becomes specified as border cells, and delaminates from the epithelium. In this process the cells lose their normal epithelial morphology and undergo a shift in polarity organization. This cluster then extends filopodia toward the oocyte and the cells migrate as a group between germline cells of the developing egg chamber to reach the oocyte at the egg chamber’s posterior. In an EMS mutagenesis screen on chromosome 2L performed to study general follicle cell development, we isolated two alleles of the gene rickets (rk) – encoding a G protein coupled receptor. The rk alleles result in border cell migration defects in a significant fraction of egg chambers. We used the FLP/FRT system to generate mosaic egg chambers containing cells with homoyzgous mutations for rk alleles. RNAi against rk, and its ligand burs was also utilized. In rk mutants, border cells are properly specified and express the marker Slbo. Yet some single border cells tend to lag behind the main border cell cluster during migration, and in some cases, the entire cluster does not detach from the anterior, or only migrates part of the distance. The border cells also show defects in localization of apical polarity markers during migration. Our screen has thus identified a receptor with a previously unknown role in border cell migration that appears to regulate polarity and detachment of the border cell cluster.

Program Abstract #265
Aquaporin-3b participates in convergent extension
Aquaporins constitute a highly conserved family of proteins that form pores in cell membranes to facilitate rapid cellular water movement along osmotic gradients. Very little is understood concerning the roles of aquaporins during embryonic development. We have found that the Xenopus laevis aqp3b gene is expressed in a highly specific manner during early embryogenesis, both during gastrulation and neurulation. In gastrula embryos, aqp3b expression is specifically restricted to the deep cells (sensorial layer) of the blastocoel roof and the dorsal margin. Inhibiting Aqp3b translation with morpholino oligos (MO) determined that the Aqp3b protein is necessary for proper blastopore closure. Confocal microscopy revealed a lack of tissue organization and abnormal cell shape in aqp3b MO-injected gastrula embryos, where the boundary between involuted and non-involuted cells was particularly disorganized. In neurula embryos, aqp3b is expressed exclusively in the neural folds. Lower doses of aqp3b MO result in defective neural tube closure, resulting in abnormal cell shapes. Convergent extension is critical to drive mesoderm involution during gastrulation and cell movements during neurulation. Since our results indicate a role for aqp3b in both processes, we examined its role in convergent extension movements in Keller explants. aqp3b MO treated Keller explants showed convergent extension defects in which convergence was more highly affected than extension. The involvement of noncanonical Wnt signaling was demonstrated by rescue with Disheveled (DvlΔDIX). Further rescue experiments showed that Aqp3b exerted its influence through Wnt/Ca2+ signaling and not via the Wnt/PCP or Wnt/Ror2 pathways. We are exploring the role of this signaling and our continued studies are focused on defining the role of Aqp3b at the molecular and cellular level, including imaging during cell migration.

Program Abstract #266
Parallel regulation of convergence and extension by Planar Cell Polarity and notochord boundary signaling
Margot Williams, Atsushi Sawada, Chunyue Yin, Terin Budine, Lila Solnica-Krezel
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All animal embryos form an elongated body axis with a head on one end and a tail on the other. This process of axial elongation is accomplished during gastrulation by convergence and extension (C&E) of embryonic tissues, a process driven by polarized cell migration and mediolateral (ML) intercalation. Although molecular regulation of ML cell polarity in embryonic tissues is largely attributed to planar cell polarity (PCP) signaling, there is evidence that signals from the notochord boundary are also involved. The nature of this signal and its relationship to PCP are not understood. 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 body axis, abnormal notochord boundaries, and reduced ML polarity 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. Cells in PCP mutant kny/glypican4 -/- 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. MZ udu embryos also exhibit mesoderm patterning defects and reduced paraxial mesoderm. spt/tbx16 mutant embryos, which lack paraxial mesoderm, exhibit notochord boundary and cell polarity defects similar to MZ udu. Loss of spt function exacerbates axial extension defects in kny/- embryos to a similar degree as loss of udu, but does not further reduce axis length in MZ udu embryos, suggesting that udu and spt may disrupt cell polarity via a similar mechanism. We propose that Udu/Gon4l is required for proper mesoderm patterning and subsequent notochord boundary formation, which mediates a polarity cue that acts in parallel to PCP to regulate ML cell polarity underlying C&E. Funding: W.M. Keck Postdoctoral Fellowship, NIGMS

Program Abstract #267
Fibronectin is required for endoderm convergence and myocardial migration
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Fibronectin (Fn)—a major component of the extracellular matrix (ECM)—is known to promote migration of myocardial cells by regulating their epithelial organization. Here we show that Fn is also required for convergence of the endoderm, a previously unappreciated role. Our previous study revealed that the endoderm undergoes three distinct phases of movement: initial rapid convergence, which reduces the width of endodermal sheet (5-13s); slow convergence, which produces little change in endoderm width (14-16s); and slight expansion, which leads to an increase in endoderm width (after 18s). The endoderm in Fn-depleted embryos, in contrast, converged more rapidly from 7s onward, and failed to slow at 13s, such that the sheet was extremely narrow at 18s. Furthermore, we found that myocardial precursors exhibited complex and dynamic associations with the endoderm and displayed different stage-dependent migratory modes.
respectively: co-migrate with the endoderm prior to 13s, subduct from the dorsal to the ventral side of the endoderm at 13-15s, and migrate actively towards the midline from 16s. Notably, in Fn mutant embryos, the myocardial cells failed to co-migrate with the rapidly converging endoderm. Intriguingly, we found that throughout medial migration, Fn assembles the areas between myocardial cells. These findings suggest that Fn-mediated cell-ECM adhesion is critical for the association of myocardial cells with the endoderm. Furthermore, Fn-depleted myocardial cells failed to undergo subduction and the subsequent changes in cell polarity and tissue architecture required for active myocardial-cell migration. Collectively, our findings indicate that Fn is essential for myocardial migration because it regulates both endoderm convergence and interactions between myocardial cells and the endoderm. Thus, our study provides new insights into how the ECM orchestrates endoderm convergence and myocardial migration during heart-tube development.

Program Abstract #269
Grainyhead-like 2 Suppresses Epithelial-to-Mesenchymal Transition During Neural Tube Closure and Cancer Progression through Activation of Novel EMT Suppressors
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During embryonic development, epithelial cells frequently undergo an epithelial-to-mesenchymal transition (EMT) in order to leave an epithelial sheet and migrate to distal sites. This process must be highly regulated to ensure that only the correct number of cells migrate while neighboring cells are maintained as epithelial. During neural tube (NT) closure, cells fated as neural crest undergo EMT, delaminate and migrate away while the adjacent neural ectoderm and non-neural ectoderm (NNE) cells remain epithelial. Neural crest EMT has been well studied however the mechanisms preventing the neural ectoderm and NNE from also undergoing EMT are not known. Our studies show that loss of the NNE-specific transcription factor Grainyhead-like 2 (Grhl2) causes NNE cells to lose epithelial integrity and aberrantly exhibit mesenchymal characteristics. Live mouse embryo imaging shows changes in NNE structural integrity affecting the migrating neural crest cells and preventing the neural folds from meeting in the midline, resulting in a neural tube defect. To define the mechanism of Grhl2 action, NNE cells were isolated and subjected to RNA-sequencing, which identified 6 potential EMT suppressors. Knockdown of each individual gene in a cell culture system induces a phenotypic and functional EMT, suggesting that Grhl2 promotes NNE epithelial integrity in part through activating a network of EMT suppressors. Additionally, there are broader implications for human disease, as EMT in cancer is molecularly similar to that which occurs during development. Indeed, high expression of three of the genes identified here correlate with increased survival in breast cancer patients. On-going studies are using a breast cancer model in mice to determine relevance of these novel EMT suppressors as possible therapeutic targets. This work highlights not only how EMT is regulated during embryonic development, but how studies of developmental EMT can identify new genes involved in cancer progression.

Program Abstract #270
RhoA is involved in multiple events during neural tube closure
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In mammals, the neural ectoderm (NE) forms the neural tube, the non-neural ectoderm (NNE) closes over the NE. The NNE starts off surrounding and adhered to a flat NE. As closure proceeds, the NE curls up with the NNE moving to cover the closing tube. The process finishes when the NNE and NE separate from each other on each side to adhere instead with themselves. The NNE appears to reach across the gap to make first contact through a zippering process. The RhoA pathway regulates the actin cytoskeleton and is known to be involved in several well studied epithelial closure events, including neural tube closure. However, there are two epithelial closure events occurring during neural tube closure. The specific roles the RhoA pathway plays the closure of the NE and NNE are not completely understood. To test the hypothesis that the RhoA pathway has roles in both the NNE and NE, we used pharmacological inhibitors of multiple steps in the pathway in time-lapse microscopy on mouse embryos in culture. We find that inhibition of actin polymerization and myosin activation prevent normal neural tube closure and cause a reopening of the NE and NNE. Unexpectedly, we found that RhoA inhibition prevented neural tube closure and also caused partial dissociation of the NNE. Our data suggest the RhoA pathway is involved in multiple epithelial remodeling events during neural tube closure. Funding: NICHD #1R01HD081117-01A1
Program Abstract #271
Claudins coordinate signalling events at the apical cell surface during neural tube closure
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During neural tube closure regulated changes at the level of individual cells are translated into large-scale morphogenetic movements that convert the flat neural plate into a closed tube. This extensive remodelling requires the coordinated activity of ROCK signalling to regulate actin-myosin contractility that drives apical constriction at the midline and the planar cell polarity (PCP) pathway to direct the polarized cell rearrangements that drive convergent extension. During this process the integrity of the neural epithelium is maintained by intercellular junctions, the most apical of which are tight junctions. In eukaryotic epithelial cells, the claudin family of integral tight junction proteins regulate paracellular permeability, apical-basal cell polarity and cell adhesion, and link the tight junction to the actin cytoskeleton. Recently, we discovered that claudins regulate molecular and morphological changes that are essential for neural tube morphogenesis in the chick embryo. Specifically, we determined that targeted removal of 3 of the 14 claudins expressed during neurulation caused open neural tube defects in 100% of embryos. These embryos exhibited significantly reduced convergent extension in the neural plate, defects in apical constriction of cells at the median hinge point and failure of the epithelial remodelling events required for fusion of the lateral edges of the neural folds to form the closed neural tube and a continuous overlying layer of surface ectoderm. Although the neural epithelium cells still exhibited apical-basal polarity, apical accumulation and/or localization of Rho-GTPase and PCP signalling components were dramatically reduced/altered. Finally, these defects could not be rescued by folic acid. We hypothesize that the cytoplasmic tails of claudins interact with components of Rho-GTPase signalling and polarity complexes to coordinately regulate changes in cell movements and cell shape that are required for neural tube closure.

Program Abstract #272
Using zebrafish to search for enhancers regulating BMP signaling in cranial bone development
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Craniosynostosis (CS) or premature closure of the cranial sutures is a common congenital defect, which can occur as part of several syndromes, but more often is nonsyndromic. Several signaling pathways have been implicated in syndromic forms of CS, including BMP signaling. Bmp2 is expressed in osteogenic areas during suture development and inhibition of Bmp impairs suture closure while increased Bmp signaling leads to CS. In humans, activating mutations in the transcription factor MSX2, a target of BMP signaling, cause Boston type CS, while haploinsufficiency leads to delayed suture closure. However, for most cases of nonsyndromic sagittal CS, the genetic basis is not known. Genome-wide association studies (GWASs) can identify genetic risk factors in complex diseases of unknown etiology. Recently a GWAS for nonsyndromic sagittal CS identified two risk–associated loci, one near BMP2 and one within BBS9. The susceptibility locus near BMP2 likely indicates a regulatory element controlling BMP2 expression during cranial suture development. While BBS9 has no obvious biological link to CS, it has a conserved syntenic association with BMPER, which regulates Bmp signaling. We hypothesize that the risk locus within BBS9 is associated with an enhancer for BMPER. Therefore, we are evaluating conserved sequences within the 167kb association region for regulatory activity in transgenic zebrafish. Using a set of vectors incorporating ΦC31 integrase, to allow quantitative assessment of enhancer activity in a single genomic context we have identified two conserved sequences which show expression in developing cartilage. We will thus be able to evaluate candidates for the causal mutation by determining the effect of disease related SNPs on enhancer activity. This approach will be generally useful to find enhancers associated with disease risk in the large majority of GWASs in which no coding sequence changes have been identified. This work was funded by NIH grant number R01DE022955.

Program Abstract #273
A three–dimensional view of skull development and growth in normal and mutant zebrafish.
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Skull shape and function are specified by coordinated growth of multiple bones throughout development. Although the general pattern of the skull is specified during early development, the final form of the skull is established later during post-embryonic stages. In the zebrafish, some determinants of cell migration, gene function, and tissue interaction underlying the early patterning of the skull have been defined. However, later post-embryonic events that control growth and differentiation have not been well studied. To define the spatial and temporal pattern of post-embryonic skull
development and growth, we have developed novel confocal imaging techniques to monitor the dynamics of frontal and parietal bone growth in live fluorescent transgenic zebrafish. Frontal bone growth initiates around 5.7mm standard length (SL) along the taenia marginalis cartilage at the lateral boundary of the skull. The paired frontal bones grow along the existing cartilage before extending inward toward the midline to overlap and form the interfrontal suture. Parietal bone growth initiates later at 7.0mm SL along the tectum synoticum. Similar to the frontal bone, the parietal bone first grows along the tectum synoticum cartilage before expanding toward the midline. We hypothesize that skull bone growth occurs in three phases: 1) Initiation of bone growth near cartilage; 2) Directional growth of the frontal and parietal bones toward the center of the skull vault, and; 3) Suture formation which slows bone growth and links it to brain expansion. We are taking a genetic approach to screen for new mutations that affect skull development. Our description of normal bone growth will provide a critical knowledge base for characterization of mutant phenotypes identified in the screen. Funded by FaceBase / NIDCR.

Program Abstract #274
Altered methylation near Wnt9b affects facial shape and leads to cleft lip and palate in the mouse.
Rebecca Green1, Eric Schmidt1, David Aponte1, Trish Parsons1, Ralph Marcucio2, Benedikt Hallgrimsson1
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Cleft lip and palate is one of the most common human birth defects, but the underlying etiology is poorly understood. One hypothesis to explain the development of clefting is based on extreme variances in facial shape. Most embryos would fall in a normal distribution of facial shape, but embryos that fall at the extremes of this distribution would cleft. Therefore, this model predicts an association between variation in craniofacial morphology and elevated risk for cleft lip. To test this prediction, we examined craniofacial morphology in A/WySn mouse embryos during early facial development, because this line has a clefting incidence of about 20%. We did not find a normal shape distribution with clefted embryos at the extremes of population, instead we find early evidence of facial shape differences between clefting and non-clefting embryos. Further, cleft lip correlates with decreased methylation state of an A/WySn specific IAP retrotransposon insertion near Wnt9b, which is normally expressed in the midfacial primordia during upper jaw development. Variation in the retrotransposon’s methylation state also correlates with this early embryonic craniofacial shape difference. These results suggest that early alteration of key signalling pathways results in altered shape differences that lead to cleft lip and palate, likely due to alteration of proliferation and apoptosis leading to altered growth and ultimately shape. If this is the case, then various genetic and epigenetic perturbations may act through a common morphogenetic axis to produce variation in the manifestation and penetrance of orofacial clefting. Funding sources: NIH 5R01DE019638 to RM and BH and 5R01DE021708 to RM and BH and NSERC 238992-12 to BH.

Program Abstract #275
Spatial expression patterns of highly differentially expressed genes in developing mouse teeth
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Understanding the functional roles of, and interactions among, specific genes within and between dental epithelial and dental mesenchymal tissues first requires detailed characterization of their spatiotemporal expression patterns during tooth development. Recent transcriptional profiling studies of mouse molars and incisors have identified several genes previously unknown in tooth development, but their spatial locations within the tooth germ (and the cell types where these genes are expressed) are still unknown. In this study, we examine the spatial expression patterns of a set genes newly identified in developing mouse teeth. Data sets were validated by characterization of genes previously reported to have differential expression between incisor and molar teeth. For example, cntn6 was detected in the dental mesenchyme of the molar tooth germs. Expression domains of other genes, however, did not match the patterns expected from transcriptome data sets. For example, nefl is reported to be a marker of molar tooth germs, however, mRNA localization analysis in situ did not reveal expression in the molar tooth germs. By comparing spatial patterns of gene expression between incisor and molar teeth in Mus, we may begin to understand the genetic differences underlying development of single-cusped (incisors) vs. multi-cusped (molar) teeth. This work was funded by an NSF DDIG to EDW.

Program Abstract #276
Conditional loss of GPR126 in chondrocytes in mice models human idiopathic scoliosis and pectus excavatum
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Adolescent Idiopathic Scoliosis (AIS) and Pectus Excavatum (PE) are common musculoskeletal disorders, yet little is known of their pathogenesis. Scoliosis is a complex rotational deformity of the spine affecting an estimated 3% of children worldwide. PE is a congenital deformity of the anterior chest wall due the dorsal depression of the sternum, occurring in 1 in 400 births; interestingly, PE and AIS have high concomitant incidence in humans. Large-scale Genome wide association studies (GWAS) have begun to implicate important genetic loci for both AIS and PE including many candidate genes (e.g. GPR126, CHL1, LBX1, GAL3ST4). Functional validation of these candidate loci is critically important yet sorely lacking. Here we report the functional validation of a recent genome wide association study implicating GPR126 (encoding the Adhesion G protein-coupled receptor 126) in AIS. We generated mice in which GPR126 is specifically ablated in chondrocytes using Col2Cre. Col2Cre;GPR126^{fl/fl} mice are phenotypically normal at birth and begin to present with scoliosis and PE beginning at 20 days of age. Mechanistically, we observe increased apoptosis and the presence of acellular clefts in the vertebral body cartilage of Col2Cre;GPR126^{fl/fl} prior to the manifestation of the phenotype. These findings emphasize the critical role of Gpr126 function in axial cartilage tissues and provide a direct genetic link for the pathophysiology of AIS and PE.

Program Abstract #277
Study of chondrocyte intracellular density change by quantitative Stimulated Raman Scattering microscopy (qSRS)
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The major determinant of growth rate in long bones is the increase in cellular volume (hypertrophy) of chondrocytes. Recent analysis using quantitative phase microscopy (QPM) revealed that chondrocytes in growth plates undergo distinct phases of hypertrophy, where an increase of volume by swelling is followed by a stable, low intracellular density phase. The volume to density relationship is consistent throughout a variety of appendicular skeletal elements; whether the trajectory holds true for growth plate chondrocytes in axial skeletal elements, or for chondrocytes that undergo hypertrophy outside the epiphyseal plate, remains unknown. QPM requires dissociation of live chondrocytes. As a result, chondrocytes exposed to the dissociating medium are vulnerable to changes in intracellular density and damage, necessitating quick processing as well as relatively large number of cells for statistical analysis. Moreover, the spatial information is lost, and cross-examination of intracellular density with other markers from traditional tools such as immunohistochemistry is limited. Here we present a complementary method, quantitative Stimulated Raman Scattering microscopy (qSRS). qSRS directly gauges the intracellular density of protein and lipids in situ. It can measure samples in both live and fixed condition, and its label-free approach enables acquisition of additional information from conventional fluorescence microscopy from the same specimen. We performed measurements of cellular density and volume by qSRS on variety of epiphyseal plates from both appendicular and axial skeletons. qSRS results show good match to the data obtained by QPM. Moreover, it reveals subcellular features that give insight to the mechanism of swelling of hypertrophic chondrocytes; it enables direct association of intracellular density to known immunohistochemical marker expression. Finally, qSRS suggest distinct modes of hypertrophy of chondrocytes in different compartments of the cartilage.

Program Abstract #278
The role of the transmembrane proteins Lrig1 and Lrig3 in the development of the ribcage
Misty Riddle, Tony Del Rio, Lisa Goodrich, Clifford Tabin
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The Lrig gene family consists of three members in vertebrates that encode conserved single-pass transmembrane proteins with closely related extracellular domains and distinct intracellular domains. The Lrigs have been shown to regulate multiple signaling pathways (BMP, ErbB, Wnt, FGF) and can have redundant or independent functions depending on the context. Mutations in Lrigs result in a variety of developmental abnormalities but not much is known about how they regulate signaling during development. We found that mice with homozygous mutations in both Lrig1 and Lrig3 have defects in the rib cage. The ribs develop from the scleretome component of the somite and signaling from the myotome to the scleretome is important for proper rib patterning. In the Lrig1/3 mutant, the costal cartilages of the ribs are truncated, fused, and do not properly connect to the sternum. We observe aberrant sternum ossification and shape that likely results from delayed or absent connection with the costal cartilages. Ossification of the ribs appears normal in the mutants but defects in the costal cartilages are visualized as early as E13.5. While Lrig1 mutants appear similar to wild type, Lrig3 mutants have mild defects in the rib cage, including a small thirteenth rib and abnormal xiphoid process. Our current results suggest that Lrig1/3 function semi-redundantly during rib cage formation and could regulate signaling between the myotome and scleretome. We are currently analyzing the expression of Lrigs during rib specification and examining
somite differentiation in the double mutant. We will discuss our findings in the context of known regulators of skeletal development in order to understand a previously unknown role for Lrig family members in the formation of the skeleton.

Program Abstract #279
Examining the regulation of Lunatic fringe by mir-125a in the mouse segmentation clock
Kanu Wahi, Susan E. Cole
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Segmentation in vertebrates occurs as somites bud from the pre-somitic mesoderm (PSM), giving rise to the axial skeleton and skeletal muscle. This process is regulated by a "segmentation clock" that times somite formation. Genes, such as Lunatic fringe (Lfng), that are linked to the clock exhibit cyclic expression with a period that matches the rate of somite formation. However, the oscillations of clock genes slow as cells enter the anterior PSM, and no mechanism has been identified that contributes to this phenomenon. Changes in the post-transcriptional regulation of RNA turnover along the PSM provide an attractive potential regulatory mechanism. We have identified mir-125a as a regulator of Lfng expression and mRNA turnover in the chicken PSM. Interestingly, mir-125a expression appears to describe a gradient in the chicken and mouse PSM, with its expression gradually decreasing in the anterior PSM. It is possible that graded mir-125a expression could influence the changes in the frequency of Lfng oscillations in the PSM. To test effects of the mir-125a on mRNA stability in mouse embryos, we are examining the expression of Venus reporter constructs that contain either wildtype or mutant Lfng 3’UTR sequences in cell lines and in mouse embryos. To test the effects of mir-125a on mouse segmentation, we are using the CRISPR-Cas9 system to delete part of the mir-125a locus, and to target the mir-125a binding site within the Lfng 3’UTR. Our early data suggest that the Lfng 3’UTR can destabilize an exogenous transcript and that mir-125a affects turnover of transcripts that contain the Lfng 3’UTR. Venus expression is also found to differ between transgenic embryos with wildtype versus mutant Lfng 3’UTR. This work is supported by the NSF and a Pelotonia fellowship to K.W.

Program Abstract #280
Modulating miR-206 expression is essential for proper somite formation in early Xenopus laevis Development
Gabriel Peixoto, Carmen Domingo, Julio Ramirez
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Somites are bilaterally paired blocks of mesoderm that form along the anterior-posterior axis during development. In Xenopus laevis, somites consist primarily of myotome fibers that are aligned parallel to the notocord. MicroRNAs (miRNA) are small non-coding RNA molecules that function in post-transcriptional gene silencing. They inhibit translation or promote mRNA degradation generally by associating with the 3’-UTR of target mRNAs. miR-206 is a muscle specific miRNA, which has been shown to play a fundamental role in regulating muscle proliferation and maintenance in vertebrates. Preliminary results from our lab has shown that either knocking down or overexpressing levels of miR-206 disrupts muscle formation in Xenopus laevis embryos. We used a chemically synthesized duplex to test whether the miR-206 duplex can rescue the miR-206 morphant phenotype. We injected different concentration of miR-206 duplex along with miR-206 morpholino. We allow these embryos to develop and then fix and perform immunocytochemistry using 12/101, a muscle specific marker and beta-integrin antibodies. Confocal imaging of these embryos show that the miR-206 mimic does in fact rescue the miR-206 morphant phenotype thus, confirming the specific of the morpholino approach. These results indicate that fine-tuning the expression levels of miR-206 plays is important for proper muscle morphogenesis in Xenopus laevis. This research was funded by NIH MBRS 1SC3GM111118-01 and MARC fellowship.

Program Abstract #281
Signaling integration in regulating cellular specification and morphogenesis
Jia L. Song, Nadezda Stepicheva, Priya Nigam, McCann Tyler
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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 \(\beta\)-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 \(\beta\)-catenin of the cWnt pathway did not affect skeletogenesis, blockage of miRNA regulation of \(Dvl\) led to severe defects in PMC patterning, suggesting that non-canonical Wnt signaling pathways regulate skeletogenesis. 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.

Program Abstract #282
Optic cup morphogenesis requires the extracellular matrix component laminin-\(\alpha1\)
Chase Bryan, Kristen Kwan

Department of Human Genetics, University of Utah, United States

Proper morphogenesis of the eye is absolutely critical for vision, but the cell biology underlying ocular morphogenesis is poorly understood. In all vertebrates, a complex layer of extracellular matrix (ECM) has long been known to surround the optic vesicle during optic cup morphogenesis, but the role of any specific ECM component during eye development has remained elusive. In zebrafish, disruptions to the laminin-1 heterotrimer impair evagination and establishment of apicobasal polarity of the optic vesicle; retinal misorganization and lens degeneration are seen at later stages of ocular development. To determine the role of the ECM protein laminin-\(\alpha1\) during optic cup morphogenesis, we utilized 4-dimensional timelapse imaging of the zebrafish \(lama1uv1\) mutant and demonstrate that laminin-\(\alpha1\) is required for multiple steps of optic cup formation. The optic stalk does not fully constrict and the retina fails to enwrap the lens in \(lama1\) mutants, resulting in a flat retina with an aberrantly wide connection to the brain. \(lama1\) mutants display multiple apical surfaces at ectopic locations throughout the developing optic cup, suggesting that laminin is necessary to construct a single coherent apical surface. These defects could arise due to loss of cell-ECM adhesion through focal adhesions. Surprisingly, however, cell-ECM adhesion appears quantitatively unchanged in \(lama1\) mutants: measurements of cortical recruitment of the focal adhesion protein EGFP-Vinculin indicate that adhesion is unaffected by loss of \(lama1\), and is not sufficient to drive optic cup morphogenesis. We propose that laminin has a non-redundant signaling role during optic cup morphogenesis that cannot be compensated for by adhesion to other ECM proteins. Experiments are ongoing to determine how laminin signals to the developing optic cup to induce precise morphogenetic events in a spatiotemporally specific manner. This work was funded by grants from the Knights Templar Eye Foundation and March of Dimes.

Program Abstract #283
Protein Kinase A Coordinates Dorsoventral Polarity Signals Driving Inner Ear Regional Patterning
Sho Ohta, Gary Schoenwolf

University of Utah, USA

The inner ear consists of two otocyst-derived, structurally and functionally distinct regions, the dorsal vestibular and ventral auditory components. Two signals, BMP from the dorsal neural tube, and SHH from the ventral neural tube and notochord, are required to establish dorsoventral (DV) polarity of the otocyst. However, how these extracellular signals are coordinated with intracellular signals to effect DV polarization is unknown. We show that BMP and SHH activate and inactivate, respectively, protein kinase A (PKA), forming a DV gradient of activated PKA. This in turn establishes a dorsal-high to ventral-low ratio of GLI3 repressor to GLI3 full-length activator, regulating the regional expression of genes driving DV-specific morphogenesis. Thus, we identify a molecular mechanism that links extracellular and intracellular signals, coordinating DV polarity of the inner ear. Our findings offer a new understanding of how PKA activity mediates DV polarity, providing insight into the coordination of polarity signals in multiple organ rudiments, including the neural tube and somite, whose development is regulated by DV gradients of signaling molecules. Moreover, our results identify a common intersection point for coordinating BMP and SHH signaling and reveal that BMP as well as SHH signaling can be mediated by GLI.

Program Abstract #284
FGF, BMP and SHH signaling pathways regulate inner ear morphogenesis
Edgar Gutierrez, Suzanne Mansour, Lisa Urness
The vertebrate inner ear forms from a small region of thickened epithelial cells during embryogenesis. This epithelium invaginates, forming a cup-like structure on either side of the developing head that deepens and pinches off from the overlying ectoderm to form the hollow sphere of cells called the otocyst. This simple structure gives rise to the two major components of the ear, the vestibular and auditory chambers that sense movement and sound, respectively. The otocyst provides a unique system for analyzing the morphogenetic events that transform a simple epithelial embryonic rudiment into a highly complex three-dimensional organ such as the ear. Failure to undergo perfectly choreographed morphogenesis is a well-recognized cause of congenital hearing loss in humans. Bone Morphogenetic Protein (BMP), Sonic Hedgehog (SHH), and Fibroblast Growth Factor (FGF) mediated-signaling are critical for many aspects of inner ear development. We hypothesize that these signaling pathways function both in parallel and via intersecting pathways to regulate a repertoire of epithelial cell behaviors, including changes in cell shape, location, division rate, and survival, and that these behaviors drive morphogenesis to ultimately sculpt the complex inner ear labyrinth. My summer research involves characterizing the expression patterns of SHH and BMP signaling read-out genes in the otocyst. I have found several useful probes for BMP and SHH signaling, and I am testing a BMP reporter mouse, with which I will begin to examine the changes in BMP and SHH signaling in FGF-deficient otocysts. This work will provide new insight into mechanisms underlying the morphogenesis of the inner ear and ultimately be applied toward improving the prospects for diagnosis and treatment of patients with inner ear dysfunction.

**Program Abstract #285**

**FGFR2b ligands in cochlear non-sensory specification and inner ear morphogenesis**

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The vertebrate inner ear is a morphologically complex sensory organ comprised of two compartments, the dorsal vestibular apparatus and the ventral cochlear duct, required for motion and sound detection, respectively. Signaling by FGFR2b ligands is required for many aspects of inner ear development. Fgf3 and Fgf10 are required for the earliest stage of otic placode induction, but continued expression of both genes suggests additional roles during morphogenesis of the labyrinth. Loss of Fgf10 alone was implicated previously in semicircular canal agenesis. We showed that Fgf10+/embryos also exhibit a reduction or absence of the posterior semicircular canal, revealing a dosage-sensitive requirement for FGF10 in vestibular morphogenesis. In addition, we found that Fgf10−/− embryos have previously unappreciated defects of cochlear morphogenesis, including a somewhat shortened duct and, surprisingly, a substantially narrower duct. The mutant cochlear epithelium lacked Reissner’s membrane and a large portion of the outer sulcus—two non-sensory domains. Marker data indicated a dual role for Fgf10 in cochlear development: to regulate outgrowth of the duct and subsequently as a bidirectional signal that sequentially specifies Reissner’s membrane and outer sulcus non-sensory domains (Dev Biol (2015) 400:59). To address post-induction roles for FGFR2b ligands in otic development, we conditionally inactivated Fgf3 and Fgf10 using Tg-Pax2-Cre and also induced a soluble (dominant-negative) form of FGFR2b at various times and are evaluating epithelial morphogenesis, proliferation and patterns of gene expression. Our data show that Fgf3 and Fgf10 are required together to initiate both cochlear and vestibular outgrowth from the otocyst and that FGFR2b ligands are required continuously between E8.5 and E13.5 for otic morphogenesis. Progress in identifying the specific roles and targets of FGFR2b signaling in this process will be presented. Supported by NIH R01DC011819.

