ABSTRACTS

Program Abstract #1
CRISPR-based genome editing strategies for Caenorhabditis elegans
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Over the past 18 months, the CRISPR/Cas9 system has emerged as a powerful genome editing tool with the potential to revolutionize experimental biology. The Cas9 endonuclease is targeted to its substrate by a guide RNA that base pairs with the target, and thus can easily be configured to produce a DNA double strand break at a defined site. Cas9 has been used to produce a variety of genome modifications in C. elegans, ranging from small indels and single-nucleotide substitutions, to insertion of GFP, and even replacement of entire genes (~10 kb) with modified versions. I will discuss practical considerations for the use of Cas9 in C. elegans, including construct designs, screening strategies, and the use of selectable markers. I will also share some examples from our own work that illustrate how Cas9-mediated genome engineering is being used to gain new biological insights. Although our group’s focus has been on developing tools for C. elegans, we anticipate that the broad outlines of our strategy will be applicable to other genetically tractable organisms as well.

Program Abstract #2
Systems developmental biology of cell migration
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Cell migration programs are crucial to normal animal development and are coopted by metastatic cancer cells. We seek a comprehensive understanding of cell migration programs and are thus studying several migratory cell types in C. elegans. One migration is of the single male “linker cell (LC),” which drags the developing vas deferens. We used single cell RNA-seq to profile transcripts made by the LC at two stages in wild-type and at one stage in animals that fail to execute part of the migration program (defective in nuclear hormone receptor nhr-67I). Individual, staged migrating cells were microdissected and their mRNA converted to cDNA, amplified and sequenced. About 1000 genes are expressed specifically in this stage of the migration, and we are using molecular genetics to analyze their function. Several proteins are necessary to keep the linker cell attached to the developing vas deferens, including a conserved membrane protein, TAG-256. We used mass spectrometry to identify proteins that interact with a tagged version of the human ortholog, and then tested the function of their orthologs in C. elegans. We thus define a potential new conserved protein complex necessary for the adhesion during migration. We are analyzing the role of neuronal ion channels enriched in the LC. Another migration is of the uterine-seam cell (ute), an H-shaped cell that undergoes a dramatic outgrowth and connects the uterus to the body wall. Uterine epithelial cells, the anchor cell, and developing uterine muscles are necessary for proper ute outgrowth. The ute is highly sensitive to reduction of gene function, and we have found over 100 genes expressed in the ute, the uterine epithelium, the anchor cell and the sex muscle lineage necessary for ute outgrowth, e.g., the RASEF ortholog rsef-1. Lastly, we have found that two secreted astacin proteases and a variety of secreted protease inhibitors are necessary for proper ute outgrowth.

Program Abstract #3
Mechanisms of mitochondrial inheritance in germ line stem cells
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Mutations in mitochondrial DNA (mtDNA) are a major cause of maternally inherited human disease. Mothers often contain a mixture of mutant and wild-type mtDNA (a condition known as heteroplasmy) and