**Program Abstract #286**

**The impact of shear stress and reverse flow on cardiac morphogenesis and gene expression**

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Missteps in formation of the embryonic heart can have drastic consequences, making cardiac malformations a common human birth defect. During development, biomechanical factors including shear stress and reverse flow impact cardiogenesis. Shear stress is an epigenetic biomechanical force acting upon endothelial cells. Normally, a short period of reverse flow occurs prior to atrioventricular valve formation during ventricle systole and atrial diastole. The goal of our research is to investigate how altered biomechanical forces acting on endocardial cells lead to genetic responses by the heart. The mammalian zinc finger transcription factor Krüppel-like factor 2 (KLF2) responds to shear stress signals. Here, we explore the zebrafish KLF genes: klf2a, klf2b, and klf4. Whole embryo RT-PCR indicates that the three genes are expressed throughout early development, with cardiac expression present by 48 hpf. To evaluate how changes in biomechanical environments trigger altered gene expression in endocardial cells, we will use comparative qPCR to
Program Abstract #287

Trim33 is required for lineage potential of cardiac progenitor cells in the embryonic mouse heart

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Canonical Tgf-β signaling via Smads 2/3 and Smad4 is known to be essential for heart development. In vivo models of altered expression of canonical Tgf-β signaling components and progress in in vitro cardiac differentiation methods represent a useful system to investigate potential fine tuning mechanisms underlying congenital heart disease. Tripartite motif (TRIM) 33, a Smad binding protein and E3 ubiquitin ligase is shown to regulate Tgf-β signaling. Trim33 interacts competitively with pSmad2/3 affecting transcription of poised Tgf-β- dependent genes by altering epigenetic landscape and allowing Smad2/3-Smad4 complex to access Smad binding elements. In addition, Trim33 is shown to negatively regulate Wnt signaling by depleting nuclear β-catenin and countering cell proliferation in human cancer cell lines. Epiblast-specific Trim33 mouse mutants show ventricular septal defects, myocardial thinning and die around embryonic day (E)13. While mesodermal induction itself is intact in epiblast-specific Trim33 mutants, progressive differentiation of mesodermal subset is affected as shown by in vivo phenotype in myocardial tissue and upregulation of Mesp1, a marker of cardiac progenitor cells. Cardiac-specific Trim33 mutants lack obvious cardiac phenotypes indicating that Trim33 function is required between E6.5 to E7.5 in mice, when cardiac progenitor cells have relative plasticity. I have developed an in vitro system using tamoxifen inducible, Trim33-Floxed, UB Cre ERT2+ ES cells, mimicking in vivo temporal requirement of Trim33 in cardiac precursor lineage. In vitro ES cell differentiation shows that Trim33 loss results in sustained levels of pluripotency gene Oct4, indicating that Trim33 regulates progenitor cell differentiation. I am currently investigating molecular mechanisms by which Trim33 regulates Tgf-β targets and cross talk between Tgf-β and Wnt signaling in cardiac progenitor cell differentiation. (NIH R01DE013085 & NIH R01HL074862 to VK)

Program Abstract #288

Blood vessel lumen formation is regulated by Rasip1-mediated Rho GTPase signaling

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Understanding blood vessel formation at the cellular and molecular level is essential to the development of pro- and anti-angiogenic therapies. GTPases have been shown to control basic behaviors of endothelial cells (ECs) during blood vessel morphogenesis. However, the question remains open as to what processes the different GTPases control and how the GTPases are regulated. Our lab has previously discovered a protein called Rasip1 that is necessary for endothelial cell (EC) polarity and blood vessel lumen formation. We showed that Rasip1 and its binding partner Arhgap29 inhibit the activity of the small GTPase RhoA and its downstream actomyosin contractility effector non-muscle myosin IIA (NMIIA) in ECs. In addition, Rasip1 and Arhgap29 were found to positively regulate Cdc42 and Rac1 GTPases and their corresponding signaling pathways during vascular lumen formation in vitro. In this study, we test how Rac1, RhoA and Cdc42 regulate vascular lumen formation downstream of Rasip1 during vasculogenesis in vivo by creating conditional knockouts of each gene. RhoA deficient embryos were found to create large vessels with larger lumens, whereas Cdc42 deficient embryos were unable to develop lumens during vasculogenesis similar to Rasip1 null embryos. Further work demonstrates that RhoA and Cdc42 largely regulate the cytoskeleton to control blood vessel morphogenesis and polarity. Rac1 deficient embryos developed blood vessels lumens normally but possessed vascular remodeling defects. This study affirms that a balance between Rho and Cdc42/Rac1 GTPase signaling is necessary to regulate downstream signaling pathways that control blood vessel lumen formation, size, and morphology. This balance is regulated by Rasip1 via the GTPase activating protein Arhgap29 and possibly Guanine nucleotide exchange factors. This work lays the ground for future therapies that need to prevent or enhance lumen size in blood vessels to regulate blood flow. R01 HL113498-03 and R01 HL113498-03S1
The Epidermis is a Template for Development: The role of JNK organizing centers
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During development of multicellular organisms, cells within seemingly uniform cellular fields are marked for unique fates. Thus, while two cells may appear physically identical, one may be molecularly distinct from its neighbor. Understanding how differential signaling domains are partitioned within cell fields is important for appreciating how morphological processes are coordinated during development. We and others have shown that mutations in the JNK signaling antagonists (raw, ribbon [rib], and puckered [puc]) exhibit several shared loss-of-function phenotypes affecting both the embryonic epidermis (dorsal closure defects and hypotrophy of ventral denticle belts), and the peripheral nervous system (PNS; fasciculation defects and thickened and misguided axons). Given the well-documented role for raw-group genes and JNK signaling during dorsal closure, we speculated: (1) that the additional phenotypes shared by raw-group mutants are also consequences of dysregulated JNK signaling and (2) that the epidermis plays an instructive role in development, containing signaling centers mediating cell-cell communication within epidermal subdomains as well as between cells in the epidermis and adjacent tissues. Jun-active leading edge cells function as a signaling center for closure of the epidermis and for heart morphogenesis in the underlying mesoderm. We also show that Jun accumulates at previously unrecognized sites in the embryonic epidermis, including single epidermal cells lying directly above the PNS. Our demonstration that Jun activity is required for the nervous system defects observed in both raw and rib mutants indicates that as for the heart, cell signals from the epidermis to an adjacent tissue (in this case nervous system) is integral to proper development. In understanding the mechanism of action of multiple JNK signaling antagonists and their pleiotropic mutant phenotypes, we have found the epidermis itself plays an instructive role during development.

Molecular and cellular pathways triggering zebrafish fast muscle maturation
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What triggers a muscle cell precursor to mature into a muscle fiber? Zebrafish fast muscle cell (FMC) activation is triggered by slow muscle cells (SMCs), and time-lapse imaging confirms that FMCs elongate as they are passed by SMCs. Consistent with an SMC trigger, we have identified two FMC markers (EB165 and mylpfa) that are activated during SMC migration and whose expression is delayed in embryos that lack SMCs. However, other FMC markers do not require SMCs for activation. Hence, we propose that multiple cues work in concert to trigger FMC maturation. To test FMC maturation pathways, we mutated several candidate genes (brag2, cdh15, crk, crkl, kirrel3l, lbx2, mylz3, nhp211a, nhp211b, nr5a2, six1a, six1b, six4a, six4b, and tmem8c) predicted by literature to be required for early events in FMC morphogenesis. Some mutants (cdh15, mylz3, nhp211a/nhp211b, tmem8c) show defects in muscle morphology at 26 hours post fertilization (hpf), a time point after wildtype embryos complete FMC morphogenesis, yet others appear dispensable at least at this time point. We are now testing muscle morphology in our mutant collection at 20 hpf, because some mutants showing overt early defects (20 hpf) can later (26 hpf) show full or partial recovery. We also expect that some genes that are dispensable during embryonic development will be required in adult muscles. For instance, we looked at both embryonic timepoints in six1b mutants and have observed no defects, however six1b mutants show severe defects in adult muscle tissues. To test for redundancy between six-family members, we have deleted all four muscle expressed six genes (six1a, six4a, six1b, six4b), and will analyze quadruple mutants in the coming months. Our analysis has changed the way we view early FMC development, and future experiments will further test pathways that regulate the initiation of zebrafish FMC maturation. Support: NIH R01GM88041, NINDS T32 NS077984 and Pelotonia Fellowship.

Bioelectrical signal controls skin pattern formation of zebrafish
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Zebrafish (Danio rerio) have characteristic stripe pattern on their skin. This pattern is mainly composed of two types of pigment cells, black-pigmented melanophores and yellow-pigmented xanthophores. In wild-type zebrafish, black and yellow stripes are obviously separated and the boundary is distinct. Recent studies have revealed that melanophores escape from xanthophores in a contact-dependent manner, so two types of cell populations are segregated. When a melanophore contacts with xanthophores, membrane potential of the melanophore is depolarized and this membrane...
potential change is essential for escape behavior of the melanophore. In this study, to elucidate molecular mechanisms of electrical signal in the skin pattern formation, we utilized optogenetics techniques to control membrane potential of pigment cells. Light-gated cation channel, Channelrhodopsin2 (ChR2), induces depolarization in the expressing cell when stimulated by blue light. In blue light illuminating rearing condition, ChR2 expressing melanophores are inferred to be continuously depolarized and hardly change their membrane potential even when they contact with xanthophores. In fact, these transgenic melanophores did not escape from xanthophores and two-types of cell populations were intermingled in that condition. Next, we switched lighting conditions during skin pattern formation. In early stage, initial pattern formation was disturbed by blue light illumination. Then, in late stage, pattern was reconstructed in dark condition. The resulting labyrinth-like pattern is implicating that a reaction-diffusion system is viable in zebrafish skin pattern formation. In addition, here we show that artificial control of membrane potential by optogenetics tools is useful technique to analyze functions of bioelectrical signal in the field of morphogenesis, as in neurophysiology or electrophysiology.

Program Abstract #292
Emergence of a dermal cell ring in early feather development
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Avian feathers are arranged in a hexagonal pattern on the skin, which has been studied as a good model for periodic pattern formation. Feather primordia emerge from embryonic skin composed of epidermis and dermis. Histological studies have suggested that dermal cells beneath epidermis migrate and aggregate to make dermal condensations that grow into feather primordia. However, actual behavior of dermal cells during this process is largely unknown. Here we show that a particular type of dermal cell does not participate in the dermal condensation, but actively forms the dermis around the condensations. To label dermal cells in the chick skin, we electroporated Tol2 plasmids that expresses fluorescent genes under the control of CAGGS promoter into the epiblast of anterior primitive streak that differentiates into somites. Surprisingly, dermal cells in the center of dermal condensations failed to be labelled, whereas the labelled cells were detected in both inter-primordia and the periphery of dermal condensations. This result implies that dermal cells that constitute feathered skin arise either from two lineages or from cells change properties quickly in different topological position. Furthermore we observed the behavior of labelled dermal cells in the skin explant. Before dermal condensations clearly emerge, the labelled dermal cells were homogeneously distributed within the skin. As the condensations mature, the labelled cells migrated out from the center of condensations and aligned in periphery of the condensations. The configuration appears like a ring. Our results raise the possibility that dermal condensations are formed by the segregation between different types of dermal cells. Funding: JSPS fellowship (KAKENHI 13J06412).

Program Abstract #293
Dynein arms assembly and transport in mammal
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There are two types of mammalian cilia: motile and immotile cilia. Most of motile cilia, like the ones found in the trachea and brain, have a 9+2 structure with nine peripheral microtubule doublets arranged around two central microtubules. On the other hand, immotile cilia lack the central pair apparatus (9+0). Cilia have diverse functions in signaling and fluid dynamics. Immotile cilium can sense extracellular signals. Motile cilium can generate fluid movement. Defects in the function and structure of cilia result in several disorders, known as ciliopathies like bronchiectasis, male infertility, and situs inversus. To better understand the cilia functions as well as cause of cilia related diseases, we deleted the Lrrc6 (Leucine rich repeat containing 6) gene in the mouse. This gene was first identified as a gene causing cystic kidney disease in a mutagenesis screen in zebrafish. Lrrc6 null mice displayed aberrant Left-Right (LR) axis. Mutant mice also showed hydrocephalus, and exhibited immotile cilia in the node, trachea, and brain. Lrrc6 null cilia morphology was not different from WT ones, but inner structure of the cilia showed defects, such as cilia in the node and trachea lacked some outer and inner dynein arms. Dynein arms are the molecular motors essential for the ciliary beating. Both outer and inner dynein arms are composed of several subunits heavy, light, and intermediate chains. They are assembled in the cytoplasm and carried into the cilia by intraflagellar transport (IFT). Lrrc6 was located in the cytoplasm. And lacking dynein arms in Lrrc6 null cilia suggested that Lrrc6 has important roles for this dynein arm assembly and transport procedure. Recently it was reported that dynein arm assembly and transport is mediated by chaperones in cytoplasm. But the way to assemble inner and outer dynein arm components mostly remains elusive. Further analysis of Lrrc6 functions will reveal more detailed mechanisms of dynein arm assembly and transport.
Program Abstract #294

Identifying a Conserved Set of RNA-Binding Proteins Required for Dendrite Morphogenesis
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Dendrites are neuronal processes that receive and integrate synaptic or sensory signals. Thus, dendrite branching is important for covering receptive fields and establishing myriad neural connections that determine learning, memory, behavior, and adaptive responses to the environment. Defects in dendrite morphology are associated with neurological disorders, thus elucidating the molecular mechanisms that govern dendrite morphogenesis is critical. While several studies implicate transcriptional control in dendrite development, a growing body of evidence highlights the importance of posttranscriptional regulation. Since RNA-binding proteins (RBPs) mediate many posttranscriptional mechanisms, we decided to investigate the extent to which conserved RBPs contribute to dendrite development across phyla. Previously, Olesnicky et al. (2014) conducted a genetic screen of all RBP genes in the Drosophila genome and found 63 RBPs required for normal morphology in dendritic arborization sensory neurons. Building on this work, we identified 54 orthologous RBP-encoding genes in C. elegans and tested each for a role in dendrite development using the multidendritic PVD sensory neuron as a model. We found that loss or RNAi-mediated knockdown of 12 conserved RBP-encoding genes produces a reduction in the number of dendritic termini. We found that these RBPs are expressed in the PVD neuron and most regulate dendrite development in a cell autonomous manner. We infer the molecular function of each RBP from their subcellular localization and gene ontology information. Our results suggest that RNA processing, splicing, localization, stability, and translational control are all important mechanisms that contribute to dendrite morphogenesis, and we present a conserved set of RBPs that regulate these processes in diverse animal species. Since homologs of these genes are expressed in the human brain, these RBPs are candidate regulators of dendrite development in humans.

Program Abstract #295

Apoptotic Morphogenesis of Urogenital System
You Chi Tang, Katie Stewart
McGill University, Canada

Programmed cell death is a crucial and tightly regulated process during embryonic development. Among different types of programmed cell death, apoptosis is critical for the shaping of tissues (apoptotic morphogenesis). Previous research has described an exquisite system of apoptosis involved in early stages of urogenital system (UGS) maturation, in which the appropriate elimination of common nephric duct (CND) results in normal ureter-bladder connection. In this system, progressive CND elimination has been shown to strictly depend on regulated apoptosis, reaching to the highest level of 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, elsewise one would expect the duct to rupture by cell elimination, which is never observed. So far, no robust system of the CND maturation process has been described in the literature. We therefore hypothesize that an active mechanism of apoptotic cell resolution acts as a driving force during ureter maturation with three possible mechanisms that are not mutually exclusive: cell extrusion, cell rearrangement, and E-cadherin homophilic interactions. A series of experiments are then performed to visualize cell behaviors with respect to apoptotic cells during CND elimination and to deduce molecular mechanisms of ureter apoptotic morphogenesis. Since inappropriate CND apoptosis is directly linked to disease states, our findings will additionally provide better mechanistic understanding of urinary tract diseases by identifying the main driving force of ureter apoptotic morphogenesis. The project was funded by Kidney Foundation.

Program Abstract #296

Apoptotic programmed cell death in urinary tract morphogenesis
Maxime Bouchard, Katherine Stewart, Maxwell Shafer, Maya Saleh
McGill University, Canada

Programmed cell death is essential for normal tissue morphogenesis and its failure is associated with important developmental defects. In recent years, we and others have shown that the appropriate connection of the ureter to the bladder (ureter maturation) requires the removal of the intervening common nephric duct (CND), a process driven by apoptosis. We further identified LAR-family tyrosine phosphatases as necessary for this process. Embryos deficient for LAR-family phosphatases fail to correctly eliminate the common nephric duct, resulting in vesico-ureteral junction

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obstruction, hydroureter and hydronephrosis. We have now identified the molecular pathway regulated by LAR-family phosphatases in this process. We find that mouse embryonic fibroblasts (MEFs) derived from LAR-family triple knockout animals are resistant to both intrinsic and extrinsic apoptotic stimuli. This phenotype is associated with deregulated activity of the cellular inhibitor of apoptosis protein (cIAP) family, as apoptosis is rescued by co-administration of a cIAP1/2 small molecule antagonist in LAR-family deficient MEFs. Co-IP experiments demonstrate that LAR-family phosphatases directly interact with cIAP1. Importantly, the regulation of the IAP pathway is crucial during ureter maturation as both the treatment of urogenital systems with cIAP1/2 antagonist or the genetic inactivation of cIAP1 in vivo, results in an acceleration of apoptotic CND elimination associated with elevated levels of caspases 3/7. As predicted from our model, cIAP1-deficient animals result in the retrograde flow of urine from the bladder to the kidney, known as vesicoureteral reflux. In summary, the identification of disease states resulting from both reduced and increased apoptosis highlights the importance of apoptotic pathway regulation by the LAR phosphatases-cIAP1 pathway during ureter maturation. This work is supported by the Canadian Institutes for Health Research (CIHR).

**Program Abstract #297**

**Mitogen-Activated Protein Kinase (MAPK) Pathway Regulates Branching and Nephron Differentiation**

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Renal development is a multistage process where branching of the ureteric bud (UB) and nephrogenesis are tightly linked. Inductive interactions between the epithelium and mesenchyme 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 mesenchymal (MM) processes. MAPK pathway has been linked to UB branching and proliferation but regulation of extensive amount of cytoplasmic and nuclear targets suggests a more elaborate role during kidney development. 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, nephron differentiation and maintenance of MM progenitor population. We have characterized the UB epithelium specific MAPK pathway deficiency in vivo where branching, proliferation and adhesion defects, leading to kidney hypodysplasia, were found. Furthermore, our observations of MAPK pathway activity in the MM and during nephrogenesis has led us to deplete the pathway in vivo from the MM where its functions have not been previously described. MAPK pathway depletion resulted in hypoplastic kidneys with reduced nephron number and impaired kidney morphology. Our fundamental goal is to understand the vital process of nephron morphogenesis and differentiation that will ultimately determine the functional capacity of the whole kidney and the viability of the individual. Funding: Jane & Aatos Erkko Foundation, Sigrid Juselius Foundation, Munuissäätiö

**Program Abstract #298**

**Gain-of-function genetic studies for the role of Ret signaling in the behavior of ureteric bud tip cells during branching morphogenesis.**

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The renal collecting system develops from a primary ureteric bud (UB), emerging from the caudal end of the nephric duct (ND) in response to cues from the adjacent mesenchyme. The UB then branches numerous times at its tips, to generate the complex architecture of the mature collecting system. One cue necessary for kidney development is GDNF (expressed by the mesenchyme) and its receptor, Ret (expressed by the ND & UB tips), since in their absence no kidneys form, or they are severely hypoplastic. During primary UB formation, variations in the levels of Ret signaling regulate complex cellular rearrangements. Mosaic loss-of function studies show that Ret/-cells are unable to contribute to the primary UB tip (Chi et al., 2009) and, separately, that Ret/-cells lose the ability to remain at the UB tips during later rounds of branching (P. Riccio and F.C., submitted). To further investigate the role of Ret signaling in UB cell behaviors, we are performing gain-of-function studies to complement our loss-of-function experiments. We have generated a series of transgenic mice to conditionally express 1) wild type Ret, 2) a constitutively active form (Ret-Men2a), or 3) a constitutively active, ligand-insensitive form (Ret-PTC2). These alleles are knocked-in to the *Rosa26* locus, allowing unrestricted, conditional expression in recombined cells. We can also track each manipulated cell, and its progeny, using a nuclear-localized reporter (H2B:tdTomato) – which we introduced by a viral 2A peptide. Using these mice, we ask how
forced expression of Ret (the various forms) influences UB cell behavior. Preliminary data indicate that the forced expression of Ret-PTC2 (beginning at E12.5) in rare UB tip cells generates large clones, nearly all of which remain exclusively at the UB tips (at birth). Further analysis of clones expressing each of the Ret variants should allow us to address the potential role of chemo-attraction by GDNF, in the behavior of Ret-expressing UB tip cells.

Program Abstract #299
Dynamin-mediated endocytosis is required for tube closure, cell intercalation, and biased apical expansion during epithelial tubulogenesis in the Drosophila ovary
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For metazoans to attain a complexity beyond a few hundred cells and support differentiated tissues, multicellular, epithelial tubes are indispensable. Epithelial tubes serve critical roles both during embryonic development and as the infrastructure for mature organs and tissues. To characterize the cellular behaviors and molecular mechanisms required for the morphogenesis of epithelial tubes (i.e., tubulogenesis), we have turned to the D. melanogaster ovary. In this context, somatic epithelia surrounding each developing egg chamber pattern, form, and extend a pair of unelaborated epithelial tubes in the absence of cell division or apoptosis: the dorsal appendage (DA) tubes. This genetically tractable system allows us to assess the relative contributions that coordinated changes in cell shape, adhesion, orientation, and migration make to epithelial tubulogenesis. We find that Dynamin, a conserved and critical regulator of endocytosis and the cytoskeleton, serves crucial, endocytic roles throughout DA tubulogenesis. We demonstrate novel requirements for Dynamin in 3 distinct aspects of DA tubulogenesis: DA-tube closure, DA-tube-cell intercalation, and biased apical-luminal expansion. We provide evidence that Dynamin promotes these processes by facilitating endocytosis of both apical, E-Cadherin-based, cell-cell adhesions, and basal, Integrin-based, cell-matrix adhesions. Furthermore, we find that regulating precise levels of E-Cadherin and specific Integrin subunits impact DA tubulogenesis, and account for distinct features of DA tube morphology. Thus, our studies identify novel roles, and expand upon established roles, for Dynamin in epithelial tubulogenesis, and emphasize the crucial importance of regulating and remodeling cellular adhesions during this process. This work was supported by NIH R01 GM079433, NSF GRF DGE-0718124, and UW Provost Bridge Funds.

Program Abstract #300
Glandular morphogenesis in the mouse uterus
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The focus of this study is on the development of the endometrial glands of the mouse uterus. Endometrial glands secrete substances that are essential for uterine receptivity to the embryo, implantation, conceptus survival, development and growth. In models where uterine glands have been knocked out, females are infertile due to defects in implantation and early pregnancy loss, suggesting its role for fertility. Adenogenesis is the formation of glands within the stroma of the uterus. Uterine glands form 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 thought to be highly coiled and branched. However, the morphology of mouse glands is poorly understood because it is based on two-dimensional histology. We are using various imaging methods to determine the 3D structure of forming glands and gland morphology during the estrous cycle. By labeling the uterine epithelial cells with an antibody and/or genetically with a conditional lacZ reporter (R26R-lacZ; Wnt7a-Cre), we have generated 3D models of uterine glands, using Optical Projection Tomography. We have shown that SOX9 is expressed in the glandular epithelium in mouse and human uterus. Analysis of Sox9-eGFP in the mouse uterus has provided insights into the structure of developing glands and their distribution. Cumulatively from our reconstructions, we conclude that glands are “buds” at P8 and become elongated tubes at P11. By P21, these elongated tubes will be curved, whereas in adults, glands will be branched and coiled, depending on the stage of the estrous cycle. To examine glands at single cell resolution, we are using a conditional fluorescent reporter (Wnt7a-Cre; R26R-RG) to quantify the number of cells in developing and mature glands. Our studies should provide insights into the cellular mechanisms that result in the development of uterine glands.

Program Abstract #301
Porcupine Dependent Wnt Signaling Plays an Essential Role in Murine Uterine Gland Development and Stromal Maintenance.
Program Abstract #302
Quantifying spatial patterns of cell proliferation during monopodial branching in the developing chicken lung
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Several organs in the body consist of branched networks of epithelial tubes. During development these ramified structures form via a process known as branching morphogenesis. In the embryonic lung, airway branching is highly stereotyped and involves reciprocal signaling between the airway epithelium and surrounding mesenchyme. Many of the molecular components important for the branching process have been identified, but the physical mechanisms which link these molecules to actual changes in tissue morphology remain unclear. During airway branching, it is generally thought that localized cell proliferation within the epithelium initiates the outgrowth of new branches, but clear experimental evidence in support of this mechanism is lacking. Using embryonic chicken lungs, we created 3D reconstructions of confocal immunofluorescence stacks to quantify patterns of proliferation in both the branching airway epithelium, as well as the surrounding mesenchyme. The nuclei of proliferating cells were labeled via EdU incorporation, and epithelial proliferation was distinguished from that of the mesenchyme by staining for E-cadherin. Our data do not show elevated proliferation in the bud-forming region of the epithelium. Rather, increased rates of cell division were observed in the mesenchyme adjacent to newly forming epithelial buds. These data will be combined with mechanical modeling to determine how these spatial patterns of cell proliferation might contribute to the branching process. This work was supported by the NSF (IOS-1239422), the NIH (GM083997, HL110335, and HL118532), the David and Lucile Packard Foundation, the Alfred P. Sloan Foundation, and the Camille & Henry Dreyfus Foundation.

Program Abstract #303
Notum, a Wnt/Beta-catenin modulator, is dynamically expressed in developing conducting airways.
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The trachea, which is part of the conducting airways, is patterned across the dorsal ventral axis, presenting cartilage in the ventral side and muscle in the dorsal aspect. Tracheomalacia is a congenital disorder in which the supportive cartilage rings of the trachea are missing. Patients usually have trouble breathing, decreased airflow and in some cases the condition causes death. Therefore, by defining the molecular mechanisms underlying tracheal cartilage development, better treatments and diagnostic procedures can be implemented. Wntless (Wls) and its associated Wnt pathways are critical for embryonic development, including the patterning of the upper airways. Deletion of Wls in respiratory endoderm results in tracheomalacia and ectopic muscle due to loss of dorsal-ventral patterning. We identify Notum, a deacetylase required for Wnt ligand morphogen gradient, as a target of endodermal Wls. Notum’s role in respiratory tract is
unknown. *Notum* expression is reduced after deletion of *Wls* from the pulmonary epithelium, and simultaneously, expression of Bmp ligands and modulators required for cartilage formation was altered. The expression of *Notum* in pre-chondrogenic mesenchyme overlaps with *Bmp4* and *Wnt5a*, sites where tracheal cartilage will be formed; *Notum*'s mesenchymal ventral expression contrasts to the circumferential expression of Wnt ligands in tracheal epithelium. *Notum* expression in tracheal mesenchyme is also diminished after mesenchymal deletion of Sox9. As tracheal development proceeds, *Notum* expression becomes restricted to sub-epithelial mesenchymal and to the periphery of the developing cartilage. We proposed that Notum influences the dorsal ventral patterning of the trachea by generating a barrier for Wnt ligands produced and secreted by the tracheal epithelium. Thus, Notum may promote cartilage formation and prevent ectopic smooth muscle formation in the ventral side of the trachea. This work was partially supported by NIH K01HL115447 to DS.

**Program Abstract #304**

**Mechanical coupling to extra-embryonic tissues directs progenitor cell migration and protects cell fate**  
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Tissue-tissue interactions are critical in development as they can dictate the position and fate of progenitor cells. Here we show that mechanical coupling between progenitor cells of the zebrafish Kupffer’s vesicle (KV) and two extra-embryonic tissues, the yolk syncytial and enveloping cell layers, serves a dual role in development: to direct progenitor cell migration and protect cell fate. Pulling forces exerted by the actomyosin motor driving epiboly of extra-embryonic tissues combined with Cadherin-1 interprogenitor cell-cell adhesion drive the collective migration of KV progenitors towards the posterior end of the embryo. Notably, when KV progenitors escape from the collective cell group they transform into endoderm. Our findings reveal a tissue-guided developmental strategy that secures the correct positioning and specification of progenitor cells in a region of the embryo where they differentiate into a functional organ.

**Program Abstract #305**

**Reverting Lateral Line Supporting Cells to a Migratory Mesenchyme Capable of Reconstituting Neuromasts and Generating New Hair Cells**  
Jason Meyers, Joseph Porco, Eleanor Walker, Parker Lewis  
Colgate University, USA  
The lateral line is a collection of small sensory organs called neuromasts that run along the body of fish and amphibians. The initial deposition of neuromasts occurs from a primordium that begins near the ear and migrates down to the tail between 24-48 hours post fertilization. Cellular proliferation and the deposition of clusters of cells as the primordium migrates are coordinated by Wnt and FGF signaling. Wnt signaling is also important in controlling proliferation of cells in the deposited neuromasts during initial differentiation into hair cells and support cells, ongoing-growth, and regeneration following lesion. Given the interaction between Wnt and FGF signaling in initial patterning of the primordium and protoneuromast deposition, we have examined whether FGF signaling interacts with the Wnt pathway to coordinate later stages of neuromast development, growth and regeneration. Simultaneous activation of Wnt signaling and inhibition of FGF signaling caused supporting cells within the neuromast to revert to a highly proliferative migratory mesenchymal state. The supporting cells lost N-cadherin expression, and the neuromasts lost their compact morphology as the cells migrated bidirectionally along the lateral line. During the dissolution of the neuromasts, hair cells were extruded from the epithelium, and the supporting cells re-express genes associated with the migratory primordium. Upon washout of the drugs, the cells reconstituted neuromasts along the lateral line, and produced new cohorts of hair cells, though the positioning and polarity of the neuromasts are abnormal. These data suggest that Wnt and FGF signaling work together in later stages of neuromast differentiation to maintain the epithelial organization of the neuromast and the balance between proliferation and differentiation. It also indicates that supporting cells within the deposited neuromast can be reprogrammed to an earlier migratory mesenchymal state by manipulating these signals.

**Program Abstract #306**

**Trans-cellular activation of transcription in biological systems**  
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University of Utah, USA  
Visualization and manipulation of neural circuitry has remained a vexing problem in neuroscience. Our goal is to translate
and implement a novel methodology, trans-cellular activation of transcription (TCAT), to both genetically label cell-cell interactions, as well as to induce gene expression in interacting cells to assemble a functional circuit map of connectivity during development. TCAT is based on components from the receptor/ligand pair of Notch/Delta. Upon ligand binding to receptor, the intracellular domain of Notch is cleaved and translocates to the nucleus. We replaced the intracellular domain of Notch with the yeast transcriptional activator Gal4, so that we can express transgenes at the Gal4-binding site UAS. For TCAT we used the homologs LAG-2 (Delta) and LIN-12 (Notch) from the nematode C. elegans to prevent cross-reactivity with the endogenous zebrafish proteins. We overcame species-incompatibility of the protease cleavage reaction necessary for TCAT by developing a chimeric system: receptor-ligand binding specificity is maintained using C. elegans LAG-2 and LIN-12 binding domains, but with substitution of the zebrafish Delta and Notch signal sequences and transmembrane domains. In proof-of-principle experiments, we found that chimeric LAG-2/Delta tagged with red fluorescent protein (LADR) and LIN-12/Notch (LINch) activates transcription in different cell types in transient injections. To demonstrate the wide applicability of TCAT for a range of biological questions we have generated constructs expressing LINch or LADR in different cell types including retinal ganglion cells, dermal progenitor cells, epidermal cells, and in different neuron sub-types. TCAT can be used to drive expression of any desired gene; the method allows both labeling and manipulation in a variety of biological systems. We anticipate that TCAT may represent a significant technical innovation for mapping and understanding brain circuits in zebrafish and other vertebrate systems.
Program Abstract #309
Generating morphological variation in the gut
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The gastrointestinal (GI) tract is composed of an essential set of organs necessary for digestion. Beginning as a simple tube of endoderm surrounded by mesenchyme, the early gut is patterned along the A/P axis into the foregut, midgut, and hindgut. During development, these compartments acquire unique topologies on their luminal surfaces that are associated with distinct digestive functions. While many studies have addressed how each compartment is specified, it is unknown how distinct luminal topologies are generated along the A/P axis of the digestive tract. Work from our lab has recently showed that smooth muscle within the small intestine is required to constrain the growth of the endoderm and mesenchyme to form villi. We are now addressing if physical forces within the gut tube are also directing the morphogenesis of unique luminal topologies in the foregut and hindgut. We have begun characterizing mesenchymal development of these compartments and measuring physical properties within the different tissue layers over time. Our initial results highlight the differences in smooth muscle development along the A/P axis of the GI tract and show a correlation between luminal topology and smooth muscle formation. Additionally, analysis of circular and longitudinal residual strain within segments of the GI tract reveals that the physical forces within the gut tube vary along the A/P axis and with time, suggesting that as in the midgut, smooth muscle may be constraining epithelial and mesenchymal growth within these other compartments. In order to directionally constrain growth, the layers of smooth muscle must be arranged into both circumferential and longitudinal layers at specific times during development, and our ongoing experiments indicate potential molecular and mechanical factors that are directing this process. Further investigation into these findings will provide insight into the developmental mechanisms that shape the gut tube.

Program Abstract #310
Iroquois 3/5 regulate cell division and morphogenetic cell movements
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The relationship between morphogenetic mechanisms that generate organ primordia and subsequent pattern formation is intriguing but unclear. Iroquois (Irx) 3 and -5 TALE class homeodomain proteins are best understood as transcription factors that regulate developmental processes including skeletal pattern formation. Interestingly, we found that Irx3/5 also help to shape the early limb bud. To identify binding partners of Irx3 and -5 that might regulate morphogenesis, we performed in vitro BioID mass spectrometry. We took a particular interest in multiple proteins associated with Irx3 and -5 that regulate sister chromatid segregation, including subunits of the Cohesin complex (eg. Smc1a), Cux1 and Kifc1. These proteins colocalised and physically associated with Irx3 and -5 in vivo as shown using STED super-resolution microscopy and proximity ligation assay, respectively. Surprisingly, abundance of Smc1a and Cux1 was dependent upon Irx3/5 in a transcriptionally independent manner. Consistent with a role in Cohesin function, live imaging of Irx3/5 mutant mouse embryos that express an H2B-GFP transgenic reporter revealed the presence of anaphase chromatin bridges in early anterior limb bud mesoderm where Irx3/5 are normally expressed. These findings suggest that Irx3/5 stabilise a complex that regulates sister chromatid segregation. Some of these chromatin bridges resolved, resulting in lengthened cell cycle time and disorientation of the plane of division, and some failed to separate altogether, resulting in apoptosis. We found that mesodermal daughter cells normally intercalated among their neighbours, but not where chromatin bridges were present. As a result, morphogenetic cell movements were impaired in early mutant limb buds. The data suggest that Irx3/5 perform an unexpected nontranscriptional function that ensures appropriate sister chromatid segregation that helps to shape the limb field prior to overt pattern formation.

Program Abstract #311
c-Fos targeting by the Piwi-piRNA pathway regulates Drosophila ovarian germline
Jamy Peng, Jonathon Klein, Chunxu Qu, Chunlao Tang
In the germline, germ cells produce gametes and contribute genetic material to the offspring, and somatic cells form tissues to house and enable gamete production. Drosophila oogenesis requires both germline (GSC) and somatic stem cells (SSC) and presents a unique model to study the interplay of the two populations. GSC function requires Piwi activity in the GSC and the somatic niche. Piwi proteins associate with a class of small RNAs, Piwi-interacting RNAs (piRNAs), to execute their molecular activities. While the role of piRNAs in suppressing transposon activity in the germline has been well studied, the possible involvement of piRNAs in Piwi-mediated developmental regulation has been largely unexplored. We showed that c-Fos, a classic proto-oncogene regulating many cell and developmental processes, is involved in Piwi-mediated regulation of GSC. Reduction of c-Fos, by ways of heterozygous mutations or shRNA-mediated knockdown, partially rescues piwi phenotypes in Drosophila ovarian germline. RNA-Seq and immunofluorescence imaging showed Piwi suppresses c-Fos. Bioinformatic analysis followed by molecular biology validation and analysis of GFP-c-Fos UTR reporter animals revealed that Piwi regulates piRNA production from the c-Fos 3′ untranslated region (UTR). We disrupted Piwi-piRNA suppression of c-Fos by replacing the c-Fos 3′ UTR to show that (i) the 3′ UTR alone is sufficient to induce gene suppression and piRNA production and that (ii) c-Fos deregulation results in ovarian somatic cell disorganization, tissue dysmorphogenesis, oocyte maturation arrest, and complete infertility. We identified c-Fos as part of Piwi-mediated regulation of GSC maintenance and differentiation and a target of the Piwi-piRNA pathway to regulate SSC and somatic cell organization. These data expand our understanding of the role of non-coding RNAs in coordinating stem cell populations and tissue morphogenesis. Our work is supported by ALSAC and an NIH grant (R00HD071011).

Program Abstract #312
Oocyte polarization is coupled with the chromosomal bouquet configuration in meiosis
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The source of symmetry-breaking in vertebrate oocytes is unknown. Animal-vegetal oocyte polarity is key to forming the embryonic body axes, as well as the germ line, and is established by the Balbiani body. The Balbiani body is an aggregate of specific mRNAs, proteins and organelles, which specifies the oocyte vegetal pole. However, how Balbiani body formation is regulated and how its asymmetric position is established are unknown. Here we trace oocyte symmetry-breaking in zebrafish 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, facilitating chromosomal pairing and meiotic recombination. We show that Balbiani body precursor components first localize with the centrosome to the cytoplasm adjacent to the telomere cluster of the bouquet. They then aggregate around the centrosome in a specialized nuclear cleft, that we have discovered, assembling the early Balbiani body. Thus the animal-vegetal axis of the oocyte is aligned to the nuclear axis of the bouquet. We show that the Balbiani body and the chromosomal bouquet, two universal oogenic features, are linked during oocyte polarization, and propose that a meiotic-vegetal center couples meiosis and oocyte patterning. Our findings reveal a novel mode of cellular polarization in meiotic cells whereby cellular polarity is aligned to nuclear polarity.

Program Abstract #313
A Novel Pathway for Germ Cell Differentiation in the Nematode
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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. Here we present our studies 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 germelines, reduced brood sized, and a subset are sterile due to complete lack of germ cells. Based on similar expression patterns and phenotypes, we explored xnd-1’s epistatic relationships with the Nanos homologs, nos-1 and nos-2. While xnd-1 and nos-2 each display only a minor “no germ cells” phenotype, double mutants display synthetic sterility with nearly half of the animals developing without a germ line. The additional removal of nos-1 leads to near complete sterility with the vast majority of animals absent germ cells. The sterility in xnd-1 and the double and triple
mutants is preceded by an increase in the activating histone marker, H3K4me2, within the PGC. These data strongly suggest that xnd-1 defines a new branch for PGC development that functions redundantly with nos-2 and nos-1 to promote germ line fates by maintaining transcriptional quiescence and regulating germ cell proliferation.

Program Abstract #314
Characterization of xnd-1 Function during Caenorhabditis elegans Germline Development
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Proper development of germ cells is critical to prevent infertility, birth defects, and other germline abnormalities. The nematode, Caenorhabditis elegans, is a model system for studying germ cell development, as we can observe germ cells in situ from birth to adulthood, as they develop and divide to form gametes. Despite the critical importance of proper germ cell development for fertility, the genes influencing their specification and differentiation are poorly understood. Our lab is studying this problem through the characterization of the xnd-1 (X chromosome nondisjunction factor-1) gene. Originally identified for its role in meiosis, we recently discovered that xnd-1 is expressed in and required for primordial germ cell development. Accordingly, xnd-1 mutants display sterility, reduced brood size, abnormal oocyte development, and stunted germline growth. Many of the mutant phenotypes of xnd-1 animals share striking resemblance with that of mitogen activated protein kinase (MAPK) mutants, therefore, we hypothesized that xnd-1 may be modulating MAPK signaling. Consistent with this hypothesis, immunostaining revealed low MAPK signaling in the germline of xnd-1 mutants. To further understand the relationship between xnd-1 and MAPK signaling, we have been examining a set of double mutants. Specifically, we focused our efforts on two regulators of the MAPK pathway, let-60 and lip-1. Contrary to prior studies with these regulators, we found that let-60(gof) and lip-1(lof) mutations gave opposite results, with the former decreasing fecundity and the latter increasing in the xnd-1 background. This research was supported by Choose Development!, - an NSF sponsored program through the SDB (grant IOS-1239422).

Program Abstract #315
The effect of germ granules on gene expression in the C. elegans germline
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Germ granules (P granules) in C. elegans are required for fertility and function to maintain germ cell identity and pluripotency. Sterility in the absence of P granules is often accompanied by the mis-expression of soma-specific proteins and the initiation of somatic differentiation in germ cells. To investigate whether this is caused by the accumulation of somatic transcripts, we performed mRNA-seq on dissected germ lines with and without P granules. Strikingly, we found that somatic transcripts do not increase in the young adult germline when P granules are impaired. Instead, we found that impairing P granules causes sperm-specific mRNAs to become highly overexpressed. This includes the accumulation of major sperm protein (MSP) transcripts in germ cells, a phenotype that is suppressed by feminization of the germline. A core component of P granules, the endo-siRNA-binding Argonaute protein CSR-1, has recently been ascribed with the ability to license transcripts for germline expression. However, impairing CSR-1 has very little effect on the accumulation of its mRNA targets. Instead, we found that CSR-1 functions with P granules to prevent MSP and sperm-specific mRNAs from being transcribed in the hermaphrodite germline. These findings suggest that P granules protect germ line integrity through two different mechanisms, by 1) preventing the inappropriate expression of somatic proteins at the level of translational regulation, and by 2) functioning with CSR-1 to limit the domain of sperm-specific expression at the level of transcription. This research is supported by NIH grants P20GM103423, P20GM104318, and R01GM113933.

Program Abstract #316
Mechanisms of action for TGFß signaling in C. elegans germline stem cell development
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An appropriate molecular environment, consisting of a niche as well as global signaling cues, is essential for stem and progenitor cells to decide whether to remain undifferentiated or to undergo differentiation. We are using the C. elegans germ line as a model for understanding how this decision is influenced by external environmental conditions. In the C. elegans germ line, progenitors accumulate during larval development to form an adult pool from which gametes are continuously produced. Previously, we found that members of a TGFβ-like signaling pathway modulate the balance of proliferation versus differentiation in the larval germ line in response to sensory cues, independent of previously defined roles for this pathway in the dauer decision and lifespan regulation. daf-7 encodes a TGFβ-like ligand the level of which
in ASI chemosensory neurons provides a link between environmental changes perceived by the animal during development and the number of adult proliferative germ cells. The TGFβ-receptor (TGFβR) complex and its downstream transcriptional regulatory complex act in the distal tip cell (DTC), the germ stem cell niche (Dalfó et al., 2012). We are exploring the molecular mechanism of how changes in TGFβR signaling within the DTC result in an appropriate response in the germ line. The Notch signaling pathway maintains the proliferative pool of germ cells with ligands produced by the DTC and the receptor expressed on germ cells. Previously, we found that TGFβR signaling can act in a Notch-independent manner (Dalfó et al., 2012). More recently, we found that TGFβR signaling also promotes the expression of one of the Notch ligands in the DTC. We identified a 0.5 kb promoter region, including several conserved elements, that responds to changes in TGFβ signaling. We are currently testing which elements are necessary and sufficient to modulate ligand expression, and whether the response is direct. Funded by NIH R01GM102254.

Program Abstract #317

**Importin a1 is required for maintaining germline stem cells in Drosophila melanogaster testes**

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Importin α (Impα) proteins are required for transporting proteins from the cytoplasm into the nucleus via interaction with Importin β (Impβ) in all tissues but also have roles in transcriptional regulation and organisation of chromatin. The Drosophila melanogaster genome encodes four Impα genes (1-4). We have identified a specific requirement for Impα1 in maintenance of male germline stem cells and spermatogonial differentiation. Germline stem cells (GSCs) are restricted to the apical testis where the stem cell niche responsible for regeneration is located. 8-10 GSCs surround a group of somatic niche cells termed the hub. Mitotic division of a GSC results in a daughter remaining attached to the hub that maintains GSC identity and a daughter displaced from the hub that becomes a gonialblast, or primary spermatogonial cell. The gonialblast undergoes four more rounds of mitosis characterised by incomplete cytokinesis to produce a cyst of 16 interconnected spermatogonia which then proceed through pre-meiotic S-phase and differentiate as spermatocytes. Loss of function Impα1 mutants lose GSCs, this loss can be rescued by germline specific expression of Impα1 but the rescue animals are still infertile indicating a secondary role of Impα1 in spermatogenesis. The Impα1 mutant phenotype can be phenocopied by germline expression of a dominant-negative Impα1 protein that lacks the Impβ binding domain. This phenotype can be characterized by a loss of early germline cells and an increase in the size of the hub. We are currently screening molecules that may be transported by Impα1 into germ cell nuclei to regulate GSC maintenance and differentiation. The screen has identified the gene CG12909, an ortholog of the mammalian gene LYAR present in the human and mouse genomes.

Program Abstract #318

**High-resolution analysis of maternal RNAs reveals gene pathways specifically enriched at the vegetal pole in Xenopus oocytes**

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The Xenopus oocyte is a highly polarized cell along the animal/vegetal axis. During oogenesis, specific maternal RNAs are localized to the vegetal pole that will determine germ layer identity, dorsal/ventral patterning, and the germ cell lineage in the embryo. Using RNA-seq, we have identified RNAs highly enriched at the vegetal pole. In situ hybridization and qPCR served to validate localized RNAs. In this study, vegetal and animal pole tips were cut from stage VI oocytes and frozen immediately. Samples from 3 different frogs were pooled and comprised one sample set; a total of 3 sets were made from a total of 9 frogs. Data were aligned to the Xenopus laevis (v7.1) and Xenopus tropicalis (v7.1) annotated genomes. Using the 0.05 q-value cutoff determined by cuffdiff, 5,720 total transcripts were found differentially expressed between the animal and vegetal pools. Reads aligning to a ribosomal specific reference or mitochondria sequences represented <5% and 1.28% respectively. The top 197 RNAs enriched >4-fold over the animal pole sample were selected for GeneGo analysis. Of these, protein-modifying enzymes, receptors, ligands, and 4 key transcription factors defining hubs will be functionally tested in future experiments. The maternal transcription factor Creb1 was found in diverse gene pathways involving localized mRNA, suggesting its function as an embryonic determinant is based on its regulating expression of localized RNAs. Known genes in neurogenic pathways were well represented. miRNA analysis identified eight uniformly distributed in the oocyte. Interestingly, all are up-regulated in cancers and found clustered on the human X chromosome., Sixteen vegetally localized mRNAs contained at least one recognition sequence conserved between Xt.
and humans for these miRNAs. Our data suggest that early embryonic patterning is not regulated by localized maternal miRNAs, but rather localized mRNAs. Funding from NIH GM102397; HD072340.

Program Abstract #319
Analysis of the function of FNDC3A in mouse spermatogenesis
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Fibronectin type III domain-containing 3a (Fnnd3a) null mice are sterile due to defective spermatogenesis. FNDC3A is a member of a family of three novel membrane anchored cytosolic proteins in mice. The presence of multiple conserved WW-domain binding motifs in the N-terminal proline rich region (PRR) of all FNDC3 family members suggests these proteins function by interacting with WW-domain containing proteins. Arrays of WW domain peptides were used to determine the relative affinity of the PRR of FNDC3A for each WW domain. Spermatids in mice homozygous for the Fnnd3a<sup>907</sup> null allele do not develop normally beyond step 8 of spermiogenesis and form symplasts (i.e. multi-nucleated cells) present in seminiferous tubules and vasa deferentia, suggesting defective adhesion between spermatids and Sertoli cells. Intercellular bridges between spermatids in Fnnd3a<sup>907/907</sup> mice widen prior to symplast formation, suggesting Fnnd3a functions in the maintenance but not establishment of germ cell intercellular bridges. Leydig cells in Fnnd3a<sup>907/907</sup> adult mice appear swollen and oil red O staining indicates the presence of increased lipid, suggesting a possible defect in lipid homeostasis. As Fnnd3a is expressed in both germ cells and somatic cells of the mouse testis, we used conditional mutagenesis to demonstrate that Fnnd3a is required in a germ-cell intrinsic manner, but not in Leydig cells for spermatogenesis. Models are presented as to how Fnnd3a functions in mouse spermatogenesis. This work was funded in part by the GAANN fellowship and grant from the U.S. Department of Education, CFDA Number: 84.200.

Program Abstract #320
Tamas mediated paternal mitochondria DNA elimination during Drosophila sperm development
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Maternal inheritance of mitochondrial DNA (mtDNA) is typical in animals and uniparental inheritance is widespread among eukaryotes. While this pattern of inheritance is often attributed to the size asymmetry of male and female gametes, and the consequent preponderance of the cytoplasmic contribution of the female to the zygote, it has become clear that active mechanisms enforce the uniparental transmission pattern. In some organisms paternally derived mitochondria are eliminated in the fertilized zygote, but in others the mitochondrial genome appears to be reduced or eliminated in the sperm prior to fertilization. We previously showed that the Drosophila Endonuclease G (EndoG) promotes elimination of mtDNA during spermatid development while leaving the mitochondria intact. However, mutation of EndoG delayed but did not prevent mitochondrial DNA disappearance. Here, we report that germ line reduction of the levels of the Drosophila mtDNA polymerase, Tamas, result in persistence of mtDNA nucleoids in both late elongating and individualizing spermatids, phenotypes that are augmented by the EndoG mutant. Since the DNA polymerase has a proofreading exonuclease that resects available 3’ ends, we are testing the idea that this nuclease acts in concert with the end producing activity of the EndoG to eliminate the DNA. Artificially cutting mitochondrial DNA during spermatogenesis is shown to advance Tamas dependent mtDNA elimination, indicating that Tamas has a destructive capacity prior to its usual time of action. We are exploring mechanisms that might control the activity of EndoG and Tamas to guide the precise developmental timing of the mtDNA elimination in spermatogenesis. This work is supported by a grant from NIH GM086854 to Patrick O’Farrell.

Program Abstract #321
Transcriptomic analysis reveals molecular similarity between the male germline of parasitic and free-living flatworms
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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. While significant progress has been made in the
understanding of schistosomiasis immunobiology, the reproductive biology of these parasites remains poorly understood. To better understand the reproductive biology of schistosomes, we used RNA-seq to enrich for mRNAs associated with the male germline of Schistosomamansoni. We find 400 transcripts (>1.5X, p≤ 0.05) putatively enriched in the S. mansoni male germline. 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 transcripts we have tested are expressed in S. mansoni testes, and interestingly, also in the ovaries. Knockdown of several of these genes results in a range of male germ cell defects in the parasite. Furthermore, our studies reveal that many parasite germ cell-enriched genes have orthologs in the free-living flatworm Schmidtea mediterranea. In situ hybridization and RNAi of the S. mediterranea orthologs show that these genes are expressed and function in the planarian male germline, indicating molecular conservation between free-living and parasitic flatworms. We are currently taking advantage of the molecular resources available for planarians to understand schistosome germ cell biology. 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. Funding sources: NIH R01 HD043403, R21 AI099642, Howard Hughes Medical Institute.

Program Abstract #322
The Analysis of Whole Transcriptome of Human Preimplantation Embryos Using the Third Generation Sequencing Technology
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Human preimplantation embryos mark the first stages of development. Understanding how this program gets established is paramount to more broadly understand early events of differentiation, pluripotent stem cells biology and ultimately early gestational failures. Though much is known about mouse embryo development we know very little about the specifics of human preimplantation development. We have recently developed a new and sophisticated “hybrid” RNA-seq method based on the combined analysis of short reads and long reads generated, respectively, by second- and third-generation sequencing technology. By combining the strengths of the two sequencing platforms it is possible to identify the full length of long transcripts and at the same time gain deep information on exon-intron boundaries, splicing variants and isoforms abundance. We applied this method to obtain a comprehensive characterization of the transcriptome of human embryonic stem cells and identified hundreds of novel pluripotency-specific genes for which we have detailed information both at the genomic and at the transcriptomic level. The aim of this project is to adopt a similar approach on single embryos and single blastomeres, with the goal to build a transcriptome map of human preimplantation development to accurately determine the transcriptional program from the zygote (soon after fertilization) to the blastocyst stage. This work will give us invaluable information about early development in humans and will be instrumental to the development of early diagnostic tools to assess the quality of embryos obtained by Assisted Reproductive Technology. Funded by California Institute of Regenerative Medicine(CIRM).

Program Abstract #323
Genetic Dissection of Ventral Folding Morphogenesis
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Upon gastrulation, the Early Head Fold (EHF) stage mouse embryo initiates ventral folding morphogenesis (VFM), a multi-step process that in amniotes orchestrates gut endoderm internalization, linear heart tube formation, ventral body wall closure and encasement of the fetus in extraembryonic membranes. Irregularities in VFM during the fourth week of human embryonic development underline birth defects associated with incomplete body wall closure, such as gastrochisis (intestines outside the abdominal wall) and ectopia cordis (heart outside the thoracic cavity). VFM and the etiology of body wall closure defects remain understudied despite prevalence similar to that of neural tube closure defects. A factor contributing to this dearth of knowledge is that severe VFM defects in human embryos will block formation of an effective maternal-fetal interface, resulting in spontaneous abortion, often before confirmation of pregnancy. Studies using lineage-specific mouse mutant analyses identified the BMP pathway as a central regulator of VFM and genetically dissected the tissue rearrangements mediating VFM into two sequential phases: EHF to 0-to-8 somite stage and 8-to-20 somite stage during axial rotation. In situ hybridization analysis of Bmp2, combined with characterization of mutant embryos lacking Bmp2 exclusively in visceral endoderm (VE) or all epiblast derivatives, point to Anterior Visceral Endoderm (AVE) as the source of the BMP2 signal initiating VFM at the EHF stage. Our working model posits that by signaling to one or more distinct epiblast-derived cell types, AVE-expressed BMP2 directs and coordinates cell behaviors
that mechanistically link formation of foregut invagination with positioning of the head anterior to the heart, concurrent events during initiation of VFM. Ongoing experiments investigating this model, as well as the impact of defective early VFM on elaboration of L-R asymmetry and arrangement of extraembryonic membranes, will be discussed.

Program Abstract #324
Differentially expressed long non-coding RNAs in mouse extraembryonic tissues
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The first embryonic cell type to acquire specialized function is the trophectoderm layer of the blastocyst. The trophectoderm interacts with the maternal environment to initiate critical nutrient and gas exchange, and develops into extraembryonic tissues including the placenta, an organ essential for sustaining the fetus throughout gestation. Defective trophectoderm differentiation during the first trimester leads to improper implantation, which manifests as placental pathologies late in pregnancy. Recently, landmark studies have uncovered the functional importance of long non-coding RNAs (lncRNAs) in gene regulation. Once thought of as “junk DNA”, lncRNAs have been implicated in chromatin modification, cell cycle and growth regulation, mRNA decay, and protein translation regulation. Our hypothesis is that differentially expressed lncRNAs in extraembryonic cell types play a similar regulatory role in specification of trophectoderm lineage and in the development of a robust, functional placenta. To investigate the putative role of lncRNA involvement in extraembryonic tissue development, RNA sequencing and differential expression analysis was performed on three early embryonic tissues: epiblast, extraembryonic ectoderm, and ectoplacental cone of 6.5dpc mouse embryos. Using TopHat and Cufflinks to perform transcriptome analysis along with a pipeline to detect lncRNA transcripts, 69 differentially expressed IncRNAs were identified in the three tissues analyzed. Of these, 28 were highly expressed in the extraembryonic tissues, with some transcripts having a six-fold change in expression. Going forward, in vitro RNA pull-down assays will be used to reveal involvement in specific regulatory pathways. Additionally, in vivo over-expression studies will be performed to understand the functionality of these candidate IncRNAs in both specification of the trophectoderm lineage and in the development and maintenance of extraembryonic tissues.

Program Abstract #325
Light-sheet imaging of post-implantation mouse development
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Recent advances in imaging technology have enabled unprecedented views into the development and morphogenesis of numerous organisms and tissue systems. However, despite these advances, early mouse embryogenesis remains a unique challenge for live imaging due to its sensitive growth requirements, dramatic changes in size and tissue morphology, rapid cell movements, and complex optical properties. Because of these challenges, relatively little is known about the morphodynamic processes that attend gastrulation through to tissue formation, or how the order and migration of cells within the developing germ layers correspond with their fate. Light-sheet microscopy provides an excellent opportunity to study early mouse development in unparalleled detail and with minimal impact on normal development. We have developed a light-sheet system capable of sustaining mouse development from pre-gastrulation to early somite stages while continuously imaging from multiple angles and with multiple markers. Adaptive imaging techniques optimize optical sectioning and correct for aberrations throughout the specimen and through time, maintaining high image quality over different developmental stages. Multi-view detection combined with confocal line-scanning and bi-directional illumination provides full coverage of the embryo at rapid speeds and minimal light exposure, allowing for high temporal resolution. Furthermore, automated tracking of embryo position and size ensures the embryo remains centered and optimally focused for the duration of the experiment. As a single experiment is capable of generating tens of terabytes of data, a custom computational pipeline was designed to handle multi-view processing, visualization, and cell tracking. These technological advances have enabled us to analyze gastrulation through to early organogenesis at a cellular level, offering new insights into this critical, yet poorly characterized area of mammalian development. Funded by HHMI.

Program Abstract #326
The role of glucose metabolism in extraembryonic endoderm differentiation
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During early mouse embryogenesis, the primitive endoderm (PrE) forms from cells originating from the inner cell mass
around 4.5 dpf. Due to the difficulty of studying PrE formation in vivo, F9 teratocarcinoma cells are used as an in vitro model of differentiation. Exposure of F9 cells to retinoic acid (RA) or WNT6 induces differentiation and changes to their metabolic profile. Message and protein levels of enzymes that promote glycolysis, including lactate dehydrogenase A, pyruvate dehydrogenase kinase isozyme 1 and phospho-pyruvate dehydrogenase (LDHA, PDK1, P-PDH, respectively) are reduced in cells treated with RA, thus promoting oxidative phosphorylation (OXPHOS). Whether or not this shift from glycolysis to OXPHOS is sufficient for differentiation is unclear. We have used chemical inhibitors to block LDHA and PDK1 activities and examined their effects on PrE differentiation. Results using LDHA inhibitors GSK 2837808A and Sodium oxamate revealed that LDHA activity is sufficient for PrE formation. Similarly, chemical inhibition of PDK1 with dichloroacetic acid is sufficient to induce PrE. PDK1 was examined further as it is a known canonical WNT/β-catenin signaling target. XAV939 (Wnt signaling inhibitor) and CHIR99021 (Wnt signaling activator) were used to investigate if canonical WNT/β-catenin signaling regulates LDHA and PDK1 expression in F9 cells. LDHA and PDK1 mRNA and protein levels were measured using qPCR and immunoblot analysis, respectively. LDHA and PDK1 levels increased and differentiation perturbed when cells were treated with XAV939. In contrast, CHIR99021 treatment reduced LDHA and PDK1 levels and differentiation proceeded. Together, these results highlight the importance of canonical WNT/β-catenin signaling on affecting metabolic changes leading to PrE formation. Research supported by NSERC of Canada.

Program Abstract #327

_Xenopus_ TRIL augments BMP signaling and dampens nodal signaling to ensure normal patterning during gastrulation

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Primitive hematopoiesis is the earliest production of blood cells that deliver oxygen to the embryo. Mesodermal cells commit to a primitive blood cell fate during gastrulation and, in order to do so, the mesoderm must receive non-cell autonomous signals transmitted from other germ layers. In _Xenopus_, these signals come from the ectoderm and the transcription factor GATA2 is required to generate them. Using microarray analysis, we identified TRIL, a transmembrane protein, as a target of GATA2 in _Xenopus_ ectoderm. TRIL is expressed in ectoderm but also throughout the mesoderm during gastrulation. When expression of TRIL was knocked down in either ventral ectoderm or ventral mesoderm, markers of hematopoietic specification were reduced. Thus, TRIL is required in both germ layers for red blood cell commitment. BMP function is required in both ectodermal and mesodermal cells for blood formation and we asked whether BMP signaling is impaired in TRIL morphants. Expression of BMP target genes and levels of pSmad1/5/8 were reduced in TRIL morphants during gastrulation, but levels of Smad7, a BMP and Nodal inhibitor, were elevated. These results suggest that TRIL enhances BMP signaling by targeting Smad7 for degradation, and this is required for hematopoietic commitment. TRIL is also strongly expressed in dorsal mesoderm during gastrulation. When antisense TRIL morpholinos were targeted to dorsal cells, morphants showed defects in gastrulation and head development, phenotypes that could result from Smad7 mediated inhibition of nodal signaling. Surprisingly, we found that expression of nodal target genes and levels of pSmad2 were elevated in TRIL morphants, despite the high levels of Smad7 protein. Overall our data demonstrate that TRIL enhances BMP signaling by targeting Smad7 for degradation, and this is required for hematopoietic commitment. TRIL is also strongly expressed in dorsal mesoderm during gastrulation. When antisense TRIL morpholinos were targeted to dorsal cells, morphants showed defects in gastrulation and head development, phenotypes that could result from Smad7 mediated inhibition of nodal signaling. Surprisingly, we found that expression of nodal target genes and levels of pSmad2 were elevated in TRIL morphants, despite the high levels of Smad7 protein. Overall our data demonstrate that TRIL is required to promote BMP signaling but to dampen Nodal signaling during gastrulation and that this is essential for normal gastrulation, head development and hematopoietic commitment. Funding source: NIH (T32 DK007115 and R01HD067473).

Program Abstract #328

Posttranscriptional Regulation of Symmetry Breakage in _Xenopus_: Flow and micro-RNA dependent Repression of Dnad5

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Symmetry breakage in fish, amphibian and mammalian embryos depends on motile cilia, which cause a leftward flow of extracellular fluids across the ciliated epithelium of the left-right organizer (LRO). LROs transiently form in the embryonic midline during early neurulation at the posterior pole of the notochord; they are represented by the spherical Kupffer's vesicle in bony fish, the gastrocoel roof plate (GRP) in the frog and the posterior notochord/"node" in mammals. Flow is sensed by lateral mechanosensory cilia. On the left side of the LRO, the Nodal inhibitor Dand5 is repressed due to leftward flow, resulting in the transfer of the co-expressed Nodal signal to the left lateral plate mesoderm and heart primordium. How Dand5 is regulated has remained enigmatic. Here we show, that micro-RNAs (miRs) mediate Dand5 repression in a flow-dependent manner. Knockdown of the miR-processing enzyme Dicer resulted in specimens which failed to induce the Nodal cascade. Importantly, laterality was rescued by a parallel knockdown of Dand5 on the left side,
demonstrating that Dand5 is the decisive miR-target in symmetry breakage. Analysis of the 3'-UTRs of the two Dand5 genes in *Xenopus* revealed several candidate miR binding sites. Knockdown of miR genes and protection of target sites in Dand5 3'-UTRs through injection of target protector morpholino oligonucleotides demonstrated a role for two miR genes, whose specific roles will be discussed. Together, these miRs mediate the efficient flow-dependent repression of Dand5 and thereby regulate the biased breakage of the bilateral symmetry in the vertebrate embryo. Symmetry breakage therefore is a unique case in which miRs provide a all-or-nothing switch, in contrast to the buffering functions ascribed to most miR-dependent regulations of gene expression.

**Program Abstract #329**

**LvTsg is required for dorsal specification via modulation of LvBMP2/4 signaling**

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Spatial restriction of bone morphogenetic protein (BMP) signaling is critical for dorsal-ventral axis specification in developing embryos. Extracellular BMP proteins relocalize to spatial regions distinct from those in which BMP is expressed in both sea urchin and fly embryos. In *Drosophila* embryos, extracellular BMP movement requires interaction with the secreted BMP inhibitors Sog/Chordin and Twisted Gastrulation (Tsg). In the sea urchin embryo, BMP2/4 is expressed in the ventral ectoderm, but signals broadly in the dorsal ectoderm. Chordin is likewise expressed in the ventral domain. We have characterized LvTsg, which is expressed with no apparent restriction along the dorsal-ventral axis, in sea urchin embryos. Perturbation of Tsg expression affects both the spatial restriction and strength of dorsal BMP signaling, indicating that Tsg is required for normal BMP movement. Tsg loss of function (LOF) ventralizes embryos, indicating that Tsg is required for dorsal specification. Tsg gain of function (GOF) does not have the reciprocal effect: global GOF results in widening of the pluteus arms, an apparent partial ventralization. Analysis of BMP target genes at late gastrula stage shows that global Tsg GOF contracts the dorsal BMP signaling domain and weakens the strength of BMP signaling. This weak BMP signaling is not sufficient to clear ciliary band gene expression from the dorsal region, and these boundary genes continue to be expressed alongside low levels of dorsal genes. We interpret these effects of Tsg GOF as promoting excessive BMP movement. Interestingly, both Tsg LOF and GOF have negative effects on BMP signaling in sea urchin embryos, demonstrating a requirement for Tsg promoting BMP signaling in a dose-dependent manner.

**Program Abstract #330**

**Localization and movement of axis induction and germ cell determinants in the early zebrafish embryo**

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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. In *Xenopus* and zebrafish, factors involved in both axis induction and primordial germ cell (PGC) development are initially localized to the vegetal pole of the egg, and previous studies have shown functional overlap of vegetally localized factors in these processes. However, axis induction and PGC determining factors experience different modes of transport after egg activation, namely an asymmetric off-center shift and symmetric animaly-directed movement, respectively. We therefore examined the spatial relationship between the proposed dorsal determinants wnt8a and grip2a and the PGC factor dazl, to understand their localization at the vegetal cortex with respect to cortical rotation. We find that these two types of factors localize to different cortical depths, with the PGC-determining component at a deeper cortical level than axis-inducing factors. This supports a model in which differential RNA localization within the vegetal cortex, coupled to a cortical rotation-like symmetry-breaking mechanism, underlies differential transport behaviors for determinants involved in two key processes in early embryogenesis: axis induction and germ cell specification. Diversity supplement: RO1 GM065303-10A1S1.

**Program Abstract #331**

**Temporal and spatial requirements for Nodal-induced head mesendoderm in anterior neurulation**

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Neural tube defects (NTD) such as anencephaly are among the most prevalent human birth defects. Zebrafish lacking Nodal signaling have an open anterior neural tube phenotype similar to anencephaly. Previous work indicated Nodal acts through induction of head mesendoderm/mesoderm. When these tissues are rescued, NTD are also corrected. The developing neural tube of Nodal mutants is already abnormal by neural plate stages (late gastrulation), suggesting that Nodal and mesoderm/mesendoderm act before the onset of neurulation. To gain further insight into the role of mesoderm/mesendoderm, we defined the temporal requirement for Nodal signaling. When treatment with a Nodal signaling inhibitor started before or at sphere stage (mid blastula) embryos had open neural tubes. In contrast, embryos treated at or after dome stage (late blastula) had closed neural tubes. This timing is consistent with prechordal plate mesendoderm and anterior mesoderm but not endoderm being involved in neurulation. To further identify the tissues involved in neural tube closure, we used sqt and lefty1-overexpressing embryos, which have incomplete penetrance of NTD and variable mesendoderm/mesodermal deficiencies. One-tailed Fishers exact tests and Phi coefficients of association indicated a strong, positive correlation for a closed neural tube and the presence of mesendoderm/mesoderm derived tissues, specifically hatching glands, cephalic paraxial mesoderm, notochord, and head muscles. However, none of these tissues were always present in embryos with a closed neural tube or always absent when it was open. This suggests no single mesendodermal/mesodermal tissue is required. Instead, we propose a model in which a critical amount of mesendoderm/mesodermal precursors are required during gastrulation to promote formation of the neural plate and its subsequent closure into a neural tube. Funded by NIH 1 R15 HD068176-01A1 and a University of Minnesota Grant in Aid of Research, Artistry, and Scholarship.

Program Abstract #332
Characterization of the role of Wnt5a in neural tube
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Spinal cord development is a tightly regulated process. Improper closure of the spinal cord causes neural tube defect. The Wnt family of secreted morphogens plays a significant roles in the formation of the early embryo. In order for Wnt ligands to be secreted and biologically active, they must first be modified by Porcupine (PORCN). The importance of Wnt ligands in neural tube closure is highlighted by the observation that inhibition of PORCN causes improper neural tube closure in early stage chick embryos. However, the identity of the Wnt ligands that control neural tube closure is unknown. Our In situ studies indicate that Wnt5a is present during neural tube closure in the developing chick. Previous reports suggest a possible link between Wnt5a and neural tube closure. Thus, we hypothesized that the Wnt5a ligand participates in neural tube closure. To test this hypothesis, exogenous Wnt5a will be used to rescue neural tube closure in embryos in which the processing of endogenous Wnts has been inhibited using a pharmacological inhibitor of PORCN. Out of four inhibitors, IWP12 treatment showed a consistent abnormal phenotype. Inhibition of PORCN with IWP12 caused neural tube defects. Before attempting to rescue these phenotypes by adding exogenous Wnt5a protein, we first catalogued the effects of adding Wnt5a to streak stage embryos. Embryos treated Wnt5a all exhibited abnormalities that overlapped with those found in IWP12 embryos, including neural tube defects. Further analysis of the phenotypes showed that cells in the spinal cord of Wnt5a treated embryos failed to properly elongate and had increased levels of apoptosis as compared to controls. IWP12 with Wnt5a did not rescue phenotypes associated with IWP12 treatment alone. We then determined an optimal concentration of Wnt5a to use for rescue experiments. Our preliminary results indicate that addition of Wnt5a to IWP12 treated embryos did not rescue spinal cord closure.

Program Abstract #333
RhoA-dependent disassembly of actomyosin is necessary for mouse spinal neural tube closure
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The cytoskeleton is widely considered essential for neurulation and yet mouse spinal neural tube closure can occur despite disruption of actin microfilaments by Cytochalasin D. To investigate this apparent contradiction, we treated mouse embryos in culture with a panel of cytoskeletal inhibitors. Preventing actomyosin cross-linking, F-actin assembly or myosin II contractile activity had no effect on spinal neural tube closure. In contrast, inhibiting Rho kinase signalling or blocking F-actin disassembly resulted in spinal neural tube defects, with apical F-actin accumulation and abnormal adherens junctions in the neuroepithelium. Cofilin I-null embryos closely yielded a similar phenotype, supporting a key role for actin turnover. Blebbistatin could rescue the neural tube closure defects caused by ROCK inhibition. This study
identifies a requirement for precise regulation of RhoA/Rho kinase/LIM kinase/cofilin signalling in mouse spinal neurulation and shows that, while actomyosin assembly and myosin ATPase activity are not limiting factors for mouse spinal neural tube closure, there is an essential role for actin turnover and actomyosin disassembly within the neuroepithelium. This work is supported by the Wellcome Trust.

Program Abstract #334
The role of miR-206 in the formation of the dystrophin-glycoprotein complex during somitogenesis
Melissa Adams, Julio Ramirez, Carmen Domingo
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MicroRNAs are a recently discovered class of regulatory RNAs which specifically bind regions in the 3’ UTR of their target mRNAs to block their translation. A muscle specific family of these microRNAs, the myomirs, are important for early muscle development and muscle maintenance. The dystrophin-glycoprotein complex, which spans muscle cell membranes, has been shown to play an important role in muscle maintenance in vertebrates. Myomirs, and the members of the dystrophin-glycoprotein complex are often deregulated or mutated in a variety of muscular diseases including many muscular dystrophies. Furthermore, when a member of the dystrophin-glycoprotein complex, dystroglycan, is knocked down or mutated, somitogenesis is disrupted in *Xenopus laevis* (Hidalgo et al 2009). Preliminary work from our lab has shown that somitogenesis is disrupted in *Xenopus* when the skeletal muscle specific myomir, miR-206, is knocked down using morpholinos. Through immunohistochemistry, we show that expression of dystroglycan, and one of its intracellular binding partners, utrophin, is disrupted when mirR-206 is knocked down as well. Using bioinformatic analysis, we have determined that dystroglycan has no predicted miR-206 binding sites, thus it is unlikely that it is a direct target of miR-206. However, miR-206 has been shown to directly target utrophin A in several cell lines and in mice. However, this has not been investigated in *Xenopus* (Rosenberg 2006, Basu 2011, Amouche 2013). To confirm that utrophin is a target of miR-206 in *Xenopus*, we will use a GFP reporter assay. Together this work will help us to understand the possible link between miR-206 and the dystrophin-glycoprotein complex in somitogenesis. Furthermore, because these molecules are involved in muscular dystrophies, this study will shed light on the molecular pathways involved in these muscle diseases. This research was funded by NIH MBRS 1SC3GM111118-01.

Program Abstract #335
Characterization of the overexpression of miR-206 during early muscle development in *Xenopus laevis*.
Rebecca Blandino, Carmen Domingo
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MicroRNAs (miRNAs) are small, non-coding sequences of RNA that regulate genes post-transcriptionally. They bind to specific messenger RNAs and block translation to silence protein synthesis. miRNAs have been shown to play a role during embryonic development and in diseases. Of the wide variety of miRNAs, our lab is interested in miR-206 because it is exclusively expressed in cells that will give rise to the skeletal muscle of vertebrates. To understand the role that miR-206 plays in muscle development we used a mimic to overexpress miR-206 in the embryo. We hypothesize that increased levels of miR-206 may lead to changes in gene expression levels that are important in muscle development. To test this hypothesis, we injected miR-206 mimic into one of the two blastomeres at the 2-cell stage along with rhodamine dextran amin, which acts as a lineage tracer for the miR-206 mimic. We allowed the embryos to develop to the late tailbud stage and then fixed, stained, and imaged them using a confocal imaging system to analyze the muscle cell morphology. We find that over-expression of miR-206 disrupts somite formation. In particular, many muscle cells within the somite are disorganized. Furthermore, the expression level of the transmembrane protein, Beta-dystroglycan, an important for muscle cell attachment to the intersomitic boundary, is significantly lower in the presence of miR-206 mimic. Similarly, the expression level of the extracellular matrix molecule laminin, which is present in the intersomitic boundary, is also lower in the presence of the miR-206 mimic. These results suggest that miR-206 may play a role in regulating the dystroglycan adhesion complex important for muscle cell attachment during vertebrate development. Acknowledgments: MARC: Minority Access to Research Careers. Member ID: 5T34GM008574-18 NIH MBRS 1SC3GM111118-01

Program Abstract #336
Roles of intracellular calcium mobilization in intercellular signaling, cell-type specification, and tissue patterning.
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The Ryanodine Receptor (RyR) intracellular calcium release channels (CRC) regulate release of calcium from intracellular stores in the ER/SR. Though expressed in many cell types, they are best known for their roles in muscle
contraction; indeed loss-of-function mutations are associated with myopathy in humans. We propose this narrow view of the RyR function fails to account for the full range of phenotypes observed in humans carrying RYR1 mutations, which include slow muscle cell defects. Here we elucidate unexpected functions of RyR in the zebrafish embryo. We demonstrate that RyR function is needed for asymmetric expression of southpaw and subsequent left/right patterning. Further we show multiple Hedgehog-dependent cell specification events, including generation of somite muscle cells and dorsal root ganglia, require RyR-mediated calcium mobilization. With blocked RyR function, adaxial precursor cells develop into fast muscle cells, as they would in the absence of Hh signaling. We present these findings and report the results of our efforts to identify the specific combinations of ryr genes that support these developmental functions. We have i) reinvestigated the expression of ryr genes, ii) generated null ryr alleles, and begun to analyze mutant embryos carrying combinations of the mutations.

Program Abstract #337
The Role of NADPH Oxidase in ROS-Mediated Differentiation
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Mouse teratocarcina F9 cells differentiate into primitive endoderm (PrE) when treated with retinoic acid (RA) and these changes are accompanied by an up-regulation of Wnt6 and activation of the canonical WNT/β-catenin pathway. Data from our lab shows PrE differentiation is accompanied by an increase in reactive oxygen species (ROS), which induces a conformational change in Nucleoredoxin (Nrx) preventing its ability to bind and inhibit dishevelled. This in turn positively impacts on the WNT/β-catenin signaling pathway leading to differentiation. Treating F9 cells with H2O2, in the absence of RA, is sufficient to induce F9 cells to differentiate into PrE. Furthermore, treating cells with antioxidants attenuates this RA-mediated differentiation. The source of endogenous ROS seen following RA treatment was investigated and members of the NADPH oxidase (NOX) family were identified as candidates. Nox1-4 and Duox2 genes are up-regulated by RA and in silico analysis revealed the Noxl promoter is regulated by Gata6, a master regulator of extraembryonic endoderm differentiation. Interestingly, Nox1 and Nox4 are up-regulated when Gata6 is overexpressed in F9 cells. Furthermore, the pan-NOX inhibitor VAS2870 and NOX1-specific inhibitor ML171 significantly reduced the ability of RA to induce PrE differentiation. Overexpression of Nox4 in F9 cells, however, is not sufficient for differentiation. Thus, the data would suggest that the ROS produced during the differentiation of F9 cells into PrE is the result of an increase in NOX1 activity and this is being explored using a genetic approach. Research supported by NSERC of Canada.

Program Abstract #338
Role of primary cilia in the developing chick
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Introduction: Impairment of cilia function underlies a number of human diseases including hydrocephalus. However, the role of cilia in the developing embryo is not well understood. Chloral hydrate is known to have an adverse effect on the cilia formation. In this study, the possible role of cilia in developing chick embryos will be described mainly by focusing on the formation of the nervous system. Materials and Methods: White Leghorn chicken eggs were incubated until embryos reached Hamburger and Hamilton stages 4 to 10. Chick embryos were prepared for both in vitro culture and in ovo culture. Embryos were treated with a chloral hydrate solution for 20 minutes, and re-incubated for 24 hours. Embryos were collected from the incubator and examined morphologically after approximately 24 hours. Results: In vitro: The embryos treated with chloral hydrate developed neural tube defects, reversed side heart looping, and an abnormally shaped primitive cerebral ventricle in a dose dependent manner. The embryos with neural tube defects will be described mainly by focusing on the formation of the nervous system. Materials and Methods: White Leghorn chicken eggs were incubated until embryos reached Hamburger and Hamilton stages 4 to 10. Chick embryos were prepared for both in vitro culture and in ovo culture. Embryos were treated with a chloral hydrate solution for 20 minutes, and re-incubated for 24 hours. Results: In vitro: The embryos treated with chloral hydrate developed neural tube defects, reversed side heart looping, and an abnormally shaped primitive cerebral ventricle in a dose dependent manner. The embryos with neural tube defects developed a small primitive ventricular system. In ovo: With the same concentration of chloral hydrate as used for in vitro culture, embryos did not form any of the anomalies described above. However, embryos treated with higher doses showed more severe anomalies than those developoing in vitro, such as a divided cardiac system and/or maldevelopment of the rostral neural tube. Discussion: The importance of motile cilia in normal function of the cerebral ventricular system, including its role in circulation of cerebrospinal fluid, is widely recognized, but the role of primary cilia in early embryonic development is not well understood. In this study we found that embryos treated with chloral hydrate have many types of anomalies depending on the concentration and method of culture. Our results suggest that cilia also have an important role in early development of the ventricular system in addition to a role in axial development.
Program Abstract #339
Defining the role of Histidyl tRNA Synthetase in the Zebrafish Eye and Ear
Ashley Waldron, Susan Robey-Bond, Alicia Ebert
University of Vermont, United States
Aminoacyl tRNA synthetases are critical enzymes responsible for attaching specific amino acids to their appropriate tRNA molecules during protein synthesis. In humans, a point mutation in the gene for Histidine tRNA Synthetase (HARS) has been associated with Usher Syndrome Type 3b, a disease characterized by hearing and vision loss by early adulthood. This association suggests an important role for HARS in eye and ear development and maintenance. The use of antisense morpholinos to knockdown HARS expression in zebrafish results in a lack of retinal organization and loss of neuromasts – exterior sensory organs in the zebrafish that are comparable to the sensory patches in ears. These results support the hypothesis that HARS plays a particularly important role in the vertebrate eye and ear. We aim to define the role of HARS in these sensory systems using the zebrafish as a model.

Program Abstract #340
Mathematical modeling of a signal interactions network aligning neural and mesodermal tissues at the head-trunk transition
Isaac Skromne, Don DeAngelis
University of Miami, United States
The assembly of a functional Cranio-Vertebral Junction (CVJ) in vertebrates requires hindbrain-spinal cord territories of the neuroectoderm and occipital-vertebral somites of the paraxial mesoderm to align precisely. Misalignment can result in congenital malformations in humans (e.g., Chiari), although during evolution, changes have resulted in favorable head-trunk adaptations. Our previous work has demonstrated that several signaling factors including FGF, Wnt and Retinoic Acid (RA) are required at the head-trunk transition for proper neural-mesodermal alignment. However, epistatic analysis has not provided the full extent and contribution of these interactions to tissue alignment. As an alternative and complementary approach we are using mathematical modeling to uncover signaling network interactions and better understand the normal assembly of the CVJ and the etiology of CVJ malformations.

Mathematical and computational approaches can be used to systematically analyze components and interactions within and between signaling pathways that can then be re-evaluated in vivo. Models are versatile, allowing the testing of several parameters including the time, space and strength of interactions. We describe a series of equations modeling FGF, Wnt and RA network interactions spatiotemporally and their effect on the regulation of the spinal cord specification gene cdx4. These equations can recapitulate the complex expression dynamics of cdx4 and other genes observed in zebrafish during the critical tissue specification and alignment period. Further improvements to the model would provide an important bioinformatics sandbox to test the contribution of each signaling pathway to the genetic network coordinating neural-mesodermal tissue alignment at the CVJ.

Program Abstract #341
The mouse Gene Expression Database (GXD): a resource for developmental biologists
Constance Smith, Terry Hayamizu, Jacqueline Finger, Ingeborg McCright, Jingxia Xu, Janan Eppig, James Kadin, Joel Richardson, Martin Ringwald
The Jackson Laboratory, USA
The Gene Expression Database (GXD) is an extensive, easily searchable, and freely available database of mouse developmental gene expression information (www.informatics.jax.org/expression.shtml). GXD integrates data from different assay types, including RNA in situ hybridization, immunohistochemistry, and RT-PCR. It includes data from wild type and mutant mice. These data are acquired from the literature and directly from researchers, including groups doing large-scale expression studies. GXD currently contains nearly 1.5 million expression results for nearly 14,000 genes. Database entries are detailed and include the following: time and tissue of expression (or non-expression); pattern and strength of expression; numbers and sizes of detected bands (for blots); genetic background of the samples; experimental conditions used; and a description of the probe/antibody used in the assay. These data are accompanied by nearly 270,000 images, allowing users to retrieve the primary data and interpret it themselves. GXD is an integral part of the Mouse Genome Informatics (MGI) resource. This means GXD’s expression data is integrated with other genetic, functional, phenotypic, and disease-oriented data. This allows GXD to provide tools for researchers to evaluate expression data in the larger context, search by a wide variety of biologically and biomedically relevant parameters, and discover new data connections to help in the design of new experiments. Thus, GXD can provide researchers with critical insights into the function of genes and the molecular mechanisms of development, differentiation, and disease. Recent GXD
enhancements include: interactive matrix displays that provide overviews of spatio-temporal expression patterns and facilitate the comparison of expression patterns between genes; filters that enable the iterative refinement of data sets; and gene-based links to expression data from chicken, *Xenopus*, and zebrafish. GXD is funded by NIH grant HD062499.

Program Abstract #342
Large-scale discovery of embryonic lethal phenotypes in mice
Stephen Murray, James Denegre, Brianna Caddle, Candice Baker, Sarah Edie, Kevin Peterson, Matthew McKay, Adrienne Mehalow, Emily Gordon, Robert Taft, Robert Braun, Karen Svenson
The Jackson Laboratory, USA

Nearly one third of all mammalian genes are essential. Embryonic lethal genes identified and characterized through mouse knockouts (KO) have greatly furthered our understanding of gene and pathway function. The overarching goal of the Knockout Mouse Phenotyping Program (KOMP2) and its partners in the IMPC International Mouse Phenotyping Consortium (IMPC) is to generate an encyclopedia of gene function through genome-wide generation and phenotyping of knockout mice. Collectively, the consortium has produced nearly 5000 knockout strains and identified hundreds of embryonic lethal genes. As part of this effort, we have identified a large set of novel lethal genes that we have characterized using a high-throughput embryo phenotyping pipeline, which includes the use of high-resolution 3D imaging for high-throughput generation of rich datasets that are distributed to the scientific community. Additionally, our standardized approach has revealed novel phenotypes for previously described knockouts. We have also developed an atlas of gene expression through comprehensive analysis of in situ LacZ reporter activity, revealing numerous novel gene expression patterns. Together, this program reveals new embryonic lethal phenotypes on an unprecedented scale, providing novel insight into gene function and human disease.

Program Abstract #343
Modeling Developmental Disorders Using CRISPR/Cas9 Mediated Gene Modification
Candice N. Baker1, Kevin A. Peterson1, You Li2, Cecilia Lo2, Stephen Murray1
1The Jackson Laboratory, USA; 2University of Pittsburgh, USA

Advances in genome editing technologies (e.g. CRISPR/Cas9) coupled with next generation sequencing allows for rapid assessment of putative human disease alleles using animal models. Here, we establish the use of direct analysis of CRISPR/Cas9 modified G0 mouse embryos to model novel mutations potentially associated with human developmental disorders. Typically, the timescale from identification of possible causative allele to functional validation is on the order of several months to a year with current technologies. In this study, we demonstrate the feasibility of direct investigation of G0 embryos to model embryonic lethal genes as well as human congenital heart defects reducing the time scale of analysis to weeks. Our method relies on injection of RNA guides and Cas9 mRNA into zygotes, which yield G0 embryos that mimic phenotypes observed in human disease cases. In proof-of-principle studies, we tested both point mutation knock-in and gene disruption knock-out strategies. Identification of the desired mutation was confirmed via traditional cloning and sequencing approaches as well as deep sequencing to account for possible low abundant alleles. Additionally, we observed the intriguing phenomenon of regional mosaicism within tissues of different developmental origins within a single embryo. We show that these potentially confounding effects can be mitigated by engineering mouse embryonic stem cells to contain the desired mutation followed by 8-cell injection into a genetically marked host. This approach eliminates potential genetic ambiguity and enables quick assessment of embryonic stem cell contribution to the embryo. These advancements in generation and characterization of G0 embryos provide a robust and reliable method for the innovation and validation of new human developmental disease models. Funded by NHLBI Bench to Bassinet Program

Program Abstract #344
Utilizing egg biology to understand reprogramming
Christine Reid1, Taejoon Kwon2, Edward Marcotte2, Julie Baker1
1Stanford University, US; 2University of Texas at Austin, US

Oogenesis organizes the egg to efficiently reprogram a foreign nucleus, but how this organization drives the rapid chromatin modifications in reprogramming is poorly understood. To this end, we investigated RNA and protein abundance in the late stage oocyte, mature egg and early one cell embryo in *Xenopus laevis*. We find the oocyte, egg and embryo are transcriptionally and proteomically distinct, suggesting that oogenesis and early fertilization is a dynamic process that utilizes many different transcripts and proteins. Further, we find compartmentalization of a large number of transcripts and proteins to the animal or vegetal regions, suggesting that the oocyte, egg and embryo are organized to drive reprogramming. One of the most abundant protein complexes found within the oocyte nucleus is the Nucleosome
Remodeling and Deacetylase (NuRD) complex, suggesting that chromatin modifications are essential for rapid and efficient reprogramming. Current work is focused on characterizing chromatin changes during oogenesis, fertilization and reprogramming. Identifying endogenous reprogramming factors is essential to define more efficient somatic cell reprogramming.

Program Abstract #345
Global Transcriptomic Analysis of Compensatory Response to Genetic Perturbation of the Notch Signaling Pathway
Andrew Halleran, Caroline Golino, Charith Ratnayake, Brian Rabe, Margaret Saha
College of William and Mary, USA
As an organism progresses through development it encounters chemical, physical, environmental, and genetic stressors that it must overcome in order to develop appropriately. While the molecular mechanisms that govern normal embryonic development are relatively well understood, comparatively little is known about how embryos respond to perturbations over the course of development. However, knowledge of this process is critical for unraveling the embryonic origins of adult disease and for understanding the unique plasticity of embryonic tissues. Conserved across all metazoans, the Notch pathway is a juxtacrine signaling pathway that establishes a balance between differentiated and progenitor neural cells during neurogenesis. Overexpression of Notch signaling at the two-cell stage of *Xenopus laevis* embryos results in a severely reduced NBT expression phenotype by the early tailbud stage. However, as development progresses to the swimming tadpole stage, NBT expression approaches control levels. Through global transcriptome analysis of *Xenopus laevis* embryos in which Notch signaling was mis-expressed at the two-cell stage, we have identified potential regulators of this observed embryonic compensation in response to genetic perturbation of the Notch pathway. 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 #346
Domestic pigeon’s checkered past: a link between wing color patterning and hereditary blindness
Anna Vickrey, Zev Kronenberg, E.J. Osborne, Mark Yandell, Michael Shapiro
University of Utah, USA
Animals have evolved a vast array of colors and color patterns in response to natural, sexual, and artificial selection. Domestic rock pigeons (*Columba livia*) are a stunning example of this diversity because different individuals within the same species vary tremendously in color and color pattern. Classical genetics suggest that four alternative alleles (*T*-check, Check, Bar, Barless in decreasing order of dominance) at a single locus determine wing color pattern. Although the Bar pattern is thought to be the ancestral phenotype, birds with the Check and T-check patterns are more numerous in urban environments, possibly due to enhanced fitness. In this study we investigate the genetic basis of wing color pattern variation. We performed whole-genome comparisons between Bar and Check birds to identify a candidate region containing 5 genes that was highly differentiated between the two phenotypes. Surprisingly, cross-species sequence comparisons suggest that a haplotype in this region that is shared by all Check birds was introgressed into domestic pigeon from a different species, providing a striking example of cross-species transmission of a unique phenotype. Further, we find that the T-check phenotype is associated with an increased copy number of a fraction of the candidate region. Lastly, we find that many Barless birds, which have an increased incidence of vision defects, are homozygous for a nonsense mutation in a gene that is associated with blindness in humans. Remarkably, the exact mutation we find in pigeons is also observed in two human families with hereditary blindness. This study highlights unexpected links between color pattern in an emerging model organism, adaptive introgression, and genetic hitchhiking of a deleterious allele.

Program Abstract #347
Advances in Gene-Targeting in the Zebrafish
Kazuyuki Hoshijima, Mick Jurynec, David Grunwald
Department of Human Genetics, University of Utah, Salt Lake City, UT84112, USA
Gene-targeting, the replacement of specific genomic sequences with exogenously provided sequences, allows for designed modifications of the genome. In yeast and mouse, gene-targeting via homologous recombination (HR) has served as a powerful strategy for eliminating gene function, controlling gene expression conditionally, or visualizing cells that expressed an endogenous gene. As HR events are stimulated by Double Strand Breaks (DSBs) in host chromosomes, we explored the use of engineered nucleases such as TALENs and CRISPR/Cas9 for stimulating gene-targeting in the zebrafish. Here we describe generation of five types of designed modifications of the zebrafish genome. Gene-targeting
was accomplished by injecting zebrafish fertilized eggs with TALEN mRNA to induce targeted DSBs and donor dsDNA with homology to the targeted locus. In a manner dependent on TALEN activity, we achieved: 1) accurate sequence modifications of a targeted locus, 2) sequence modifications accompanied by the concurrent nearby integration of a reporter gene used to mark acquisition of the modification, 3) 'knock-in/knock-out' mutations in which host gene function is destroyed and GFP reporter sequences are productively expressed from the targeted locus; and 4) in-frame insertion of sequences encoding antigen peptides resulting in the generation of viable epitope-tagged alleles of an endogenous gene product. All gene-targeting events were stable and heritable, and integration efficiencies were high enough to establish gene-targeted fish lines. We will report on factors that affect integration efficiency. Our approach to gene-targeting stimulated by targeted DSBs should be applicable in many model organisms. This work has been supported by grants from NIH (1R21OD018323-01 and 1R01HD081950-01).

Program Abstract #348
A genomic approach to investigate the interactions between somatosensory neurons and skin
Fang Wang1, Shawn Cokus2, Alvaro Sagasti2
1California State University, Dominguez Hills, USA; 2University of California, Los Angeles, USA
Somatosensory neurons detect mechanical, thermal, and chemical stimuli, which is crucial for animals to sense their environment and respond appropriately. Many studies have suggested that the interactions between somatosensory neurons and skin cells not only play an important role during development but also may be critical for neuron function. Thus, I have undertaken a genomic approach to investigate the interactions between touch-sensing neurons and skin cells. Peripheral axons of somatosensory neurons first arborize between the two epithelial layers (periderm and basal cells) that compose larval zebrafish skin, starting at the ~18 somite stage. Electron microscopy has revealed that the free endings of somatosensory axons become ensheathed by basal cells between 54 and 78 hours post fertilization (hpf). To identify genes that may be involved in axon/skin interactions, I examined the gene expression profiles of skin cells in zebrafish at three different stages: 20 hpf, 52 hpf, and 72 hpf. To accomplish this goal, I purified periderm cells, basal cells, and non-skin cells with fluorescent activated cell sorting of krt4:DsRed transgenic fish that express DsRed in both epithelial layers and krt5:GFP transgenic fish that only express GFP in periderm cells. I isolated mRNA from these cells and performed RNA-Seq. This method provides comprehensive and quantitative gene expression data. I also performed RNA-Seq on skin cells in wildtype fish and fish lacking somatosensory neurons to gain a genomic view of transcriptional changes in skin cells as they respond to axon innervation. Currently, we are analyzing sequencing data in order to figure out which genes highly express in which tissues at what developmental stages under what conditions, and what are the relationships among these genes. The bioinformatics analyses will reveal many genes that are likely involved in neuron development and function, which will pave the way for further biological analyses.

Program Abstract #349
A screen for Tribbles interacting genes using FijiWings, a toolkit for semi-automated wing morphometric analysis
Anna Shipman, Leonard Dobens
University of Missouri-Kansas City, United States
The Drosophila wing is an ideal tissue to examine how cell proliferation is coordinated with cell growth and proper tissue size. Wing features, including veins and trichome structures, provide distinctive landmarks useful to measure tissue and cell size. We have developed FijiWings, a set of macros that automate plug-ins in the ImageJ/Fiji software, to accomplish morphometric analysis of any wing photomicrograph. FijiWings uses pattern recognition to outline wing veins and image processing to identify individual trichomes to calculate tissue and cell size, respectively. FijiWings is used here to conduct a high-throughput wing misexpression screen for genes that interact with the conserved kinase-like protein Tribbles. A previous screen using the Basler collection of growth regulatory genes, which includes genes involved in Wnt, Myc and insulin signaling pathways, indicates that Tribbles is able to suppress Hedgehog and insulin stimulated growth and enhance Wingless stimulated growth. We tested the Basler collection of genes in a Tribbles sensitized background, and the resulting wings were analyzed using FijiWings. In the wing, Tribbles misexpression decreases wing tissue size but increases the size of wing cells. Co-misexpression of Tribbles with candidate genes would modify these phenotypes, by either restoring the wing tissue and cell size to wild-type, increase the wing tissue size and decrease wing cell size, or make the Tribbles phenotype more severe. Here we describe ongoing work to identify new genes that potentially act in a conserved Tribbles signaling pathway regulating cell proliferation and growth. We want to acknowledge the UMKC School of Graduate Studies Research Award Program, UMKC Women’s Council Graduate Award Fund and NSF IOS-0920613 for funding this research.

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Program Abstract #350
Genome-wide measurement of Bcd binding states at single positions along the AP axis
Colleen Hannon1,2, Shelby Blythe1,2, Eric Wieschaus1,2
1Princeton University, United States; 2Howard Hughes Medical Institute, United States
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, thereby activating at least twenty target genes at different positions along the AP axis. We have developed a method to use chromatin immunoprecipitation followed by high throughput sequencing to measure Bcd binding on a genome-wide scale in small samples of precisely staged embryos. To measure the Bcd binding state at ‘single positions’ along the AP axis, we have developed a series of transgenic lines that express consistent single, uniform concentrations of Bcd. We find that Bcd binding to enhancers that drive expression in anterior regions of the embryo is reduced in embryos expressing a low concentration of uniform Bcd, while binding to enhancers controlling posterior expression is maintained or increased. In embryos lacking posterior and terminal maternal patterning systems, despite dramatic differences in zygotic gene expression patterns, Bcd binding to the majority of its target enhancers is unchanged. These preliminary results support a model in which the positional information provided by Bcd is dependent upon its local concentration in the embryo, and is largely independent of inputs provided by other maternal patterning systems. Research support: Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health, Award Number F31HD082940.

Program Abstract #351
The role of Gata5 in regulation of mesodermal and endodermal progenitor movements in early zebrafish embryos
Anastasiia Aleksandrova1, XueFei Yuan1,2, Ian Scott1,2
1Hospital for Sick Children, Canada; 2University of Toronto, Canada
Gata5 is a transcription factor playing multiple key roles in the embryonic and adult heart. During embryonic development Gata5 regulates cardiac progenitor cell differentiation and formation of the outflow tract and heart valves. In adults, Gata5 participates in the cardiac hypertrophic response and in cardioprotection against ischemia. Gata5 expression initiates very early in embryogenesis in mesodermal cells (precursors of multiple organs including heart and vasculature), and in endodermal progenitors, which form the gastrointestinal and respiratory systems, and endocrine glands. However, functions of Gata5 during the earliest embryogenesis stages remain unknown. We used transgenic zebrafish lines gata5:gfp and smarcd3:gfp to visualize mesendodermal progenitors in vivo. We investigated the effect of morpholino-mediated Gata5 knockdown on cell motility via time-lapse microscopy and cell tracking, and found a significant reduction in mesendoderm migration speed in Gata5 morphant embryos compared to controls. RNA in situ hybridization analysis of genes marking embryonic anatomical landmarks revealed patterning changes in Gata5 morphant embryos suggestive of convergence extension defects. Whole embryo level Gata5 overexpression induced pathological aggregation of mesendoderm. Upon transplantation of Gata5-overexpressing cells into wild type embryos the former assemble into large multicellular clusters. These results suggest that Gata5 may act to regulate expression of surface adhesion molecules in mesendodermal progenitors, subsequently influencing their motility. Ongoing RNA-Seq analysis will compare transcriptional profiles of mesendoderm in control embryos and Gata5 morphants, and reveal the molecular identity of downstream targets of Gata5. In summary, our results suggest a potential role for Gata5 in regulating gastrulation movements of mesodermal and endodermal progenitors.
Funded by HSRLCE (University of Toronto) Fellowship (AA) and CIHR grant MOP 123223 (IS).

Program Abstract #352
Expression of myogenic regulatory factors in the non-contractile electric organs of Gymnotiform electric fish species
Iliana Hernandez, Michael McDowell, Robert Güth, Graciela A. Unguez
New Mexico State University, United States of America
Electric organs of electric fishes are comprised of cells called electrocytes that are specialized to generate electricity. In all species known to date, electrocytes are modified striated muscle cells that disassemble their contractile apparatus and downregulate of most of the myogenic program. Although not contractile, mature electrocytes are multinucleated, receive cholinergic innervation, and continue to express some muscle proteins. Previously, we reported that electrocytes in the gymnotid Sternopygus macrurus express the myogenic regulatory factors (MRFs) MyoD, myogenin, Myf5, and MRF4 at levels similar to those found in skeletal muscle cells despite their incomplete muscle phenotype (Kim et al., 2008, J Exp
Cdx4 leads to reduction of axis length and posterior somite size. Three possible, non-exclusive explanations for the axis reveals that the rate of somite formation is indistinguishable from their wild type siblings. However, in the tailbud, the rate of the region in which the tissue is competent to segment (determination front). Analysis of Cdx4 deficient embryos increased rate of apoptosis, (2) a faster rate of somite generation by the segmentation clock, and/or (3) changes in the size reduction phenotype can be attributed to (1) abnormal tissue growth due to decreased rate of cell proliferation and/or for a transcription factor known to regulate cdx4 candidate likely to coordinate segmentation and patterning is paraxial mesoderm (PSM) is coordinated with patterning mechanisms that bestow each segment it's unique identity. One unanswered question in developmental biology is how the sequential and rhythmic generation of somites in vertebrate somitogenesis that pattern muscles are largely abnormal and defective in multiple human syndromes. Patients with ulnar-mammary syndrome, caused by mutations in Tbx3, have known congenital limb skeleton defects. Tbx3 is a transcription factor expressed in the anterior and posterior margins of the developing limb. To determine the role of Tbx3 in development of the limb musculoskeletal system, we conditionally deleted Tbx3 in mouse in either the lateral plate mesoderm or muscle progenitors. Conditional deletion of Tbx3 in the lateral plate mesoderm recapitulates skeletal phenotypes of ulnar-mammary syndrome. Furthermore, we show that Tbx3 in the muscle connective tissue, but not in muscle, is critical for development of a subset of muscles by E12.5. Muscle precursors and myoblasts are present where these muscles should develop, but the corresponding muscles fail to form. This demonstrates that Tbx3 in muscle connective tissue non-cell-autonomously regulates the differentiation of a subset of muscles. In addition, we show that patients with ulnar-mammary syndrome have previously unidentified muscle defects equivalent to those found in Tbx3 mouse mutants. These data provide the first direct, genetic evidence of a transcription factor required specifically in muscle connective tissue for muscle morphogenesis. In conclusion, Tbx3 in muscle connective tissue is required for vertebrate limb musculoskeletal morphogenesis

Tbx3 in muscle connective tissue is required for vertebrate limb musculoskeletal morphogenesis
Mary Colasanto¹, Payam Mohassel², Michael Bamshad³, Carsten Bonnemann³, Anne Moon¹, Gabrielle Kardon¹
¹University of Utah, USA; ²National Institutes of Health, USA; ³University of Washington, USA
Development of a functional musculoskeleton requires myofibers to be correctly patterned into distinct muscles and then assembled with muscle connective tissue, tendon, and bone. In the limb, connective tissue, tendon, and bone derive from the lateral plate mesoderm-derived limb bud, while muscle derives from migratory somitic precursors. The mechanisms that pattern muscles are largely unknown and defective in multiple human syndromes. Patients with ulnar-mammary syndrome, caused by mutations in Tbx3, have known congenital limb skeleton defects. Tbx3 is a transcription factor expressed in the anterior and posterior margins of the developing limb. To determine the role of Tbx3 in development of the limb musculoskeletal system, we conditionally deleted Tbx3 in mouse in either the lateral plate mesoderm or muscle progenitors. Conditional deletion of Tbx3 in the lateral plate mesoderm recapitulates skeletal phenotypes of ulnar-mammary syndrome. Furthermore, we show that Tbx3 in the muscle connective tissue, but not in muscle, is critical for development of a subset of muscles by E12.5. Muscle precursors and myoblasts are present where these muscles should develop, but the corresponding muscles fail to form. This demonstrates that Tbx3 in muscle connective tissue non-cell-autonomously regulates the differentiation of a subset of muscles. In addition, we show that patients with ulnar-mammary syndrome have previously unidentified muscle defects equivalent to those found in Tbx3 mouse mutants. These data provide the first direct, genetic evidence of a transcription factor required specifically in muscle connective tissue for muscle morphogenesis. In conclusion, Tbx3 in muscle connective tissue is required for vertebrate limb musculoskeletal morphogenesis.

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Program Abstract #353

Cdx4 transcription factor controls the ‘determination front’ rather than the ‘segmentation clock’ during somitogenesis
Saptaparni Bandyopadhyay, Vanessa Fleites, Mejdi Najjar, Isaac Skromne
University of Miami, United States
An unanswered question in developmental biology is how the sequential and rhythmic generation of somites in vertebrate paraxial mesoderm (PSM) is coordinated with patterning mechanisms that bestow each segment it's unique identity. One candidate likely to coordinate segmentation and patterning is cdx4, a gene expressed in the unsegmented PSM that codes for a transcription factor known to regulate hox gene expression that confers somites their identity. We show that loss of Cdx4 leads to reduction of axis length and posterior somite size. Three possible, non-exclusive explanations for the axis reduction phenotype can be attributed to (1) abnormal tissue growth due to decreased rate of cell proliferation and/or increased rate of apoptosis, (2) a faster rate of somite generation by the segmentation clock, and/or (3) changes in the size of the region in which the tissue is competent to segment (determination front). Analysis of Cdx4 deficient embryos reveals that the rate of somite formation is indistinguishable from their wild type siblings. However, in the tailbud, the rate of cell proliferation is decreased and the rate of apoptosis is increased in early segmentation stage embryos. Furthermore, the determination front is affected, as shown by a reduction of FGF transcription posteriorly with a concurrent expansion of Retinoic Acid signaling domain. Thus, by regulating the determination front that controls somite size and the expression of hox identity genes, Cdx4 coordinates paraxial mesoderm segmentation and patterning. Funding

Program Abstract #354

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An unanswered question in developmental biology is how the sequential and rhythmic generation of somites in vertebrate paraxial mesoderm (PSM) is coordinated with patterning mechanisms that bestow each segment it's unique identity. One candidate likely to coordinate segmentation and patterning is cdx4, a gene expressed in the unsegmented PSM that codes for a transcription factor known to regulate hox gene expression that confers somites their identity. We show that loss of Cdx4 leads to reduction of axis length and posterior somite size. Three possible, non-exclusive explanations for the axis reduction phenotype can be attributed to (1) abnormal tissue growth due to decreased rate of cell proliferation and/or increased rate of apoptosis, (2) a faster rate of somite generation by the segmentation clock, and/or (3) changes in the size of the region in which the tissue is competent to segment (determination front). Analysis of Cdx4 deficient embryos reveals that the rate of somite formation is indistinguishable from their wild type siblings. However, in the tailbud, the rate of cell proliferation is decreased and the rate of apoptosis is increased in early segmentation stage embryos. Furthermore, the determination front is affected, as shown by a reduction of FGF transcription posteriorly with a concurrent expansion of Retinoic Acid signaling domain. Thus, by regulating the determination front that controls somite size and the expression of hox identity genes, Cdx4 coordinates paraxial mesoderm segmentation and patterning. Funding
Acknowledgement: Kushlan Research Award, Department of Biology, University of Miami and Sigma Xi Grants in Aid of Research.

Program Abstract #355
Molecular mechanisms of Presomitic mesoderm specification
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The presomitic mesoderm (PSM) is a mesenchymal tissue made of muscle/bone progenitor cells, that is modulated by signals from the Wnts, Fgfs and Retinoic acid (RA) pathways. These pathways exhibit a graded distribution along the anterior-posterior axis during development of the PSM and the somites. 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. 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. As Bmp4 is not expressed in PSM cells but in the underlying tissues (lateral plate mesoderm), these results suggest that Bmp4 sustains the PSM progenitor fate during development.

Program Abstract #356
Mesodermal loss of Stat3 causes mild campomelic dysplasia through the deregulation of Sox9
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To date, alterations of the distant upstream enhancer or within the coding region of the SOX9 gene are the sole genetic lesions underpinning human campomelic dysplasia (CD). While pathological coding-region mutations typically result in a reduced or non-functional SOX9 protein, little is known about what mechanisms control normal SOX9 expression, and subsequently, which signaling pathways may be interrupted by alterations within the upstream sequence. Here, we report the identification of Stat3 as a key modulator of Sox9 expression during chondrocyte maturation and bone growth plate development. We find Stat3 expression to be predominant in tissues of mesodermal origin in post-gastrulation mouse embryos. As such, conditional ablation of Stat3 using mesoderm-specific TCre, in vivo, causes dwarfism and skeletal defects characteristic of CD. Specifically, Stat3 loss results in the expansion of growth plate hypertrophic chondrocytes and deregulation of normal endochondral ossification in all bones examined. 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 its proximal promoter following activation. These findings illuminate a novel role for Stat3 in chondrocytes during skeletal development through modulation of the chondrogenic master regulator Sox9. Importantly, they further demonstrate the first evidence for a gene or signaling pathway, other than Sox9, capable of modeling the pathology of campomelic dysplasia and underscore a potentially valuable therapeutic target for patients with the disorder.

Program Abstract #357
Characterizing the Role of Conserved Wnt targets: sp5 and sp5-like genes, in Neural Patterning in Zebrafish
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Wnt/β-catenin signaling has a conserved role in anterior-posterior patterning of the remarkably complex vertebrate brain from a very basic primordium, the neural plate, by differentially expressing downstream target genes to induce differential neural fates. sp5 and sp5l genes are direct targets of Wnt/β-catenin signaling that encode zinc-finger transcription factors and are hypothesized to modify the transcription patterns of target genes to effect changes downstream of Wnt signaling. Despite the existing strong evidence of the sp5 genes being downstream effectors of Wnt signaling, very little is known about how they mediate the Wnt signal to pattern the neural plate. Also, we have little knowledge of the target genes that the Sp5 transcription factors act on to effect neural posteriorization. In order to determine the function of sp5 and sp5l genes, we have targeted the sp5 locus using TALENs and CRISPR-Cas9 systems and have observed brain abnormalities and axis defects in injected embryos. In addition to our mutant analysis, we will use Chromatin Immunoprecipitation-sequencing to identify the binding sites of sp5 and sp5l in the Zebrafish genome in order to determine the direct targets of
sp5 genes and analyze the downstream transcription networks that effect neural posteriorization. These experiments will reveal how sp5 genes work during development, their interaction with the Wnt signaling pathway and overall understanding of Wnt dependent cell fate specification in the vertebrate brain.

Program Abstract #358

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Ventral spinal interneurons are essential for vertebrate locomotion. As they become post-mitotic each interneuron population expresses a distinct combination of transcription factor (TF) genes. The conservation of these expression patterns between zebrafish and mammals highlights the significant role TFs play in neuronal specification, yet so far only very few of the TFs expressed by each interneuron population have been identified and little is known about the signaling cascades that they control. This critical knowledge gap impedes our ability to treat spinal cord injuries and developmental defects. Using fluorescence-activated cell sorting and transgenic zebrafish lines we have isolated pure populations of V0v, V1, V2a, V2b and Kolmer-Agduhr (KA) ventral spinal interneurons. Total RNA was extracted, converted to fluorescently labeled cDNA and hybridized to a custom-designed Agilent microarray printed with probes for each zebrafish TF. Our microarray data identify additional TFs expressed by the different ventral interneurons examined. Furthermore, we are identifying TFs that are candidates for specifying critical functional characteristics, such as axon trajectory or neurotransmitter phenotypes, by comparing TF expression profiles of populations with opposing phenotypes. Our expression data is validated using in situ hybridization on whole-mount zebrafish embryos and where possible, made available to the zebrafish community through a direct ZFIN data submission (England et al., 2014-present, ZFIN ID: ZDB-PUB-140822-10). We are now establishing a functional validation pipeline using TALEN- and CRISPR-generated mutant lines. These data will significantly increase our understanding of neuronal specification and neuronal circuitry generation - both in the zebrafish, and also (given the high degree of functional conservation of ventral interneurons) in higher vertebrates, including humans. Funding Sources: NIH R21NS073979 and R01NS077947, NSF 1257583, HFSP.

Program Abstract #359
Class 3 semaphorins and neuropilins selectively organise boundary caps at axon entry/exit points at the embryonic CNS-PNS interface
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Boundary caps (BC) are neural-crest-derived cell clusters on the surface of the brainstem and spinal cord that mark the dorsal root entry zone (DREZ) of sensory nerves and motor nerve exit points (MEPs) during embryogenesis. BCs are thought to guard the interface between the peripheral and central nervous systems (PNS/CNS), allowing axons to cross whilst constraining cell bodies of neurons and other cell types, and have been suggested as a possible regenerative therapy. Few genetic pathways have been identified that control BC organisation or function. We demonstrate that SEMA3A and its receptor NRP1 is selectively required for BC clustering and organisation at the DREZ, whilst SEMA3F and NRP2 are specifically required for this process at MEPs. These defects are mirrored by specific defects in axon patterning. Loss of both SEMA3A/3F or NRP1/2 leads to large ectopic boundary cap-like structures, which associate with ectopic axons. In addition, culturing neural crest cells with DRG induces expression of a boundary cap marker, suggesting that neuronal/axonal presence may induce the differentiation of presumptive BC. Our results suggest that SEMA3A and SEMA3F cooperate through NRP1 and NRP2 to restrict BC cells to axon exit and entry points to ensure appropriate BC function.

Program Abstract #360
GCN5 Restricts Diencephalic Size via a Novel Mechanism of Retinoic Acid Signaling
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Diencephalic defects are thought to underlie an array of neurological diseases, but there is still an incomplete understanding of the mechanisms regulating diencephalic development. Early diencephalic development relies upon sonic hedgehog (SHH) signaling in a region called the zona limitans intrathalamica (ZLI). This Shh expression is facilitated by
WNT-mediated repression of the Shh repressor Gli3. However, the mechanisms regulating diencephalic WNT signaling, as well as those required to restrict Shh to the ZLI, are still unknown. Here, we demonstrate that enzymatic activity of the acetyltransferase GCN5 is required for a novel mechanism of retinoic acid (RA) signaling in the forebrain that restricts diencephalic size through repression of WNT signaling and Shh expression. Mice lacking GCN5 activity (Gcn5^hat/hat) display significant expansion of the diencephalon that is accompanied by increased Shh, decreased Gli3, and increased WNT signaling. Gcn5^hat mice crossed with the RA reporter RAREhsplacZ indicate loss of RA signaling in regions of the diencephalon previously implicated in Shh repression. Using a neuroepithelial cell line (NE-4C) and pharmacological inhibition of GCN5, we identified a novel, non-epigenetic mechanism of RA signaling mediated by GCN5-catalyzed acetylation of the centrosome-associated protein TACC1. Co-immunoprecipitation and ChIP studies revealed that GCN5, TACC1, and RARα/γ form a complex on retinoic acid response elements in the absence of RA and that acetylation-induced dissociation of TACC1 from the complex is required for activation of RA target genes. Lastly, we show that gestational RA supplementation rescues diencephalic expansion in Gcn5^hat/hat mutants through a mechanism requiring RARβ. Together, our studies identify a novel mechanism of RA signaling required to restrict diencephalic expansion and refute prior studies that have concluded that RA is dispensable in the forebrain prior to E12.5. (Funded by NINDS Award F31NS087692)

**Program Abstract #361**
**Genetic manipulation of the nkx2.1a locus to study zebrafish hypothalamic development and function**
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The hypothalamus plays a key regulatory role in a wide variety of animal behaviors including feeding, aggression, and reproduction. To further characterize the development and function of this CNS structure, we have begun to develop several genetic tools with which to manipulate the hypothalamus in living zebrafish. *nkx2.1a* is an attractive target for genetic manipulation because the gene is expressed throughout the entire hypothalamus during early development, but is limited to only this brain tissue. Using TALENs we have obtained and recovered several lines containing in/dels that produce frameshifts in the 1st exon of *nkx2.1a* and are predicted to be null alleles. We have also targeted *nkx2.1a* for simultaneous insertion of GFP and knockout of gene function using homologous recombination. A plasmid containing genomic sequence homology arms flanking a GFP transgene was co-injected with TALEN mRNAs. GFP expression in several independent alleles suggests that the construct was inserted both as an ectopic transgene with endogenous *nkx2.1a* enhancer activity and in a targeted manner into the *nkx2.1a* locus. Individual insertion sites are currently being confirmed by analysis of flanking sequences. Our future plans are to generate a knockin of a CreERT2 transgene for temporally controlled genetic manipulation of hypothalamic cells. Together these tools will enable a wide range of studies of the developing and mature hypothalamus. R01 NS082645 - Regulation of hypothalamic radial glia by Wnt signaling

**Program Abstract #362**
**The level of COUP-TF1 expression regulates cortical regional patterning**
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The cerebral cortex represents the brain region that has undergone a major increase in size and complexity during the course of evolution. Patterning of the cerebral cortex into distinct regions is a critical developmental process for the function of mammalian cerebral cortex. From medial to lateral, the cerebral cortex consists of three regions: archicortex, neocortex and piriform cortex. Different regions in the cerebral cortex have unique anatomical and functional properties. The molecular mechanisms for cortical regionalization are largely unknown. COUP-TF1 (NR2F1), a nuclear receptor, is expressed in the cortex during development. It is expressed in cortical progenitors located in the ventricular zone of the dorsal telencephalon and in cortical neurons. The expression level of COUP-TF1 was shown to regulate the size and location of primary sensory areas. Here, we studied the function of COUP-TF1 in regional patterning of the cortex, focusing on the formation of the boundary between archicortex and neocortex. We found that the alteration of COUP-TF1 expression levels in COUP-TF1 conditional knockout or conditional overexpressing transgenic mice led to shifts in the location of the border between archicortex and neocortex. Our findings establish a genetic mechanism for determining regional-fate during cortical development.

**Program Abstract #363**
**Investigating the roles of the direct Wnt/β-catenin signaling targets sal1 and sal4 in *Xenopus* neural tube development**
The vertebrate neural plate is patterned along its anteroposterior axis in part by Wnt/β-catenin signaling activity. Though disruption of this pathway is known to lead to characteristic defects in patterning of the neural plate, few downstream factors have been identified and described in terms of a role in neural plate development. Recent work in our lab has identified direct targets of Wnt signaling involved in neural plate posteriorization, including genes encoding the transcription factors Sall1 and Sall4. Knockdown of either gene’s activity results in partial loss of posterior neural fates, consistent with a role downstream of Wnt signaling, but also causes defects in convergent extension and neural tube closure. Similar to previous reports about sall gene function in other contexts, pouV family gene expression is increased in sall1 or sall4 morphant tissue; consequent failure of these tissues to differentiate properly may be an underlying cause of both the patterning and morphogenetic defects. Recent work has focused on investigating the roles of sall1 and sall4 at the nexus of neural differentiation, patterning, and morphogenesis. In particular, confocal microscopy has been employed to further characterize the open neural tube phenotype of morphants. Ongoing work will better characterize pouV-associated differentiation defects and the consequences for patterning and morphogenesis. This work has been funded by the NIH Genetics Training Grant 5T32GM007127-38.

Program Abstract #364
Determining Skeletal Element Number in the Avian Forelimb Zeugopod
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The pattern of a single bone in the stylopod and two bones in the zeugopod is highly conserved throughout the evolution of the tetrapod limb. Natural variation as well as experimental manipulation of the Sonic hedgehog (SHH) signaling pathway can result in considerable variation in the number of digits—whereas zeugopod number only varies in the complete absence of hedgehog signaling or upon introduction of an ectopic zone of polarizing activity. Recently, IRX3/5 and SALL4 have been implicated in the development of the anterior-proximal limb skeletal elements, but the mechanisms that generate two zeugopod elements downstream of these transcription factors and SHH remain unknown. Here we address this question using classical embryological experiments and deep sequencing. Experimentally enlarging the limb bud by grafting anterior mesenchyme results in ectopic zeugopod elements in both wild type and shh null limbs, however, it cannot be ruled out that donor tissue was already patterned. Consistent with this result, early anterior limb mesenchyme segregates from posterior mesenchymal cells during micromass formation, suggesting a possible role for adhesion molecules in generating two skeletal elements in the zeugopod. Therefore, we used RNA-Seq to characterize the transcriptomes of whole wild type and shh mutant forelimbs as well as dissected anterior and posterior forelimb tissue from stages that span zeugopod development. We identified 25 transcription factors and 10 cell adhesion molecules differentially expressed across the anterior and posterior and verified their expression patterns by whole mount and section in situ hybridization. These factors are then tested for their requirement for anterior posterior segregation in micromass assays and for their sufficiency to rescue skeletal element loss in shh mutants to provide insight into how signaling pathways in the limb bud mediate skeletal patterning of tetrapod limbs.

Program Abstract #365
The SET1/COMPASS complex member wdr82 is required for positional identity in zebrafish.
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We identified a zebrafish mutant in wdr82, an essential and highly conserved member of the SET1/COMPASS histone methylation complex. wdr82 mutants exhibit specific phenotypes affecting multiple tissues, such as retinal coloboma, shortened pectoral fins, defects in kidney filtration, and patterning defects in the craniofacial cartilages. Other members of the COMPASS family are required for expression of Hox genes and other critical developmental regulators; however the role of wdr82 in vertebrate development has never been characterized in detail. Here, we find that wdr82 mutants exhibit distinctive patterning defects and gene expression changes associated with loss of positional identity in the craniofacial cartilages. Together, our results show that wdr82 is required for development in multiple tissues, and that wdr82 and the SET1/COMPASS complex have greater target specificity than has previously been appreciated.

Program Abstract #366
A Six1/Jag1/miR-106b Signaling Axis Contributes to Neural Crest Cell Identity in the Maxilla
Six1 is a homeodomain protein that, together with Eya co-activators, promotes proliferation, survival and differentiation of progenitor cells during development and cancer. Mutations in SIX1 are linked to branchiootic syndrome in humans, though its role in jaw morphogenesis is unclear. To examine this question, we examined facial development in Six1-/- mutant embryos. Six1-/- embryos die at birth with severe craniofacial malformations that include transformation of the posterior region of the maxilla into a rod-shaped bone. This transformation is preceded by expansion of Dlx3, a gene associated with lower jaw development, into the maxillary portion of the first pharyngeal arch and downregulation of maxillary-associated genes Dlx2 and Twist1 in the same region. Endothelin-A receptor (EDNRA) signaling establishes the identity of neural crest cells (NCCs) in the mandibular portion of first pharyngeal arch, due in part to exclusion of the EDNRA ligand EDN1 from the maxillary first arch. We found that transgenic overexpression of Edn1 in maxillary NCCs resulted in similar gene expression changes seen in Six1-/- embryos. Indeed, deletion of one allele of Ednra in a mutant Six1 background (Six1-/-;Ednra-/-) rescued the Six1 mutant jaw phenotype. Interestingly, overexpression of Six1 in cell culture resulted in upregulation of Jagged1 and Notch1 expression (two mediators of maxillary NCC identity). In addition, Six1 expression resulted in upregulation of miR-106b, though overexpression of a miR-106b mimic blocked Six1-mediated increase in Jagl expression Conversely, stimulating the JAG/NOTCH pathway blocked the Six1-mediated increase in miR-106b. Our results suggest that maxillary NCC identity is, in part, established by a SIX1/JAG-NOTCH/miR-106b network, in which JAG1 and miR-106b are repressing each other to refine gene expression boundaries in the developing face. Work funded by NIH/NIDCR DE018899 and DE023050.

Program Abstract #367
Spatiotemporal control of skeletal differentiation in the developing face
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Each element of the skeleton derives from skeletogenic precursors that form tightly packed condensations before differentiating into cartilage or bone. We use the craniofacial skeleton of the zebrafish to study how the early skeletal differentiation program is spatiotemporally regulated in the pharyngeal arches to ensure the reproducible formation of uniquely shaped facial cartilages and bones. Cartilage differentiation in the face initiates in the intermediate domain of the arches and then spreads ventrally and dorsally. This gradient of differentiation is also apparent at earlier stages, with differentiating sox9a+ prechondrocytes in the intermediate region partially overlapping with barx1/lhx6 expression in newly condensed cells that, in turn, are adjacent to peripheral zones of prrx1+ preskeletogenic mesenchyme. Here we use combinatorial genetics and time-lapse live imaging to show that this pattern is established in part by genetic interactions between the Notch, Endothelin-1 and Fgf pathways, which function in distinct dorsoventral domains of the arches to promote (Edn1, Fgf) or restrict (Notch) the condensation of skeletogenic precursors. We find that Jagged-Notch signaling positions nascent condensations within the dorsal domain by inhibiting barx1 expression. Edn1 and Fgf antagonize this activity by cooperatively limiting jag1b expression to a subdomain of the dorsal arches, thus allowing the initiation of barx1 expression in the reciprocal dorsal domain and in ventral cells. Fgf further drives differentiation by promoting lhx6 at the expense of the precursor marker prrx1. Differences between dorsal and ventral cells in condensation establishment and growth, imparted by these and other patterning signals, may be instrumental in sculpting the distinct skeletal morphologies of the upper and lower face. LM is supported by an A.P. Giannini postdoctoral fellowship and previously by a Hearing & Communication Neuroscience fellowship (NIH 5T32DC009975).

Program Abstract #368
Pitx1-mediated regulation of BMP signaling during the formation of the cement gland in Xenopus laevis
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Embryos of the African clawed frog Xenopus laevis are widely used for the study of early vertebrate development. The cement gland is one of the first fully functional tissues to differentiate during Xenopus development. It secretes mucus to help the tadpole attach to solid supports and thus live in relative safety. The gland develops from the outer layer of anterior ectoderm situated between the neural plate and non-neural ventral ectoderm. As a marker of anterior ectodermal fate, the cement gland is a useful model to study how signaling pathways are regionally integrated in the embryo. It has previously been proposed that an intermediate level of Bone Morphogenetic Protein (BMP) signaling promotes the formation of the cement gland. Additionally, several transcription factors have been linked to cement gland differentiation. One of these, the homeodomain-containing protein Pitx1, is both sufficient and necessary for ectopic
cement gland formation. However, the mechanisms by which Pitx1 functions in this context remain obscure. We hypothesized that the appropriate level of BMP signaling within the cement gland primordium is regulated by Pitx1. Our lab has found that injection of synthetic Pitx1 messenger RNA into the animal pole of early *Xenopus* embryos suppresses BMP-responsive target genes in a dose dependent manner. A chimeric protein, consisting of the full length Pitx1 fused to a VP16 activation domain, also inhibits BMP targets, while introduction of a Pitx1-Engrailed repressor domain fusion protein does not significantly affect expression of these genes. Injection of Pitx1 RNA also reduces C-terminal phosphorylation of Smad1/5, without affecting the stability of Smad1. These results suggest that Pitx1 activates downstream targets to inhibit BMP signaling through reduced C-terminal phosphorylation of Smad1, leading to the formation of the cement gland. This work was supported by PHS Grant R03HD077015 (to D.C.W.).

Program Abstract #369
**Establishment of Planar Cell Polarity in the Developing Mouse Utricle Requires Overlapping Functions of Vangl1 and Vangl2**
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Stereociliary bundles emanating from hair cells (HCs) of the inner ear vestibular maculae provide the most striking examples of Planar Cell Polarity (PCP) described in a vertebrate system. Two distinct regions of planar polarity exist within the maculae, separated by the line of polarity reversal (LPR). Currently, little is known regarding the requirement of the core PCP proteins in the establishment and maintenance of HC polarity across the LPR. Removal of the core PCP protein Vangl2 leads to disorganization of PCP in the medial utricle, however the LPR persists in *vangl2* KO mice and hair cells remain organized in the lateral utricle. This raises the question of whether core PCP proteins are necessary to propagate and maintain PCP throughout the utricle, or whether a second, alternative signal is acting specifically in the lateral utricle. To address this, we used an ear-specific Cre-driver to remove both mammalian homologues of Van Gogh, *vangl1* and *vangl2* from the developing ear. HC orientation was assessed using immunofluorescent markers and quantified using customized polarity analysis software. Analysis of *vangl1*;*vangl2* double KO utricles showed disruption of adjacent HC orientation throughout the medial and lateral regions, and demonstrate that core PCP signals are required for organized HC planar polarity on either side of the LPR. Interestingly, no phenotype was seen in *vangl1* conditional knockouts, but as our results suggest, Vangl1 can compensate for the loss of Vangl2 in the lateral region. In addition, further exploration has revealed micro-domains of planar polarity that are maintained in the lateral region of the double KO utricle and are composed of small groups of neighboring hair cells with coordinated alignment of stereociliary bundles. Future research will examine if the presence of these select organized groups reveal a polarity refinement process analogous to the refinement process seen in the cochlea. This research was supported by R01DC013066.

Program Abstract #370
**Spontaneous Planar Cell Polarity in Mouse Keratinocyte Culture**
Bryan Heck, Danelle Devenport
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During development, epithelial tissues organize according to a global planar cell polarity (PCP), which coordinates the alignment of cellular structures across thousands, even millions of cells. Investigations into the establishment of PCP and the interactions between PCP components have been largely limited to *in vivo* systems. Here we describe a planar-polarized mouse epidermal cell culture system - amenable to live imaging - that allows us to investigate how PCP develops *de novo* in the absence of embryo-derived organizing cues. When grown in an air-liquid interface, mouse epidermal keratinocytes develop PCP spontaneously, as measured by the asymmetric distribution of PCP proteins, which propagates locally between cells. Using stable cell lines expressing fluorescently labeled key PCP components, we describe the initiation, amplification, local, and long-range coordination of spontaneous planar cell polarity. Our findings suggest that PCP is an emergent property of cells within an epithelial sheet that both remains stable and can propagate considerable distances in the absence of any external cues.

Program Abstract #371
**Mechanical Control of Planar Cell Polarity in Mammalian Skin**
Wen Yih Aw, Danelle Devenport
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Planar cell polarity (PCP) describes the collective alignment of cells within the plane of an epithelium. A prominent example of PCP is the pattern of uniformly orientated hair follicles that decorate the surface of mammalian skin. The
establishment of PCP involves the asymmetric distribution of cortical PCP proteins, yet the global cues that direct collective PCP polarization remain incompletely understood. Here we quantitatively describe the initiation and amplification of PCP protein asymmetry in the murine epidermis. We find that the initiation of PCP asymmetry coincides, both temporally and spatially, with tissue-wide deformations in epithelial cell shape and stromal reorganization. As basal epithelial cells elongate to more than two times their width, PCP proteins asymmetrically localize perpendicular to the axis of cell elongation. This coincides with the alignment of underlying dermal fibroblasts and their surrounding ECM, suggesting the presence of anisotropic tissue-scale tension during PCP protein polarization. To examine the role of mechanical tension in the establishment of PCP, we applied exogenous mechanical force to epidermal explants. Following stretch, PCP proteins redistribute and hair follicles reorient according to the direction of applied force. We propose a model whereby tissue-scale mechanical tension promotes the long-range alignment of PCP asymmetry.

Program Abstract #372
Myc is required for initiation of radial glia dedifferentiation and programmed cell death after neural injury in an echinoderm
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Adult echinoderms can completely regenerate major parts of their central nervous system (CNS) even after severe injuries. Even though this capacity has long been known, the underlying molecular mechanisms have remained uninvestigated. The major obstacle for understanding these molecular pathways has been the lack of functional genomic studies on regenerating adult echinoderms. The echinoderm CNS is composed of a neuroepithelium, whose supporting framework is formed by radial glial cells strongly resembling radial glia of chordates in their morphological and immunocytochemical characteristics. The post-injury response in the echinoderm CNS involves extensive dedifferentiation of the radial glia. Dedifferentiated glial cells become highly proliferative and form a tubular outgrowth across the wound gap, which later becomes repopulated with neurons. The reaction of the echinoderm glia to injury thus sharply contrasts with formation of the inhibitory glial scar in the lesioned mammalian CNS. In this study, we employed RNA interference-mediated gene knockdown to characterize the role of the transcription factor Myc during the early (first 48 hours) post-injury response in the radial nerve cord of the sea cucumber Holothuria glaberrima. Our previous experiments identified Myc as the only pluripotency factor, whose expression significantly increased in the wounded echinoderm CNS. The specific function(s) of this gene, however, remained unknown. Here, we demonstrate that Myc is required for proper dedifferentiation of radial glia and for initiating programmed cell death in response to CNS injury in the sea cucumber CNS. Our study is the first implementation of RNAi methodology in adult echinoderms, and Myc is thus the first transcription factor, whose role in echinoderm regeneration was experimentally established. Funding: NIH (1SC1GM084770-01, 1R03NS065275-01), NSF (IOS-0842870, IOS-1252679), the University of Puerto Rico.

Program Abstract #373
Role of Niche in Spindle Orientation in Drosophila Germline Stem Cells
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Asymmetric division of adult stem cells generates one self-renewing stem cell and one differentiating cell, thereby maintaining tissue homeostasis. Asymmetric stem cell division within the niche is often accomplished by oriented division to place one daughter in the niche and the other outside the niche. Although stem cell-intrinsic mechanisms that orient stem cell divisions have been studied extensively, the role of stem cell niche in regulating the spindle orientation of stem cells is poorly understood. In Drosophila testis, Unpaired (Upd), a cytokine ligand, is expressed from the hub cells, a major niche component, to specify the identity of germ line stem cells (GSCs). Here we show that Upd is localized in the hub cells as puncta (likely representing vesicles), and its removal either by laser ablation or genetic knockdown leads to spindle misorientation. Further RNAi-mediated knockdown analyses reveal that Upd and its receptor Dome are required for spindle orientations of GSCs, but not their downstream components JAK kinase or STAT transcription factor, revealing the direct role of niche-derived ligand and its receptor in GSC spindle orientation. Our further analysis shows that Dome regulates spindle orientation via its interaction with Eb1, a microtubule plus end binding protein. We propose that niche signaling coordinate stem cell self-renewal and asymmetric stem cell division.
Program Abstract #374
The SWI/SNF chromatin-remodeling complexes are required for Drosophila wing disc regeneration
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Regeneration is a complex yet elegant process that some organisms use to recognize, repair and replace missing tissue. The importance of epigenetic control in development and disease is well established, however the study in regeneration has just started. We study regeneration in the genetically tractable Drosophila wing imaginal disc, using a spatially and temporally controllable tissue ablation system. Because chromatin modifiers, including Trithorax Group (TrxG) activators and Polycomb Group (PcG) repressors, control the expression of key developmental regulators, we hypothesized that they also control regulators of regeneration. We are conducting a candidate dominant modifier genetic screen of existing chromatin modifier mutants. We have found several TrxG mutants regenerate worse than wild type, and several PcG mutants regenerate better. A mutant of brahma (brm), which encodes the ATPase of the SWI/SNF chromatin remodeling complexes, showed a significant decrease in regenerative capacity. There are two Drosophila SWI/SNF complexes: the PBAP complex, which contains Bap170, and the BAP complex, which contains Osa. A bap170 mutant allele also regenerated worse than wild type, while osa mutant alleles regenerated better and showed patterning defects in the posterior part of the wings, suggesting that the two complexes play different roles in regeneration. brm2/+ mutants formed a regeneration blastema but showed defects in regenerative growth. Myc expression levels were significantly lower in brm2/+ regenerating wing discs compared to wild type. Thus the PBAP complex may regulate Myc expression to promote cell proliferation or cell growth after tissue damage. By identifying Myc and other genes regulated by SWI/SNF during regeneration and dissecting how they are regulated, we expect to learn more about the mechanisms through which chromatin modifiers respond to tissue damage and regulate regeneration. We would like to thank the Roy J. Carver Foundation for funding.

Program Abstract #375
Selective functions for core promoter factors in neural stem cell identity
Alexandre Neves, Robert Eisenman
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Tissue development and homeostasis require stem cells to balance self-renewal with differentiation yet how this occurs at the molecular level is poorly understood. Drosophila neural stem cells (NSCs) have emerged as a powerful system to study stem cell biology as they can be unambiguously identified and manipulated in vivo. While many genes and pathways have been shown to be required for NSC differentiation, much less is known about how NSCs maintain an undifferentiated state through multiple self-renewing divisions. We performed an RNAi screen for novel NSC identity genes, and have focused on a subset of TATA-binding protein associated factors (Tafs) and the TATA-binding protein related factor 2 (Trf2), since Taf mutations have been identified in human neurological diseases. In contrast to the model where Tafs function primarily with Tbp, we found that knockdown (KD) of Tafs or Trf2, but not of Tbp, in NSCs resulted in fewer NSCs that exhibited delayed cell cycle progression. Strikingly, neither Tafs nor Trf2 were required for NSC survival since blocking apoptosis failed to suppress Taf or Trf2 phenotypes. Moreover, Tafs and Trf2 were found to be required for expression of a subset of NSC and cell cycle markers, which was corroborated by analyzing Taf mutant NSC clones with the MARCM technique. We also found that Taf and Trf2 KD NSCs exhibit ectopic nuclear accumulation of Prospero, a transcription factor that is necessary and sufficient for NSC differentiation. Finally, we found that Prospero is epistatic to Tafs and Trf2 demonstrating that Tafs and Trf2 are required for the identity of normal, but not differentiation-defective NSCs. Taken together, these results demonstrate that core promoter factors are selectively required for stem cell identity in vivo by promoting cell cycle progression and preventing premature differentiation. We are currently testing our hypothesis that Tafs bind to Trf2 to form a novel core promoter transcription system.

Program Abstract #376
A key molecular link between niche signaling and the regulatory network controlling stem cell maintenance and differentiation
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Stem cells rely on signaling from their “niche”, but the key genes directly downstream of niche signaling responsible for stem cell maintenance are for the most part unknown. C. elegans GLP-1/Notch signaling from the niche is essential for germline stem cell (GSC) maintenance (Austin & Kimble 1987). Downstream, an RNA regulatory network drives stem cell or differentiated fates (Kimble & Crittenden 2007). Most relevant here, the Puf family protein FBF translational
repressor maintains GSCs by regulating >1000 genes enriched for differentiation factors (Kershner & Kimble 2010). Yet, the link between niche signaling and the regulatory network was unknown. We recently found lst-1 and sygl-1 as key redundant genes triggered by GLP-1: single lst-1 and sygl-1 mutants appear normal, but lst-1 sygl-1 double mutants are indistinguishable from glp-1 mutants (Kershner et al. 2014). Multiple lines of molecular evidence confirm that lst-1 and sygl-1 are indeed targets of GLP-1 signaling. Thus, LST-1 and SYGL-1 together fully account for the effect of niche signaling on stem cell maintenance. Both LST-1 and SYGL-1 proteins are novel, and restricted to a pool of ~50 GSCs. To ask if either is sufficient for stem cell fate, we made transgenes stripped of regulatory elements and expressed them ubiquitously. Each drives germline tumor formation on its own, confirming their importance for the stem cell fate. We now have used the unregulated sygl-1 transgene to place its activity in the network. Unregulated SYGL-1 drives germline tumors in animals lacking GLP-1, so SYGL-1 does not require other GLP-1-dependent factors to make stem cells. By contrast, unregulated SYGL-1 cannot generate germline tumors in fbf mutants, so FBF is required for the SYGL-1 stem cell mechanism. Similar experiments for unregulated lst-1 are underway. We conclude that SYGL-1 and likely LST-1 are key molecular links between niche signaling and the regulatory network driving stem cell or differentiation fates.

Program Abstract #377

Potential Link between TGFβ signaling and Notch signaling in C. elegans Germ Line Development

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A recently discovered characteristic of some stem/progenitor cells is that they can respond to environmental cues to properly regulate their numbers. The C. elegans germ line provides an ideal model to study the molecular mechanism involved in how environment influences the balance between proliferation and differentiation of stem cells. In C. elegans, germline progenitors accumulate during larval stages. The Notch signaling pathway maintains the proliferative pool of germ cells with its ligands expressed in the niche, the distal dip cell (DTC), and the receptor present on germ cells. TGFβ signaling has been shown to modulate the number of progenitors in developing germ line by influencing the balance between the proliferative vs. differentiated state, providing a link between sensory cues and germ cell fate. Preliminary results show that one possible mechanism by which TGFβ contributes to germline proliferation is regulating expression levels of lag-2, one of the Notch ligands. lag-2 is expressed in the DTC and its expression is decreased in daf-7/TGFβ and daf-1/TGFβRI mutants based on the transcriptional reporter analysis. To test the effect of TGFβ signaling on expression levels of lag-2 in an endogenous context we generated strains expressing the genomic sequences of lag-2 fused to GFP in a fosmid containing ~40kb of the C. elegans genome including native lag-2 regulatory sequences. To elucidate the regulatory sequences of lag-2 promoter that are influenced by TGFβ signaling, we generated strains expressing mCherry driven by different lengths of lag-2 promoter in TGFβ mutant backgrounds. In addition we created an alignment of orthologs of the lag-2 promoter in Caenorhabditis and located four highly conserved domains, which we will investigate further.

Program Abstract #378

Mechanisms that drive non-stem-cell fates during planarian stem cell lineage development

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Neoblasts are adult stem cells (ASCs) in planarians which sustain cell replacement during homeostasis and regeneration of any missing tissue. While numerous studies have examined mechanisms underlying neoblast pluripotency, molecular pathways driving the postmitotic fate remain poorly defined. Here we used transcriptional profiling of irradiation-sensitive and -insensitive cell populations and RNA interference (RNAi) functional screening to uncover markers and regulators of postmitotic progeny. We identified 32 new markers, which distinguish two epithelial progenitor populations, and a planarian homolog to the MEX3 RNA-binding protein (Smed-mex3-1) as a key regulator of lineage progression. mex3-1 is required for generating progenitors of epithelial, eye, and neural lineages, and concomitantly restricting expansion of the stem cell compartment. We also demonstrate the utility of using mex3-1(RNAi) animals to identify additional progenitor markers. These results show that mex3-1 promotes differentiation in multiple, if not all, lineages, and maintains the balance between ASC self-renewal and commitment.
Program Abstract #379

**Ptk7 and noncanonical Wnt signaling control A-P positional identity in planarian regeneration**

Christian Petersen, Rachel Lander

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Regeneration relies critically on robust regulation of tissue patterning to define with precision the appropriate content of the regeneration blastema. Planarians can regenerate any missing tissue and therefore must have systems that enable restoration of axial information disrupted by diverse injuries. Canonical Wnt signaling and several downstream factors establish identities of the termini of the anteroposterior (A-P) axis during head and tail regeneration, but comparatively little is known about how position along that axis is redefined after injury. We identify \textit{protein tyrosine kinase-7 (ptk7)} as a critical determinant of trunk and tail identity during re-patterning of the A-P axis through regeneration. \textit{ptk7} is expressed in an animal-wide graded fashion with highest levels in the trunk, and during axis reestablishment through regeneration, \textit{ptk7} undergoes dynamic expression changes independent of neoblast stem cells. Inhibition of \textit{ptk7} by RNAi resulted in duplication of centrally located tissues and positional displacement of regenerating organs without affecting pole identity. Ptk7 proteins are known to modulate noncanonical Wnt activity, and double RNAi experiments identify a network of axially expressed Wnt and FGFR-like genes acting with \textit{ptk7} and independently of beta-catenin signaling. Surprisingly, this signaling system operates early after injury and in a dose-dependent manner to position formation of trunk and posterior tissues prior to the restoration of A-P gradient gene expression domains. These results identify a positional gene regulatory program that controls aspects of patterning along the primary body axis during whole body regeneration. Clarifying the regulatory logic that enables restoration of positional information to an axis truncated by amputation will be an important step in understanding regenerative abilities. Funding: NRSA F32GM108395-01A1, Ellison Medical Foundation, NIDCR 1DP2OD017280-01.

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Program Abstract #380

**The role of interleukin 11-signaling in tail regeneration of *Xenopus laevis* tadpoles**

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Organ regenerative ability varies depending on animal species and/or developmental stages, but factors that determine regenerative ability are poorly understood. *Xenopus laevis* tadpoles transiently lose tail regenerative ability during a certain developmental stages called refractory period. To analyze regeneration-specific molecular processes, we previously performed RNA-seq analysis to compare gene expression profiles of proliferating and non-proliferating cells isolated from regenerating tail blastemas as well as proliferating cells isolated from tail buds. As results, we found that interleukin 11 (il-11) was expressed selectively in proliferating blastema cells, and this was confirmed by double staining of BrdU-immunohistochemistry and in situ hybridization (ISH) for il-11 [Tsujioka et al., PLOS ONE (2015)]. Here we compared il-11-expression in regenerating tail stumps between regeneration-capable and refractory periods. Quantitative RT-PCR showed that expression levels of il-11 prominently elevated 24 hr after amputation (hpa) in both periods. However, ISH revealed that, whereas il-11 was expressed at the tip of blastema 72 hpa during regeneration-capable period, it was expressed in dorsal area of notochord during refractory period, suggesting that shift in il-11-expression to the tip of blastema is important for subsequent regenerative processes. Next, to analyze possible role of il-11 in tail regeneration, we used Statick, an inhibitor of Stat3, which functions downstream of IL-11-signaling. Stat3-treatment remarkably inhibited regeneration during regeneration-capable period, suggesting that Stat3, thereby IL-11, is necessary for tail regeneration. This work was supported by Grant-in-Aids for Scientific Research on Innovative Areas ‘Homeostatic Inflammation’, and for Challenging Exploratory Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. HT is a Research Fellow of Japan Society for the Promotion of Science.

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Program Abstract #381

**miR-133a and miR-1 negatively regulate RARβ2 expression during caudal spinal cord regeneration in the adult newt.**

Amanda Lepp, Robert Carlone

*Brock University, Canada*

Adult urodèles possess the ability to regenerate lost structures, including caudal spinal cord, following tail amputation. Our lab examines the role of retinoid signaling during caudal spinal cord regeneration in the adult newt. We have previously shown that ependymal cell outgrowth and spinal cord regeneration are dependent upon retinoid signaling through the retinoic acid receptor beta (RARβ). Currently, we are identifying downstream effectors of RARβ-mediated
signaling during this process. MicroRNAs, due to their ability to regulate translation of many mRNA targets and thus coordinate many complex pathways, are candidates as downstream effectors during epimorphic regeneration. We utilized a microarray with microRNA-based profiling, which identified 18 highly conserved microRNAs that displayed significant changes in expression in tail regenerates treated with an RARβ2 selective antagonist, LE135, when compared with those expressed in control regenerates. We have chosen 7 of these microRNAs for further investigation with qPCR, in situ hybridization and functional studies using microRNA mimics and inhibitors. miR-133a and miR-1 are enriched in ependymal cells prior to injury, and significantly downregulated during the first 21 days post amputation. Both miR-133a and miR-1 were shown to target the 3’-UTR of the newt RARβ2 transcript in in vitro luciferase assays. Moreover, in vivo injections of either a miR-133a or miR-1 mimic decreased RARβ2 levels post injury. These data are suggestive, at least indirectly, of a negative feedback loop between these microRNAs and retinoid signaling within the ependyma, during spinal cord regeneration in the newt. We also examined the effects of inhibiting RA synthesis using a RALDH inhibitor, diethylaminobenzaldehyde (DEAB), on miRNA expression during tail regeneration. This microarray revealed a large number of dysregulated miRNAs whose roles during caudal spinal cord regeneration are still under investigation. Funded by NSERC.

**Program Abstract #382**

**Examining the pattern of a p21-like protein during tail and caudal spinal cord regeneration in the adult newt.**

Rachel Nottrodt, Robert Carlone

*Brock University, Canada*

Adult newts are capable of completely regenerating lost structures including limb, tail, and spinal cord tissues after injury. One mammal capable of similar regeneration is the Murphy Roths Large (MRL) mouse with the ability to fully close ear hole punctures. Examination of these mice revealed that they express very low levels of the protein p21, a cyclin dependent kinase inhibitor (CDKI), which regulates progression of the cell cycle from G1 to S phase by monitoring the cell for DNA damage. Knockout p21 mice were also able to fully close ear hole punctures validating the role of p21 in this phenomenon. Based upon these findings, our research seeks to examine p21 during newt tail and caudal spinal cord regeneration. Given that low levels of p21 allow for regeneration in these mice, otherwise considered regeneration-incompetent, it is of interest to study p21 in a regeneration-competent newt species, *Notophthalmus viridescens*. Using qPCR, we have demonstrated that p21 transcripts are significantly downregulated at 14 and 21 days post amputation (dpa). Initial protein analysis with Western Blotting indicated temporal changes in expression across regeneration time points, but at a molecular weight smaller than the predicted 21kDa. The protein recognized by the p21 antibody is highly expressed at day 0 with downregulation seen at 7 and 14 dpa followed by resurgence at 21 dpa. The discrepancy in molecular weight lead to mass spectrometry analysis indicating the presence of a p16-like interacting protein that shares 87.5% identity and 93.8% similarity with the *Xenopus* p16-interacting protein. Further in silico analysis found the p16-interacting protein shares 28.6% identity and 64.3% similarity with the human p21 sequence. Previous work has shown that *Xenopus* p16 is the functional homolog of human p21. This work represents the first study of this CDKI family in newt tail regeneration. RN funded by OGS and CGS-M NSERC grants and research funded by RC NSERC Discovery grant.

**Program Abstract #383**

**Identification and study of the miR-125 family in *Ambystoma mexicanum* limb regeneration**

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*Ambystoma mexicanum* is a neotenic organism endemic of the México valley, it has an extraordinary regenerative capacity which has made of it one of the most prominent experimental models to study the mechanisms of vertebrate regeneration. The limb regeneration is a complex process that involves cellular de-differentiation, the formation of a blastema and a well-coordinated balance between cellular proliferation and re-differentiation. It has been previously shown for other developmental processes that miRNAs play a role in modulating the expression of genes that are pivotal for development. The miRNA family “miR-125” is highly conserved between multiple vertebrate and invertebrate species and it has been previously related in the modulation of cellular proliferation and differentiation, through the regulation of multiple genes involved in these processes. Therefore the fine tune modulation of miR-125 levels defines, in some cases, the differentiation status of cells and tissues. In this study we explore the possibility of the role of the miR-125 family in
modulating cell differentiation in diverse stages of *A. mexicanum* limb regeneration. By *in silico* analyses of a library on non-coding RNAs of *A. mexicanum*, we have identified two different transcripts which we named pri-miR-125a and pri-miR-125b. We analyzed also libraries of small RNAs and found two mature miR-125 sequences, which we classified as miR-125a and miR-125b. We also predicted putative targets for both miR125a and miR125b in *A. mexicanum* and performed a GO analysis. We have found that several potential targets are involved in biological processes that are important in the distinct stages of limb regeneration. By using qRT-PCR and ISH we plan to confirm the existence of both miR125a and miR125b and to study the spatio-temporal expression pattern both miR-125a and miR125b during different stages of *A. mexicanum* limb regeneration.

Program Abstract #384
Analysis of the Cyclin D1/Cyclin Dependent Kinase 4 complex during limb regeneration of *Ambystoma mexicanum.*
Miguel Angel García-Olivares, Luis Herrera-Estrella, Luis Fernando García Ortega
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico
Cell proliferation is absolutely necessary during limb regeneration of the axolotl (*Ambystoma mexicanum*), the formation and growth of the blastema requires that somatic cells change its genetic program to proliferate, in the re-developmental stage the different tissues are formed by differentiation of the blastema cells to generate a new limb. These two major cell reprogramming events imply a tight balance among cell division and differentiation. Two molecules which are essential to re-enter the cell cycle are Cyclin D1 (CYCD1) and Cyclin-dependent kinase 4 (CDK4), this proteins form a complex to phosphorylates the pocket protein Retinoblastoma (pRB), resulting in the transcriptional activation of downstream targets that promote the G1-to-S transition. In order to know if CYCD1 and CDK4 are differentially expressed in any stage of axolotl limb regeneration. We first analyzed our axolotl transcriptome library data and identified only one putative transcript for each protein, AmCYCD1 and AmCDK4. Then, based on the mRNA sequences, we generated computationally the protein sequence and the 3D-folding of the complex. We also created phylogenetic trees which show the evolutionary importance of preserve the LxCxExE and QMALT motifs which are essential for the folding and activity of the complex. Then, we amplified by RT-PCR the CDS of the genes and cloned them. In addition, by generating prey and bait constructs we have performed Y2H assays and observed a strong *in vitro* interaction between AmCYCD1 and AmCDK4. Finally, we performed a preliminary qPCR analysis that suggests a correlation between the increase in AmCYCD1 and AmCDK4 transcripts and the phases of establishment and growth of the blastema. Our results together suggest that AmCYCD1 and AmCDK4 transcripts levels are differentially expressed during regeneration and the corresponding coded proteins interact to form a complex which may be required during limb regeneration to activate the cell cycle.

Program Abstract #385
Lizard Tail Skeletal Regeneration Combines Aspects of Fracture Healing and Blastema-Based Regeneration
Thomas Lozito, Rocky Tuan
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Lizards are amniotes with the remarkable ability to regenerate amputated tails. The early regenerated lizard tail forms a blastema, and the regenerated skeleton consists of a cartilage tube (CT) surrounding the regenerated spinal cord. The proximal CT undergoes hypertrophy and ossifies, while the distal CT resists ossification for the lifetime of the lizard [Lozito et al. 2015. *Dev Biol*]. We hypothesize that differences in cell sources and signalling account for divergent cartilage development between proximal and distal CT regions. Lizard (*Anolis carolinensis*) tail blastemas were (1) implanted with exogenous spinal cords, (2) manipulated to remove endogenous spinal cords, or (3) treated with the cyclopamine, BMP-2, or noggin. Following 2 weeks of growth, blastemas were immunostained for cartilage development markers. Exogenous spinal cord implants induced chondrogenesis in surrounding blastemal cells, effectively forming ectopic CTs. Regenerated spinal cords expressed Shh, and cyclopamine inhibited CT induction. Blastemas containing vertebrae with intact spinal cords formed CTs with proximal hypertrophic regions and distal non-hypertrophic regions, while removal of spinal cords resulted in formation of proximal CT areas only. In fate mapping studies, FITC-labelled vertebral cells were detected in proximal, but not distal, CT areas. Conversely, FITC-labelled blastema cells were restricted to distal CT regions. Proximal cartilage formation was inhibited by noggin and stimulated by BMP-2. These results suggest that the proximal CT is directly derived from vertebral cells in response to BMP signalling, while the distal CT forms from blastemal cells in response to Shh signals from the regenerated spinal cord. Thus, the proximal CT develops independently from the lizard tail blastema, resembling a cartilage callus formed during fracture repair, while the distal cartilage tube is derived from the blastema similar to regenerated salamander tails. Support: SAP 4100050913
Program Abstract #386
Loss of regenerative capacity in pectoral fin of *Polypterus senegalus*.
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The Senegal bichir (*Polypterus senegalus*) possess the particularity of being a lunged fish able to regenerate its pectoral fins. Fin regeneration in the Senegal bichir starts with the formation of a blastema. After losing its fin, it takes approximately 9 days for the differentiation process of the fin’s tissue to be noticeable, the total fin regeneration time lasting about 5 weeks. During this process, bone tissue is regenerated, which provides structure for the fin (pr-propterygium, mt-metapterygium, ms-mesopterygium, rd-radials and lp-lepidotrichia). Previous studies have shown the regenerative “accuracy” the fin displays starts to diminish as the same fin is repeatedly amputated and regenerated. In the present study, we show the skeletal defects and alterations from the repetition of said processes that affect the osseous structure conforming the fin (pr, mt, ms, rd and lp), this on 5 separate occasions where the fin was amputated and subsequently, allowed to fully regenerate. We observed alterations in the pattern of the radials at the end of the second and third regenerations. However, after the fourth and fifth regenerations took place, we noticed a reduction in some bone elements (mt, ms and rd). Finally, it’s worth mentioning that organisms fed with living food grew bigger in size and displayed a better overall regeneration than the ones that were only fed fish pellets.

Program Abstract #387
Early proximodistal specification of blastemal cells during zebrafish fin regeneration
Valerie Tornini, Greg Nachtrab, Leslie Slota, Kenneth Poss
Duke University, United States

Determining how amphibians and certain fish pattern regenerating appendages has broad implications for regenerative biology and medicine. After amputation of a zebrafish fin, each individual fin ray forms a blastema, which contains proliferating cells that generate new structures. How proximo-distal (PD) positional identities of cells are acquired and maintained in regenerating appendages remains an elusive question in the field. Two main opposing models of PD specification have been proposed to explain limb regeneration in salamanders. The early specification model suggests that appendage blastemal cells possess or acquire positional cues upon amputation and expand in their domains as growth progresses. More recently, evidence has supported a progressive specification model, which posits that cells receive positional cues from their environment progressively during growth. In this study, we identify a tryptophan hydroxylase that is induced in regenerating fin blastemas. Using a Cre recombinase-based approach, we label single early mesenchymal blastemal cells and image their progeny throughout regeneration to identify patterns of clonal contributions. Surprisingly, our data suggest that PD positional information is specified early in regeneration, rather than progressively acquired. These findings illuminate how an undifferentiated adult primordium obtains patterning information during regeneration of a complex structure. We acknowledge funding from NSF GFRP [1106401] to V.A.T., and National Institutes of Health [GM074057] and HHMI funding to K.D.P.

Program Abstract #388
Distinct groups of osteoblasts deviate from differentiation and regulate formation and maintenance of intramembranous joints in zebrafish caudal fin
Jing Zhang, Leona Probst, Marie-Andree Akimenko
University of Ottawa, Canada

Zebrafish fin rays have been used to study intramembranous bone because of their structural simplicity, easy accessibility and because fin ray regeneration mostly recapitulates ray developmental process. Zebrafish fin ray, lepidotrichia, consists of two hemirays, each of which is made of successive segments. Fin ray outgrowth is achieved by the distal addition of segments which are connected by joints. The lepidotrichia are intramembranous bones formed by deposition of bone matrix secreted by osteoblasts. How osteoblasts are spatially and temporally regulated to craft the distinct segments and joints remains unclear. In this study, we sought to examine the origin of joint-forming cells during fin regeneration. Formation of the new joint is characterized by the appearance of a small group of cells emerging from differentiating osteoblasts at locations where joints are to form. Gene expression analysis of osteoblast markers for various differentiation stages revealed that the early marker *runx2a* is continuously expressed in all osteoblasts while the intermediate marker *osterix*, late marker *ostecalcin* and *collagen Xa1* (component of lepidotrichia) are expressed in most osteoblasts but absent in the joint-forming cells. *pthrp1* and *hoxa13a* expression are activated in the joint-forming cells, suggesting their potential role in inducing osteoblast maturation arrest and/or initiating a different differentiation path for the joint cells. Immunostaining on intact lepidotrichia with Zns-5, a pan-osteoblast marker, showed that joint cells keep a round shape
compared to the flattened shape that osteoblasts acquire as they mature. *pthrp1* and *hoxa13a* expression persists in the joints of intact lepidotrichia suggesting that joint cells may always maintain the same gene expression profile as “joint-forming cells” possibly to maintain the bone surfaces and to repair bone damages caused by friction during swimming. Supported by a CIHR grant to MAA.

**Program Abstract #389**

**Positional Cues Involving Hedgehog Signaling Direct Joint Formation in Zebrafish Fin Ray Regenerates**

Stephanie McMillan, Marie-Andrée Akimenko  
*University of Ottawa, Canada*

During zebrafish fin regeneration, a blastema, a group of undifferentiated mesenchymal cells, forms underneath the wound. As regeneration proceeds, cells in the proximal blastema differentiate, giving rise to the multiple cell types that will reform the lost fin. These cells include the osteoblasts that secrete a bone matrix that will reconstruct the dermal bones making the fin rays through the sequential addition of new bone segments. During the addition of new segments, a distal cluster of cells expressing both *parathyroid-related protein 1 (pthrp1)* and *even-skipped homeobox 1 (evx1)* indicate the location of a future joint. These joint markers are lost when fins are treated with FK506, a Calcineurin inhibitor that has been shown to disrupt positional cues related to fin proximal-distal patterning. Furthermore, there is a correlation between *pthrp1/evx-1* expression and the presence of *patched-1 (ptc-1)*, a Hedgehog signal receptor, which is expressed intermittently in differentiating osteoblasts. Together, these data indicate that Hedgehog signaling and positional cues are involved in joint formation. Currently, FK506 is being used as a tool to determine the effect of a disruption of positional cues on the cyclical expression of *ptc-1*. Furthermore, the role of Hedgehog signaling in joint formation is being explored through cyclopamine and purmorphamine treatments, which inhibit and induce Hedgehog signaling, respectively. Effects on joint formation will be determined based on segmentation length and expression of joint-forming genes. This work is supported by a CIHR grant to M.-A.A.

**Program Abstract #390**

**Lineage tracing of adult-born GABAergic neurons in zebrafish**

Elyssa Mahoney, Cynthia Solek, Shengrui Feng, Marc Ekker  
*University of Ottawa, Canada*

The *dlx* gene family codes for homeodomain transcription factors that play several important roles in vertebrate development, including the specification of GABAergic interneurons in the brain. Abnormalities in the development of these neurons is associated with epilepsy, schizophrenia, and autism spectrum disorder. Although this is a subject that has been studied in other species, relatively little is known about how GABAergic neurons develop in zebrafish. Even less is known about their neurogenesis in the adult brain. We traced the lineage of *dlx*-expressing cells in the adult zebrafish brain. We accomplished this by using an inducible Cre-loxP system where an inducible Cre recombinase was driven by *dlx* regulatory elements. These fish are crossed with *Ubi Switch* reporter fish to generate double transgenic offspring that can be induced with tamoxifen for lineage tracing. Cells expressing *dlx* at the time of induction will permanently express mCherry. We have induced adult *dlx1a/2a:Cre;Ubi Switch* and *dlx5a/6a:Cre;Ubi Switch* fish and characterized mCherry expressing cells at different time points post induction. Immunohistochemistry was performed against mCherry, neuronal markers, and GABA subtype markers. Preliminary results show that mCherry labelled cells can be found throughout the brain up to two months after induction. Some of these cells express neuronal and/or GABA subtype markers as early as one week post induction. We are currently determining whether *dlx1a/2a-* and *dlx5a/6a*-expressing cells acquire exclusively a neuronal fate, or if some of these cells contribute to glia. Our work will help assess the role of *dlx-*expressing cells in adult zebrafish neurogenesis. Funding sources: the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada.

**Program Abstract #391**

**Zebrafish Hair Cell Regeneration Mutant Generated by CRISPR-Cas Genome Editing tool**

Luis Colón-Cruz, Gaurav Varshney, Normarie Herrera, Shawn Burgess, Martine Behra  
*University of Puerto Rico - Medical Sciences Campus, Puerto Rico; NHGRI, NIH, Bethesda, MD, USA*

Higher vertebrates lack the capacity to regenerate mechanosensory hair cells (HC) of the inner ear which are required for hearing and balance. By contrast, fish and amphibians can regenerate HC in their inner ears but also in an evolutionary linked sensory organ, called the lateral line (LL). This superficial organ informs fish on movements in the surrounding waters through stereotypically distributed sensory patches called neuromasts (N). Each N is composed of centrally located HC and surrounding supporting cells (SC) which contain progenitor cells that will divide to replace and regenerate lost
HC. We have previously shown that tankyrase 1 binding protein 1 (tnks1bp1) is expressed specifically in all SC of the lateral line. Its homologs have been implicated in maintenance of telomeres’ length and we hypothesized a similar role in SC and therefore this gene should be crucial in the HC regeneration process. To test this, we have generated mutant alleles using the CRISPR/Cas9 system, by co-injecting two guided RNAs designed against two distinct targets (T1 and T2) in the tnksbp1 gene. We have raised potential founders to sexual maturity and inbred them. The offspring (F1) have been genotyped for confirmation of INDELS in tnks1bp1 exonic regions. We generated those mutants in a double transgenic background, TG(tnksb1p1: GFP x atoh:TOM) which allowed us to directly visualize defective development of the LL and altered HC regeneration using in vivo techniques. Preliminary results suggest that this gene is crucial for proper development and regeneration of the LL. Future work will aim at establishing if and how this is linked to telomere length maintenance. This will expand our understanding of HC regeneration and bring us closer to translational solutions addressing HC loss and its devastating consequences on human hearing.

Program Abstract #392
Healing of large-scale bone defects does not recapitulate development and involves hybrid cartilage/bone skeletal progenitors
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The treatment of significant bone loss associated with fracture non-unions, revision total joint replacement, and tumor resection remains an unresolved problem today. Autogenous bone graft material is limited in supply and the biological activity of synthetic substitutes requires further refinement. Thus, new strategies for more physiological healing of critical sized bone defects are much needed. In particular, we are testing a novel hypothesis that the formation of a cartilage intermediate is a prerequisite for large-scale bone healing, not simply because it acts as a template for invading osteoprogenitors, but instead because repair chondrocytes are distinct from growth plate chondrocytes in that they have properties of both cartilage and bone cells. Evidence for this hypothesis has been obtained by studying extensive regeneration seen in the fish jaw and mouse rib, as well as the more modest repair of unstabilized fractures. Gene expression analysis and other assays indicate that repair does not recapitulate development. Current studies in our laboratories are determining the molecular signature of these repair chondrocytes and developing ways to generate them in large numbers for augmenting skeletal repair.

Program Abstract #393
ZF143 enhances zebrafish heart regeneration and improves mouse adult heart function after an MI injury
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Heart disease is the leading cause of morbidity and mortality in the Western world due to a limited regenerative capacity. In lieu of new muscle synthesis in response to injury, the human heart replaces necrotic tissue with deposition of a non-contractile scar. While a scar provides short-term benefits, its long-term perdurance often culminates in heart failure. Thus, there is a tremendous unmet need for therapeutic intervention that stimulates cardiomyocyte proliferation. The adult zebrafish is endowed with a remarkable regenerative capacity, capable of de novo cardiomyocyte creation and complete recovery of lost heart function when challenged with an acute injury. In this study, we employed the zebrafish to screen a limited number of compounds in order to identify molecules that may amplify genetic networks that underscore regenerative capacity. We identified ZF143, a naturally occurring compound that greatly enhances regeneration of heart tissues. ZF143 increases cardiomyocyte proliferation indices by over 200% when compared with control animals. This enhancement in proliferation is accompanied with new muscle creation and decreases in scar tissue deposition within the wounded apex. Importantly, ZF143 is able to restore regenerative capacity in a zebrafish genetic model with repressed heart regenerative capacity. Additionally, in blinded adult mice studies, ZF143 profoundly increased survival under conditions of LAD injury. This increased survival is accompanied with improved heart function and decreased infarct size following a 1-month treatment with ZF143. Collectively, this data suggests ZF143 could have strong therapeutic applications for stimulating heart muscle regeneration.

Program Abstract #394
Muscle stem cells contribute to myofibers in sedentary adult mice
Alexandra Keefe, Jennifer Lawson, Steven Flygare, Zachary Fox, Mary Colasanto, Sam Mathew, Mark Yandell, Gabrielle Kardon
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Skeletal muscle is essential for mobility, stability, and whole body metabolism, and muscle loss, for instance during sarcopenia, has profound consequences. Satellite cells (muscle stem cells) have been hypothesized, but not yet demonstrated, to contribute to muscle homeostasis and a decline in their contribution to myofiber homeostasis to play a part in sarcopenia. To test their role in muscle maintenance, we genetically labeled and ablated satellite cells in adult sedentary mice. We demonstrate via genetic lineage experiments that even in the absence of injury, satellite cells contribute to myofibers in all adult muscles, although the extent and timing differs. However, genetic ablation experiments showed that satellite cells are not globally required to maintain myofiber cross-sectional area of uninjured adult muscle. This work was supported by NIH Hematology Grant (T32 DK007115) to A.K., NIH Developmental Biology Grant (T32 HD07491) to M.C. and NIH R01 HD053728 and MDA 130903 to G.K.

Program Abstract #395
Evidence of co-chaperone STI1 role in murine embryonic stem cell maintenance
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Stress-inducible protein 1 (STI1) is known as an adaptor protein that coordinates the activity of heat shock proteins (70kDa and 90kDa) during protein synthesis. The role of STI1 in developmental biology showed that loss of STI1 expression in mice causes embryonic death in embryos at E10.5, which can be rescued by transgenic expression of STI1, demonstrating an essential role during embryonic development. Herein, we used mouse embryonic stem cells (mESCs) depleting STI1 expression as a model to evaluate its relevance for stem cell biology. Preliminary results demonstrated that downregulation of STI1 impairs ESCs proliferation in BrdU incorporation assays, loss-of-pluripotency evidenced by phosphatase alkaline assays and colony formation. Besides, cell differentiation assay assessed by embryoid bodies (EBs) formation from STI1-silenced ESCs and showed that STI1 protein is required for proper EB size and morphology of cell aggregates. However, the mechanisms that lead to this phenomenon in mESCs and its interaction with nuclear proteins are unknown. In addition, immunofluorescence assays of STI1 downregulated populations showed a weak labeling of STI1 predominantly in the nucleus, in contrast with a cytoplasmic pattern in control and parental populations. STI1 nuclear expression has been characterized in somatic cells in response to cellular stress or phosphorylation. Hence, these findings suggest an interesting role of STI1 in stemness maintenance and proliferation of mESCs. This study will contribute to establish the main functions of STI1 in early mammalian development.

Program Abstract #396
Tgif1 maintains the identity of mouse embryonic stem (ES) cells by counterbalancing the activity of ES cell core factors
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Core pluripotency factors, such as Oct4, Sox2, and Nanog, play important roles in maintaining ES cell identity by autoregulatory feed-forward loops. Nevertheless, the mechanism that provides precise control of the levels of the ES cell core factors without indefinite amplification has remained elusive. Here, we report the direct repression of core pluripotency factors by Tgif1 (TG interacting factor 1), a previously known terminal repressor of TGFβ/Activin/Nodal signaling. We show that overexpression of Tgif1 reduces the levels of ES cell core factors, while its depletion leads to the induction of the pluripotency factors. We confirm the existence of physical associations between Tgif1 and Oct4, Nanog, and HDAC1/2, and further show the level of Tgif1 is not significantly altered by treatment with an activator/inhibitor of the TGFβ/Activin/Nodal signaling. Taken together, our findings establish Tgif1 as an integral member of the core regulatory circuitry of mouse ES cells that counterbalances the levels of the core pluripotency factors in a TGFβ/Activin/Nodal-independent manner.

Program Abstract #397
NANOG binds to GLI proteins and represses Hedgehog-mediated transcription
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The Hedgehog (HH) signaling pathway is essential for the maintenance and response of adult neural stem cells. To study the transcriptional response of stem cells to HH signaling, we searched for proteins binding to GLI proteins, the transcriptional effectors of the HH pathway in mouse ES cells. We find that both GLI3 and GLI1 bind to the pluripotency factor NANOG. The ectopic expression of NANOG inhibits GLI1-mediated transcriptional responses in a dose-dependent
fashion. Similarly, in differentiating ES cells the presence of NANOG reduces the transcriptional response cells to HH stimulation. Finally, we find that Gli1 and Nanog are co-expressed in ES cells at high levels. We propose that NANOG acts as a negative feedback component that provides stem cell-specific regulation of the HH pathway.

Program Abstract #398
A novel stem-cell population play a role in visceral adipose tissue development
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The expansion of white adipose tissue (WAT) occurs through an increase in adipocytes size (hypertrophy) as well as by de novo recruitment and differentiation of adipose progenitors (hyperplasia). WAT has different anatomical location and can be found under the skin (subcutaneous) or in the viscera (visceral). These depots have been shown to have distinct developmental origins and are functionally different. Furthermore, the growth of subcutaneous fat is not associated with adverse metabolic abnormalities whereas the increase in visceral WAT is. We have recently uncovered the existence of novel populations of adipose progenitors (APs) unique to visceral (VIS) WAT of mice that exhibit distinct adipogenic and secretory capacity. We named these cells VIS low and VIS high adipose progenitors (APs) based on their low or high expression of CD34. These APs are not from hematopoietic origin as they don’t express lineage markers such as CD31, Ther119 orCD45 and failed to show any GFP labeling after bone marrow transplantation using UBC-GFP transgenic mice. However, these cells are functionally distinct based on their transcriptional profiling. Interestingly, when co-cultured with VIS low, VIS high cells seem to prevent their adipogenic differentiation. In addition, conditioned media from VIS high is able to inhibit adipogenic differentiation of both subcutaneous and VIS low APs, suggesting that they secrete factors that inhibit adipogenic differentiation. Most importantly, VIS high but not VIS low APs proliferate faster and their proportion correlate positively with hypertrophic growth of VIS WAT and the degree of insulin resistance in mice. Finally, VIS low proportions are constant during post-natal growth of VIS WAT whereas VIS high APs are only seen later in development. Taken together, these results demonstrated for the first time the existence of two APs in VIS WAT that are functionally distinct APs and could play a different role in the development of this depot.

Program Abstract #399
Gata3 regulates aPKC localization, spindle orientation and lineage specification during prostate development
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The mouse prostate is an ideal model system for studying the intrinsic and extrinsic mechanisms of asymmetric and symmetric stem cell divisions, and the origin of epithelial cell type diversity and stratification. Intrinsic mechanisms involve restricting the intracellular localization of cell type specific determinants and then orienting the plane of cell division such that these determinants are inherited by specific daughter cells, affecting their fate (stem or differentiated cells). Basal prostate stem cells are responsible for the generation of all of the cells lineages in the prostate (basal cells and/or differentiated luminal cells) through oriented cell divisions during both development of the organ, and during tissue maintenance in the adult (Wang, et al, 2014). We have recently identified Gata3 as a major regulator of prostate stem cell homeostasis in mice and of prostate cancer in humans. Loss of Gata3 during prostate development causes a disruption in apical localization of aPKC within epithelial cells, but intriguingly not of the other Par complex members, Pard6 and Pard3. Gata3 loss also causes randomization of the mitotic spindle during anaphase and leads to the generation of increased numbers of “double-positive” progenitor like cells (positive for both basal and luminal cytokeratins). This increase in progenitor cells is accompanied by gross morphological defects including ductal hyperplasia and reduced branching morphogenesis. Using 2D and 3D culturing methods for both primary mouse prostate stem cells and human prostate basal epithelial cells, we hope to further understand the role of mislocalization of aPKC in orienting the mitotic spindle in prostate stem cells, and the generation of asymmetric and symmetric divisions choices within prostate epithelial stem cells. This work is supported by a CIHR grant to M.B. and a Prostate Cancer Canada Graduate Studentship to M.S.

Program Abstract #400
Olfactory stem cells: understanding tissue homeostasis and regeneration at the single cell level
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New neurons and support cells are generated by the proliferation and differentiation of adult neural stem cells, and this
process is critical for supporting tissue homeostasis, plasticity, and regeneration. The olfactory epithelium (OE) provides an excellent model for studying neural stem cell dynamics and regulation because it undergoes adult neurogenesis and injury-induced tissue regeneration, and it is an experimentally accessible structure. To understand tissue homeostasis and regeneration, it is essential to understand the cellular dynamics and cell lineage hierarchy as well as the genetic interactions that control them. Ultimately, this requires understanding the tissue at the single cell level. We are examining the identity, dynamics, and lineage relationships of the OE neural stem cells at both the population and single cell level with transgenic lineage tracing tools, and we are coupling this transgenic fate-mapping with single cell RNA-Seq of the OE stem cells as they regenerate the tissue. Using single cell RNA-Seq together with transgenic lineage tracing has allowed us to define the heterogeneity within the different stem cell populations in the OE, the lineage relationships, and the active transcriptional networks in the OE stem cells during tissue homeostasis and injury-induced regeneration. By applying these approaches with single cell level resolution in conjunction with conditional knockouts of key regulatory factors, we are defining the genetic interactions that control the transition from quiescence to activation, stem cell self-renewal, and cell fate choice.

**Program Abstract #401**  
**Hippo-signaling in Heart Regeneration**  
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The mammalian heart regenerates poorly, and damage commonly leads to heart failure. The Hippo pathway is an evolutionarily conserved kinase cascade that regulates heart size during development and prevents adult mammalian cardiomyocyte regeneration by inhibiting the transcription cofactor Yap. To identify Yap target genes that are activated during cardiomyocyte renewal and regeneration, we performed Yap chromatin immunoprecipitation sequencing (ChIP-Seq) and mRNA expression profiling in Hippo-deficient hearts. Our findings indicated that Yap regulates genes important for cell cycle progression, genes encoding proteins that promote F-actin polymerization and link the actin cytoskeleton to extracellular matrix. Border-zone cardiomyocytes of injured Hippo-deficient mouse hearts showed cellular protrusions, indicative of cytoskeletal remodeling. Among Yap target genes were components of the dystrophin glycoprotein complex, a large molecular complex that, when defective, results in human muscular dystrophy. Mdx mutant mice lack functional dystrophin and are a model for muscular dystrophy. We found that Mdx mutant hearts had impaired cytoskeleton remodeling and heart regeneration after neonatal apex resection. Together our findings indicate that in addition to genes encoding cell cycle progression proteins, Yap regulated genes that enhance cytoskeletal remodeling and the cellular response to local mechanical microenvironment.

**Program Abstract #402**  
Prolonged mitosis of neural progenitors alters cell fate in the developing brain  
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Embryonic neocortical development depends upon balanced production of progenitors and neurons. Progenitor mitosis dynamics are posited to influence neural cell fate and brain size, however the key mechanisms remain poorly understood. Here we demonstrate that prolonged neural progenitor mitosis directly alters neuronal fate specification, by regulating the balance of neurogenic and proliferative divisions and the generation of viable progeny. First we examine progenitor mitosis in a Magoh+/− microcephaly model, using live imaging of embryonic brain slices and progenitors. We discover Magoh mutant progenitors are significantly delayed in prometaphase, and these progenitors preferentially undergo neurogenic divisions at the expense of producing progenitors. Second we use pharmacological approaches coupled with live imaging of brain slices and clonal cultures to prolong progenitor mitosis and then evaluate cell fate. We find progenitors with 2-fold longer mitosis directly produce more neurons and independently generate apoptotic progeny. Apoptotic progeny are generated in a p53-dependent manner, whereas neuronal differentiation is p53-independent. These pharmacological experiments independently recapitulate the Magoh+/- phenotypes and together support a role for mitosis duration in neural fate specification. Our findings define a new paradigm to understand how mitosis perturbations underlie brain size disorders such as microcephaly, and further suggest mitosis duration may be an important mechanism for influencing stem cell fate during development.

**Program Abstract #403**  
Culture conditions for the expansion of human midbrain dopaminergic progenitors  
Carlos Paz, Andres Bratt-Leal, Jeanne Loring
Midbrain dopaminergic (mDA) neurons have important potential as a replacement therapy in Parkinson’s disease, a neural degenerative disorder characterized by the selective loss of dopaminergic neurons located in the substantia nigra. Effective strategies for the derivation of mDA neurons mimic human development in vivo. In 2011, Kriks et al demonstrated that addition of CHIR to floor plate precursors could strongly induce roof plate signaling and resulted in true mDA cell type. In addition, Chung et al (2011) isolated proliferative mDA progenitors from mouse embryonic stem cells. Based on these studies, we hypothesized that there is a true human mDA progenitor which can be maintained in a stable, proliferative state under the right conditions. Successful culture of these progenitors would help to reduce timeframes and increase efficiency of subsequent experiments utilizing mDA neurons by separating the expansion and cryopreservation process from the experimental workflow and effectively converting it into a preparatory protocol. Optimal culture conditions would promote the expansion of a stable human mDA progenitor cell type with the following characteristics: multiple rounds of self-renewal, stable cryopreservation, and mDA neuronal cell fate. Expansion variables included day of isolation, isolation method, plating density, use of Rho Kinase inhibitor, and variation of morphogens. Temporal protein and gene expression confirmed the presence of proliferative mDA progenitors. Ki67 immunostaining confirmed visual measurement of increasing population levels. Data suggests that FGF2 affects mDA progenitor proliferation, given a minimum cell density threshold. RHOk inhibitor is crucial to cell survival after isolation, and optimal isolation timeframe occurs at day 11, as confirmed by peak co-expression of progenitor markers including LMX1A, FOXA2, and OTX2.

**Program Abstract #404**
The transcriptional state of the early ventricular-like subphenotype in human amniocyte-induced pluripotent cardiomyocytes
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Human amniocytes are a developmentally early source of cells that are clinically accessible for therapeutic intervention of congenital heart defects. Using an episomally induced pluripotent stem cell approach, followed by a timed regiment of chemically defined media (modified-CDM3), we differentiated amniocyte-derived iPS cells (A-iPS) into beating, but immature, ventricular cardiomyocytes, usually within eight days of induction. However, it is unclear what cardiogenic gene program or gene regulatory interactions regulate this early, stable ventricular cardiomyocyte subphenotype that fails to progress to normal maturation. To identify regulators of lineage subtype determination, we performed an RNA-seq time series and subsequent differential expression analysis on these immature ventricular cardiomyocytes at 0, 7, 14, 21, 35, and 49 days post-differentiation. Interestingly, we found a significant amount of inter- and intra-patient variability and heterogeneity across separate cardiomyocyte differentiation experiments. Because cardiac progenitor cells produce multiple cardiovascular cell-types, e.g. fibroblasts, smooth muscle cells, epithelial cells, and cardiomyocytes, we also performed self-organized map analysis of these temporal RNA-seq datasets and identified a number of specific transcription factors and co-regulators that can be assigned to known cell-types. To further examine the transcriptional state of A-iPS derived cardiomyocytes, we are comparing this dataset to developmental time series of cardiac differentiation in zebrafish, mouse ES lines, and fibroblast-derived human iPS lines. We are also comparing these cells to single cell analysis of heart tissue and to other atrial and ventricular lineage datasets. Supported in part by Primary Children’s Medical Foundation, T32HL007576 Cardiovascular Training Grant, NHLBI Bench-to-Bassinet Consortium grant to HJY (U01HL0981) and a core facilities support grant to New England Research Institute (U01 HL098188).

**Program Abstract #405**
Histone variant H3.3 regulates an epigenetic switch in stem and germ cells
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The epigenetic mechanisms that confer cellular plasticity upon stem and germ cells, empowering the choice to self-renew or differentiate remain largely open questions. A novel hypothesis is that histone variants such as H3.3 regulate cellular plasticity through specific epigenetic and chromatin-based mechanisms. H3.3 has particularly flexible epigenetic functions based on its replication-independent nucleosomal deposition. One of the two H3.3 coding genes, H3F3A, is frequently mutated in high-grade pediatric gliomas, raising the possibility and an oncodevelopmental biology model of aberrant plasticity in cancer driven by mutated H3.3. In testing these hypotheses we have found surprisingly that H3.3 is
induced both during cellular reprogramming to produce induced pluripotent stem cells and during embryonic stem cell (ESC) differentiation. H3.3 plays a partially conserved role as an epigenetic switch in stem and germ cells. H3.3 promotes plasticity in naïve stem and germ cells, but can switch functions in response to specific signals to help orchestrate appropriate differentiation. For example, in ESCs H3.3 loss-of-function leads to aberrant differentiation-associated gene expression both in naïve and differentiated context. Knockout of H3.3-coding genes also impairs normal germ cell functions including differentiation and the reprogramming switch from histone- to protamine-based chromatin in the male germ line. A central mechanism of H3.3 plasticity function is delimiting domains of heterochromatin and in particular the key heterochromatic mark, H3K9me3. H3.3 is also required in ESC for appropriate bivalent marks. Loss of H3.3 in addition leads to changes in global H3K4me3 by ChIP-Seq, impaired cell survival, and loss of genome integrity. These findings provide new insights into the role of histone variants and epigenetic switches in stem and germ cells with important implications for normal development, regenerative medicine, and innovative cancer therapeutics.

Program Abstract #406
Crestospheres: Long-term maintenance of multipotent, premigratory neural crest stem cells
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Premigratory neural crest cells are a transient, embryonic population that arise within the central nervous system, but subsequently migrate away and differentiate into many derivatives. Previously, premigratory primary neural crest could not be maintained in a multipotent, adhesive state without spontaneous differentiation. Here, we report conditions that enable maintenance of neuroepithelial “crestospheres” that retain multipotentiality and express early neural crest markers, (FoxD3, Sox10, Sox9, Slug, Ap2, Pax7 and c-Myc) for weeks. Crestospheres show high expression of neural crest markers and low expression of neural markers. Instead, when cells are derived from 3-6 somite stage cranial neural tubes in an equal manner to the crestosphere protocol, but cultured in traditional neurosphere conditions, neural crest characteristics drop dramatically and the expression of the neural stem cell marker Sox2 is heavily increased, thus suggesting a strong support of the neural crest cell fate from the crestosphere culture medium. Moreover, these cells can migrate and then differentiate into multiple derivatives in vitro as well as after in vivo transplantation into host chick embryos, and a clonal assay was performed to verify the presence of multipotent cells. Similarly, human embryonic stem cells directed into neural crest can be maintained as crestospheres and subsequently differentiated into several derivatives. By devising conditions that essentially “stop developmental time” in vitro, these results are the first to demonstrate that premigratory crest are capable of long-term self-renewal. This approach will help uncover mechanisms of their developmental potential, mechanisms of self-renewal capacity, differentiation, and, together with the iPS techniques, the pathology of human neurocrisopathies. This work was funded by fellowships to LK from Sigrid Juselius Foundation, Ella and Georg Ehrnrooth Foundation and The Finnish Cultural Foundation to LK.

Program Abstract #407
The coordination of cell behavior between dental lamina and replacement tooth enables physiological tooth development and regeneration
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Background: Teeth generate from crosstalk between mesenchyme and epithelial stripe (dental lamina). The ability of teeth renewal differs between species: Polyphyodonts (e.g., alligators) change their teeth lifelong, many mammals (e.g., pigs and humans) are diphyodont, and mice are monophyodont (their incisors grow continuously instead of replacing). Comparison between polyphyodonts and diphodonts provide ideal models in the modulation of dental lamina homeostasis. Niches housing ectodermal organs (e.g., hair follicles, feathers and teeth) share many common signaling pathways. Our lab had proved the stem cell niche in the alligator teeth families, so as the similar location of β-catenin expression to transient amplifying cells. Nevertheless, no studies have examined the molecular circuit regulating stem cell activation/maintenance and disappearance in diphyodonts. Materials and methods: Alligators and pigs at different developmental stages were collected for RNA-seq analysis and staining. IdU and CldU were injected for tracing cell migration. Results: In alligators, the epithelium at HERS shows a tendency of moving away from the root tip at replacement tooth 1 in initiation stage. In growth stage of replacement tooth 2, pulp cells are highly proliferating. Similar results are shown as in the odontoblasts and epithelium that the middle and cells at root apex part cells are highly proliferating without significantly migrating. In pigs, although both successional dental lamina at E50 and the dental lamina-like structure (which won’t form a tooth) at PN6 are similar in morphology and SOX2⁺ expression, PCNA⁺
significantly differs in their distribution. In alligators, the dental lamina strip shows up-regulation in Wnt families and down-regulation in BMPs when comparing to the oral epithelium according to our RNA-seq analysis. **Conclusion:** The variation within these transcription factors may cause the differences in the regulatory ability of teeth regeneration.

**Program Abstract #408**

**Loss of sir2 in Drosophila leads to age-associated insulin resistance**

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Complex metabolic disorders such as obesity and diabetes are on the rise worldwide, highlighting the importance of characterizing the factors involved in their development and progression. In large part, the complexity of these disorders arises from interactions between genetic and environmentally induced pathways that determine individual metabolic health. The highly conserved deacetylase Sir2/Sirt1 plays an important role in integrating these pathways due to its widespread effects on protein acetylation state in response to levels of NAD⁺, an important electron carrier in central metabolic pathways. Consistent with this, loss of Sir1 is associated with metabolic dysfunction and diabetes in mice and humans, although its molecular roles in these pathways remain unclear. We have shown that *Drosophila* sir2 mutants develop hyperglycemia with increasing age under both fed and fasted conditions. Subsequently these mutants become insulin resistant and glucose intolerant, all hallmarks of diabetes. Tissue-specific RNAi and rescue experiments demonstrate that Sir2 functions in the fat body, suggesting a role for this factor in regulating insulin sensitivity as opposed to secretion. Evidence from metabolomics analysis supports this hypothesis and suggests that there is altered flux through glucose metabolic pathways indicative of mitochondrial dysfunction. RNA-seq transcriptional profiling reveals significant overlaps between Sir2-regulated genes and those regulated by the HNF4 and FOXO metabolic transcription factors. Further study has demonstrated that Sir2 is important in altering the activity and stability of these regulators, potentially through direct deacetylation. Taken together, we have shown that loss of *sir2* is a genetic model for age-progressive development of insulin resistance and subsequent diabetes in *Drosophila*. Our current focus is to further characterize the molecular mechanisms of Sir2 action and identify its direct targets.

**Program Abstract #409**

**Maternal obesity compromises the blood-brain barrier in the developing fetus**

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Maternal obesity during pregnancy is associated with various metabolic and neurobehavioural diseases in the offspring. Our research investigates how altered maternal environment could differentially program the offspring brain. During mid-gestation onwards, the blood-brain barrier (BBB) is formed, with formation of tight junctions and down-regulation of fenestrations in the endothelial cells, and interaction between glial cells and brain vasculature. Our recent studies on the BBB have revealed that in the arcuate nucleus of the hypothalamus (ARC), the fenestrations in the endothelial cells persist longer than nearby hypothalamic regions. Furthermore, immunochemical analysis and RT-qPCR showed a significantly higher levels of expression of fenestrated endothelial cell markers (MECA-32 and dysferlin) in the postnatal day 0 (PN0) ARC of offspring from the maternal high-fat diet (mHFD) group compared to the normal weight control group. The increase in expression of fenestration markers in mHFD group appeared to result from an increase in the proportion of blood vessels containing fenestrated endothelial cells, rather than a generalised increase in the number of blood vessels. We then used intraperitoneal injection of Evans blue dye and quantified its diffusion into the ARC as a measure of BBB permeability in the offspring of control and mHFD groups. Consistence with our findings of increased fenestraed endothelial cells, Evans blue diffusion was significantly higher in mHFD group than the control.

Our data indicate that maternal obesity can compromise the formation of the BBB in the developing fetal brain, leading to an increase in fenestrated endothelial cells and reduced BBB function. Future work will define the physiological consequences of this altered BBB permeability.

**Program Abstract #410**

**Developing an Avian Model of Maternal PKU**

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Maternal phenylketonuria (MPKU) is a syndrome of multiple congenital anomalies including cardiovascular malformations (CVMs), microcephaly, intellectual impairment, and small size for gestational age caused when a mother with Phenylketonuria (PKU) does not control her dietary intake of Phenylalanine (Phe). High maternal serum Phe
becomes teratogenic to the fetus. In this study, we aim to establish and characterize an avian model of MPKU. We sought to determine the dose of Phe required to induce CVMs. We also examined two methods of delivery to determine which would minimize secondary developmental defects. **METHODS:** We investigated the effect of 2 experimental delivery routes and 6 dosages of Phenylalanine [200uM, 400uM, 800uM, 900uM, 1500uM, and 2500uM] upon embryo survival, growth and early avian development. The delivery routes included modified egg windowing followed by application of a vehicle or Phe to the embryo, and *in-ovo* yolk injection of a vehicle or Phe. **RESULTS:** The modified egg windowing caused significantly higher mortality and morbidity than the *in-ovo* yolk injection. Phe exposure doses 900mM, 1500mM, and 2500mM caused 100% lethality. We increased survival significantly in embryos exposed to 200mM, 400mM, and 800mM Phe. Embryos displayed gross morphological changes including developmental and growth delays, anterior and posterior abnormalities, and torsion defects. **CONCLUSIONS:** We originally started with concentrations of Phe comparable to those in the murine model, however these dosages were lethal to the avian embryo. We lowered the dosage to permit survival and we observed some gross abnormalities such as growth restriction. Histological analysis is underway to determine changes in heart development. Currently there is no data interrogating the mechanism by which Phe causes heart defects. We plan to utilize this model to define the mechanism of Phe cardiac teratogenicity which is critical for improving MPKU treatments and outcomes.

**Program Abstract #411**  
**Acetaldehyde reduces the metabolism of vitamin A to retinoic acid by inhibiting RALDH2 during embryogenesis.**  
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Exposure of human embryos to ethanol during pregnancy results in facial dysmorphology, microcephaly, short stature, central nervous system and neurodevelopmental abnormalities. Ethanol teratogenesis in humans results in Fetal Alcohol Spectrum Disorder (FASD) affecting many developmental processes regulated by the retinoic acid (RA) signaling pathway. The main hypothesis connecting ethanol and RA signaling proposed that while RA is being synthesised by oxidation of retinol to retinaldehyde (RAL) and then to RA, ethanol inhibits the first enzymatic step performed by alcohol dehydrogenases (ADH). ADH was proposed as the ethanol target based on thermodynamic considerations. In the developing embryo RA signaling starts with the localized expression of the major retinal dehydrogenase gene Raldh2. Detoxification of ethanol produces acetaldehyde, which in turn oxidizes to acetate by aldehyde dehydrogenases (ALDH). RALDH2, an ALDH, produces RA from retinaldehyde. We show that acetaldehyde recapitulates the embryonic malformations induced by ethanol in *Xenopus* embryos. Acetaldehyde inhibits RA signaling and competes for overexpressed human RALDH2 (hRALDH2) in *Xenopus* embryos. Finally, we show that acetaldehyde can inhibit the production of RA by recombinant hRALDH2 in vitro. Trying to elucidate the etiology of FASD we demonstrate that acetaldehyde is a bona fide and preferred RALDH2 substrate that competes and down regulates retinoic acid production and signaling. These findings raise the possibility that the natural genetic variation of the human Raldh genes in the population may account for the genetic predisposition to FASD.

**Program Abstract #412**  
**Hyperforin Inhibits the Anxiety and Depression Induced by Early Separation in Rats**  
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Studies report that rats suffered from maternal separation result in hormonal and behavioral changes, such as the impairment of reversal learning and spatial learning, anxiety and play-fighting. However, few studies on the treatment and its mechanisms were found. In this study, we aimed to investigate the effect of hyperforin (Hyp) on adult rats suffered from early separation (ESP). Infant Wistar rats were randomly divided into three groups: control group (CON), ESP group (ESP), and ESP treated with 3mg/kg/day Hyp group (ESP+Hyp). Postnatal rats of ESP group and ESP+Hyp group were separated from their mothers for 6 hours every day from day 7 to day 21, while rats of CON group were stayed with their mothers. Hyperforin was intragastric administrated from day 14 to day 21 in ESP+Hyp group. Rats were subjected to the open field test (OFT), novelty suppressed feeding test (NSF) and forced swimming test (FST) until they grew up. Our data showed that time spent in central grids was much shorter in ESP group compared with that of CON group in OFT. When treated with hyperforin, rats spent much more time in central area than that of ESP group. Results of NSF test showed that the feeding latency was much longer in ESP group. After being administrated with hyperforin, the feeding latency was
shorter than that of ESP group. In FST test, the score was markedly higher in ESP group than that of CON group. Interestingly, the score was obviously lower in ESP+Hyp group than that of ESP group. In conclusion, these results suggest that hyperforin is able to inhibit the anxiety and depression of ESP rats, and which is worth to be further investigated in future. Key words: Early separation from parents; anxiety; depression; hyperforin. Acknowledgments: This work was supported by grant from Tianjin Research Program of Application Foundation and Advanced Technology (14JCZDJC35000).

Program Abstract #413
Molecular interplay between BMP4 and Fibrillin in embryonic development and Marfan syndrome
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BMP4 is synthesized as an inactive precursor protein that is sequentially cleaved at two sites: an initial cleavage frees the mature ligand, which remains non-covalently bound to the prodomain until it is released by cleavage at a second upstream site. The transient prodomain/ligand complex plays a critical role in stabilizing the mature ligand since mice carrying a point mutation (Bmp4S2K) that bypasses complex formation die early in development due to reduced levels of mature BMP4. Fibrillin1 (FBN1) is an extracellular matrix (ECM) glycoprotein enriched in elastic fibers that binds to the prodomain of BMP4. We hypothesized that interactions between FBN1 and the transient BMP4 prodomain/mature ligand complex are required to deposit mature BMP4 into the ECM to promote stability. Consistent with this model, compound heterozygotes carrying a hypomorphic allele of Fbn1 and a null allele of BMP4, but not the Bmp4S2K allele, displayed early perinatal mortality and phenotypes associated with reduced BMP4 activity. These mice displayed enlarged airspaces in the lungs and fragmentation of aortic elastic microfibrils; characteristics of Marfan syndrome, a disease resulting from mutations in Fbn1. Defects in compound heterozygotes are more severe than those in Fbn1 null homozygotes, suggesting that BMP4 may be required for expression and/or function of both Fbn1 and Fbn2 during development. Levels of pSmad1, BMP4 and FBN1 protein were decreased in compound mutants relative to single heterozygotes. Collectively, these data support a model in which BMP4 and FBNs function in a positive feedback loop to regulate each other’s expression or function. We propose that FBNs bind to the prodomain of BMP4 to generate a stable, ECM anchored ligand that signals to positively regulate expression and/or assembly of FBNs into microfibrils. Funding provided by NIH (RO1HD042598 and RO1HD37976) the Shriners Hospital for Crippled Children Research Foundation, and the AHA (15GRNT2180007).

Program Abstract #414
A developmental non-endothelial cell role for Notch1 in ascending aortic aneurysms
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Ascending aortic aneurysms (AscAA) are a common complication of bicuspid aortic valve (BAV). The etiologic mechanisms of AscAA are not well understood, and treatment options are limited. We previously reported that NOTCH1 mutations are associated with familial BAV. A subset of these individuals displayed aortic dilation, which is often associated with BAV aortopathy, implicating a potential role for Notch1 in AscAA. To investigate the role of Notch1 in the AscAA, Notch1 haploinsufficiency was introduced into the Marfan syndrome mouse (Fbn1C1039G/+), a well-described model of AscAA. Serial echocardiographic, gross and histological characterization of the compound heterozygous Notch1 deficient Marfan syndrome mice (Notch1+/−;Fbn1C1039G/+ similarly) demonstrated an exacerbated AscAA phenotype when compared to Fbn1C1039G/+ mice. The compound mutant mice had increased aortic root diameter as well as a higher rate of dilation from 1-5 months of age (p<0.05). Furthermore, a small subset (4/66) of the compound mutant mice succumb to ascending aortic rupture, which was not observed in the Fbn1C1039G/+ mouse (0/63). Although endothelial Notch1 is critical for cardiovascular development, mice with heterozygous loss of Notch1 in endothelial cells that harbor the Fbn1C1039G mutation (Notch1−/−;Tie2Cre;Fbn1C1039G/) do not recapitulate the phenotype of the Notch1−/−;Fbn1C1039G/+ mice. Surprisingly, heterozygous loss of Notch1 in Myocardin-derived cells and not Myh11(smooth muscle myosin heavy chain)-derived cells in mice harboring the Fbn1C1039G mutation recapitulated the phenotype of the Notch1−/−;Fbn1C1039G/+ mice, suggesting loss of Notch1 alters a developmental process in early Myocardin-expressing cells that predisposes to AscAA. Finally, immunostaining of human AscAA tissue reveals dysregulated active NOTCH1 (NICD1) in comparison to control tissue. Our findings support a novel non-endothelial role for Notch1 and suggest that loss of Notch1 in development may contribute to AscAA.
Program Abstract #415
Molecular Mechanisms Underlying Congenital Heart Defects in a Hectd1 Mutant Mouse Line
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Congenital heart defects (CHDs) are the most common birth defect in the United States. While it is known that CHDs are caused by a combination of genetics and environmental factors, the genetic component are not well understood. A CHD genetic screen in mouse (Yu et al. 2004 Development 131(24):6211-23) identified a mutation in $Hectd1$ causing heart defects including abnormal aortic arches and ventricular septal defects (VSDs). Published and unpublished data from the Zohn lab indicates that Hectd1 is involved in retinoic acid (RA) and Wnt signaling, with both pathways being vital for proper heart development. Based on this previous work, our goal is to characterize congenital heart defects in our $Hectd1$ mutant mouse model and elucidate the molecular pathways that cause these defects. $Hectd1$ is expressed throughout heart development in the developing arteries, atria, and ventricles. Thus Hectd1 can potentially regulate heart development by acting in many cell types and developmental stages. Hectd1 mutants show hypoplastic ventricles with thin myocardial walls, as well as alterations in expression of cardiac progenitor markers that are important for ventricular and artery development. All of these defects are consistent with alterations in RA and Wnt signaling. Future work will determine if changes in these signaling pathways in $Hectd1$ mutants results in CHDs. Towards this goal we will utilize a novel allelic series of $Hectd1$ mutant mouse lines, RA reporter mice and a variety of molecular and genetic techniques. This work was funded by RO1 HD05629 from NICHD.

Program Abstract #416
A novel model of cardiac oxidative stress in Xenopus laevis reveals a unique damage response
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Oxidative stress is a leading contributor to morbidity and mortality in heart disease. The recent advent of optogenetic techniques for producing reactive oxygen species has made it easier to study the effects of oxidative stress in vivo with great temporal and spatial precision. We have taken advantage of one of these new technologies, the fluorescent protein KillerRed, to create a novel model of heart disease in the African clawed frog, Xenopus laevis. X. laevis possesses robust repair abilities in many tissues and organs, including the heart, making it a highly useful model organism for discovering novel repair pathways and strategies. We characterized the cardiac damage response to oxidative stress using a number of common physiological and molecular indicators, including apoptosis, cardiac hypertrophy, and transcription of stress markers. Interestingly, we discovered that X. laevis tadpoles undergoing oxidative stress exhibit a unique biphasic hypertrophy response following heart damage. When we looked at expression of molecular markers of cardiac damage and repair, we found that expression of early heart development genes is also correlated with this biphasic pattern. Currently our work is focused on the causative connection between these genes and the hypertrophic response, as well as the relationship between hypertrophy and the other physiological effects of oxidative stress. Funding: NSF, AHA (K. McLaughlin).

Program Abstract #417
Conditional Knockout of the Histone Chaperone Hira in Cardiomyocytes Causes Cardiomyocyte Degeneration, Fibrosis and Impaired Cardiac Function
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Nucleosome assembly is regulated by a structurally diverse group of proteins called histone chaperones. Hira is an evolutionary conserved histone chaperone implicated in both transcriptional regulation and the formation of heterochromatin. Hira is responsible for replication-independent deposition of the variant histone H3.3 into the nucleosome. Histone H3.3-containing nucleosomes are enriched within active genes and their promoters, pericentric heterochromatin, and telomeres. Deposition of H3.3 into active genes and their promoters is uniquely Hira-dependent and is thought to produce an epigenetic signature for the gene expression program giving identity to a differentiated cell type. Hira is also required for transcription restart after DNA repair since the DNA repair process requires disassembly and reassembly of nucleosomes. In this study, we tested the hypothesis that Hira is necessary for maintaining the cardiomyocyte gene expression program that controls cardiomyocyte identity and function. Surprisingly, Hira conditional
knockout mice lived to adult age with no gross developmental defects. However, investigation of cardiac pathology revealed fibrosis, which manifested mostly on the right ventricle, as early as 21 days of age. These conditional knockouts exhibited focal areas of cardiomyocyte degeneration and impaired cardiac function including decreased cardiac output, stroke volume, stroke work and increased arterial elastance. These data indicate Hira’s importance in maintaining healthy cardiovascular function by preserving the genomic integrity of cardiomyocytes.

**Program Abstract #418**

**Loss of the Muscle-Specific Methyltransferase Smyd1 Impairs Myogenesis and Causes Centronuclear Myopathy**

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The SMYD (SET and MYND domain) family of lysine methyltransferases is defined by a unique structure in which the methyltransferase (SET) domain is intervened by a protein-protein interaction MYND domain. SMYD proteins are implicated in vast biological processes including transcriptional activation/repression, elongation, DNA repair, cell cycle and apoptosis. The Smyd1 gene is unique from other family members in that it is specifically expressed in striated muscles. Smyd1 is multifunctional, harboring both transcriptional activation and repression activities and methylating both histone and non-histone substrates. Smyd1’s various activities are evident by its dynamic subcellular localization, localizing to both nucleus and cytoplasm in myoblasts, but predominantly to the M-line in myocytes. These alterations in subcellular localization led us to hypothesize that Smyd1 performs distinct functions during myogenesis and in mature fibers. Ablation of Smyd1 in myoblasts impaired myofiber development, but did not affect myoblast proliferation or gene expression. In contrast, conditional knockout of Smyd1 in myocytes produced a muscle disease similar to human centronuclear myopathy (CNM). As with CNM, mutant mice exhibited weakness, hypotrophy, prevalence of oxidative fibers, reduced triad numbers, regional myofibrillar disorganization and a high percentage of fibers with centralized nuclei. Notably, we found broad upregulation of muscle development genes in the absence of regenerating or degenerating fibers. Disease severity was greater for males than females, highlighting previously unidentified sex-specific differences in autosomal CNM. Despite equivalent expression in all fiber types, loss of Smyd1 primarily affected fast-twitch muscle. Few genes are associated with CNM and most function in membrane biology. Smyd1 is the first epigenetic transcriptional coregulator linked to CNM and the only CNM-associated protein required for proper myogenesis.

**Program Abstract #419**

**Understanding how Xenopus laevis tadpoles detect and correct craniofacial abnormalities**

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One in six hundred infants around the world are born with malformed craniofacial structures. Current medical treatment options for children with craniofacial abnormalities such as cleft palate and fetal alcohol syndrome are extremely limited. Furthermore, only the mildest craniofacial abnormalities can be improved through multiple invasive and expensive surgeries. Consequently, over the past decade numerous studies have utilized vertebrate model systems, such as *Xenopus laevis*, to investigate the underlying developmental causes of craniofacial abnormalities. A wide range of perturbations have been shown to result in the formation of abnormal facial structures, but only recently did a published study provide evidence that pre-metamorphic *X. laevis* tadpoles are capable of self-correcting altered craniofacial structures. Despite this observation, how craniofacial defects in *X. laevis* tadpoles self-correct over-time remains undetermined. In order to begin examining the self-regulating capabilities of craniofacial patterning mechanisms, we have surveyed the extent to which *X. laevis* larvae self-correct craniofacial deformities resulting from a range of mechanical, chemical, or genetic perturbations applied during embryogenesis. Our results demonstrate that *X. laevis* tadpoles are capable of correcting craniofacial defects resulting from some, but not all types of perturbations. Our current work focuses on determining the underlying mechanisms used to first detect, and subsequently correct, malformed or improperly located craniofacial structures. Knowledge of this self-correction process in *X. laevis* with craniofacial defects may provide information applicable to the advancement of new treatments for humans with craniofacial abnormalities.
**Program Abstract #420**

**A ciliopathy protein complex directs assembly and trafficking of the IFT machinery**

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Ciliopathies are human genetic disorders caused by mutations in genes regulating cilia formation or function. These diseases involve a wide range of clinical features, such as span skeletal anomalies, craniofacial defects, cystic kidneys, blindness, obesity, and more. Previously, CPLANE (ciliogenesis and planar polarity effectors) proteins were shown to govern ciliogenesis in vertebrates. However, the molecular functions for CPLANE are unknown. Here, a proteomic approach in mammalian cell culture reveals that CPLANE proteins comprise a protein complex. We find that in the absence of WDPCP, a CPLANE protein, peripheral and core IFT-B proteins are recruited to the basal bodies, but fail to traffic along axonemes and dramatically accumulate in the axonemes of *Xenopus* multiciliated cells (MCCs). Additionally, while peripheral IFT-A proteins are reduced at the basal bodies and are absent in axonemes, bi-directional traffic of the IFT-A core proteins is unaffected in WDPCP knockdown embryos. These results suggest that the IFT-A core remains intact in WDPCP knockdown embryos, and can associated with both anterograde and retrograde motors. Similar results were observed following knockdown of other CPLANE proteins such as jbts17, a protein previously associated with human Oral-Facial-Digital Syndrome Type 6 (OFD6). Exome sequencing of human OFD patients identified mutations in *jbts17* as well as other CPLANE members, including *WDPCP*. These mutated *WDPCP* alleles cause severe defects in protein stability when tested in *Xenopus* embryos. Taken together, our results suggest that the CPLANE complex plays a broad and essential role in ciliogenesis and human ciliopathies.

**Program Abstract #421**

**Understanding the Intersection between Cilia and Folic Acid in Neural Tube Closure**

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Neural tube defects (NTDs) have a complex etiology resulting from genetic and environmental causes. The mouse represents an ideal model for mammalian NT closure, and >200 mouse NTD models have been identified, many of which disrupt ciliogenesis. Aberrant cilia function is associated with numerous human inherited disorders, called ciliopathies, which can affect most organs and includes NTDs, fetal lethality, and kidney, limb, and respiratory defects. Cilia are also required for Sonic hedgehog signaling which directs NT patterning, neural fate determination, and neural stem cell proliferation. Folic acid (FA) can decrease the NTD risk, however the mechanism by which FA affects NT closure is unknown, and surprisingly only a few of the >200 mouse NTD models have been tested for FA responsiveness. Contrary to expectations, our preliminary mouse data shows that 4 out of 6 cilia mutants respond detrimentally to long-term FA fortification indicating that dose and duration of FA exposure affect the phenotypic outcome. These data suggest that cilia mutations may be a class of mutations that benefit from moderate intake of FA. Using RNA-Seq, we find significant gene dysregulation upon FA fortification, including genes important in ciliogenesis. Furthermore, we have developed an in vitro system that recapitulates the in vivo response wherein ciliogenesis appears to be adversely affected in cilia mutant cells upon exposure to high FA in the media. Using this system we are currently investigating the mechanisms underlying the FA effect on ciliogenesis. Moreover, our studies suggest FA affects epigenetic mechanisms. FA is critical for methylation reactions and we are now evaluating the impact of long-term FA on differential methylation in vivo of key targets during NT closure.

**Program Abstract #422**

**Microtubule-Associated Protein Tau Promotes the Formation of Neuronal Microtubules Containing Class II Beta-Tubulin and Axon Elongation in Xenopus Embryos**

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Much more is known about tau's roles in neurodegeneration than about its normal roles in vivo. To study its functions in developing neurons, we characterized the most prominent alternative splice forms of tau expressed in *Xenopus* embryos at the earliest stages of axon outgrowth and manipulated their expression. Tau protein initially emerged as axons underwent
the transition from neurite outgrowth to axon elongation. At these stages, *Xenopus* expressed four alternatively spliced tau mRNAs. Two resembled so-called 4R forms of regular tau, typical of CNS neurons, and two were 'big' taus, characteristic of long peripheral axons. Although knockdown of tau by antisense morpholino oligonucleotides yielded no quantifiable defects in neurite outgrowth in culture, it nonetheless compromised peripheral axon development in the intact embryo, indicating that tau is primarily required for normal axon development within the embryo's complex 3-dimensional environment. At the molecular level, tau knockdown severely disrupted neuronal microtubules containing class II β-tubulins but left other neuronal microtubules unperturbed. Interestingly, in humans, expressing high levels of this particular tubulin isoform is a contributing factor in the pathogenesis of tauopathies in select neurons. These findings suggest that the deleterious effects arising from the loss of tau are likely mediated through specific classes of microtubules. Conversely, increasing 'big' tau expression stimulated neurite outgrowth in culture significantly and enhanced tubulin acetylation more so than did boosting expression of regular tau, consistent with 'big' tau's greater abundance in long peripheral axons as compared with the shorter axons of the central nervous system, which primarily express smaller tau isoforms. These observations demonstrate the utility of *Xenopus* embryos as a tool for gaining novel insights into the normal functions of proteins linked to human neuropathologies. Supported by NSF IOS 1257449.

**Program Abstract #423**

**A Serotonin Circuit Acts as an Environmental Sensor to Mediate Midline Axon Crossing Through EphrinB2**

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Modulation of connectivity formation in the developing brain in response to external stimuli is poorly understood. Here, we show that the raphe nucleus and its serotonergic projections regulate pathfinding of commissural axons. We found that the raphe neurons extend projections towards midline crossing axons, and that when serotonergic signaling is blocked by pharmacological inhibition or by raphe neuron ablation commissural pathfinding is disrupted. We demonstrate that the serotonin receptor htr2a is expressed on these commissural axons, and that genetic knock-down of htr2a disrupts crossing. We further show that knock-down of htr2a, or ablation of the raphe neurons, increases ephrinB2a protein levels in commissural axons. An ephrinB2a mutant can rescue midline crossing when serotonergic signaling is blocked. Furthermore, we found that regulation of serotonin expression in the raphe neurons is modulated in response to the developmental environment. Hypoxia causes the raphe to decrease serotonin levels, leading to a reduction in midline crossing. Increasing serotonin in the setting of hypoxia restored midline crossing. Our findings demonstrate an instructive role for serotonin in axon guidance acting through ephrinB2a, and reveal a novel mechanism for developmental interpretation of the environmental milieu in generation of mature neural circuitry.

**Program Abstract #424**

**Modeling Port Wine Stain in the Zebrafish**

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Port Wine Stain (PWS) is a congenital developmental defect that presents as hypertrophic and dilated vasculature in the skin. Current therapies for PWS are limited to symptomatic management including laser ablation of the vasculature and drug and surgical interventions to manage complications associated with disrupted vasculature in the eyes, brain and limbs. Our understanding of PWS is limited due to the lack of an effective animal model. Our project aims to: recreate the PWS vascular hypertrophy phenotype within zebrafish, and elucidate the underlying molecular mechanisms of the disorder. A single missense somatic mutation in a guanine nucleotide binding protein, GNAQ, has been identified in many cases of PWS. This mutation is hypothesized to affect the GTPase activity of the enzyme and potentiate its activity. Injection of *in vitro* transcribed RNA of mutant *gnaq* indicates that early expression of mutant *gnaq* results in a rupture of the blastoderm similar to other mutants defective in enveloping layer differentiation. We have used this early phenotype to determine potential downstream effectors of gnaq. Potentiation of the blastoderm lysis phenotype with coinjection of *yap1* and *gnaq* indicate that activation of YAP may mediate this phenotype. To reproducibly model somatic mutation we have created a Cre/lox inducible line of transgenic zebrafish expressing wild-type gnaq, the PWS R183Q mutation, or a Q209L activating mutation found in uveal melanomas. By determining if activation of GNAQ is sufficient to induce a hypervascular phenotype, identifying the cells that require GNAQ mutation and determining what pathways are activated downstream of GNAQ activation we can inform potential therapies for PWS. Supported by Choose Development: NSF IOS-1239422
Program Abstract #425
The NICHD Zebrafish Core: Leveraging Vertebrate Homology to Explore Pediatric Disease
Benjamin Feldman1, Annabel Berthon1, Ljubica Caldovic2, Celine Cluzeau1, Fabio Fauz1, Diego Martinelli1, Stephen Kaler1, Forbes Porter1, Constantine Stratakis1, Mendel Tuchman2
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The NICHD Zebrafish Core, established in 2012, is utilized by area researchers who lack the access or expertise to work independently with the zebrafish model independently. Benjamin Feldman (Core Director) offers consultation and co-mentorship for such projects from conception through experimental design and execution. Five core-supported projects are presented here. 1) Celine Cluzeau from the laboratory of Forbes Porter (NICHD) has created zebrafish with mutations in dhcr7 - a gene whose human counterpart causes Smith Leimli Orpitz syndrome when mutated. Zebrafish dhcr7 mutants show predicted alterations in sterol levels and are viable through breeding. Although mutants are viable and display grossly normal morphology, subtle aberrations in growth, fertility, behavior and viability have been documented. 2) Ljubica Caldovic, a principal investigator at Children’s National Medical Center has developed a strategy to use zebrafish embryos for identifying small molecules able to diminish the effects of hyperammonemia. Fourteen candidate protective agents have thus far been identified from a screen of 727 candidate drugs with known human safety profiles. 3) Annabel Berthon and Fabio Fauz from the laboratory of Constantine Stratakis (NICHD) have been investigating the function of zebrafish Armc5, whose human counterpart has a potent but poorly understood role in suppressing the onset of macronodular adrenal hyperplasia. She is exploring a strategy combining transient loss of function, transient gain of function and RNA sequencing to ascertain Armc5 functions. 4) Diego Martinelli from the laboratory of Stephen Kaler (NICHD) is establishing a model of ATP7A distal motor neuropathy that will leverage the ability to visualize aberrations of fluorescently labeled motor neurons in vivo.

Program Abstract #426
Yeast Cell Recombination for Genetic Fitness and Transgenic Stability
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Transgenesis is a process that involves adding foreign genes to an animal’s DNA. The technology began in the 1980s with the implication to plants. The majority of transgenes is used for research purposes. Transgenic biotechnology allows us to test a given gene either by turning it on for a long period or controlling its gene expression. After, knowing the function of a gene, cross-pollination can be done to identify the presence with the assistance of (MAS) marker assisted selection. The selection of choosing interested genes to be passed on to the next generation. A diverse set of yeast strains was given a gene knockout; the strains were mated to generate recombinants’ to screen for healthy progeny. The lab strain that contained the knockout gene was (Tryptophan 1) which cross mated with eight wild type strains (4 clinical, 2 vineyards, 2 oak). After the eight strains had sporulated, the zygotes were picked through tetrad dissection then verified by PCR. A plate transformation was done onto a “drop out” media plate lacking tryptophan, and growth plates were made from recombinants. The following recombination were found to grow with little tryptophan. T34, T35, T38, T45, T47, T48, T56, T67, T345, T68, T34568, T4678, Tmix. In conclusion, growth of T35 in the transformant plate and T56 in the no tryptophan plate confirms recombination. A mix of two proved to be more effective than multiple ones. Yeast genetics may be used in many various areas of studies including, agriculture and industrial technology. Yeast plays a major role in making quality wines, use of probiotics, and biofuel production. If we can answer the following question, Can we congregate for the deleterious mutation through recombination? Highly resistant medicine and organ transplants can be easily made and delivered to patients.

Program Abstract #427
Collective invasion in cancer
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The tumor microenvironment supports both single-cell and collective cancer cell invasion programs, with impact on response to therapy and prognosis. Using intravital near-infrared/infrared multiphoton microscopy, we have established preclinical sarcoma and melanoma models for spontaneous cancer cell invasion and distant metastasis to lymph nodes and lungs. Using multi-parameter 3D detection with subcellular resolution, we identify the tissue niches enabling collective cancer invasion, their growth and invasion patterns, as well as niche-dependent therapy response resistance. Thereby,
invasion occurs without detectable tissue destruction along preexisting largely barrier-free tissue interfaces (“highways”) along blood vessels, myofibers, nerves, adipocytes and collagen bundles. Besides guided migration, these invasion niches support tumor cell survival in an integrin-dependent manner, and support cancer resistance to radiation therapy. Thus, beyond its function in mass movement, collective invasion supports cancer cell survival and resistance signaling which can be exploited by molecular targeted anti-adhesion therapy.
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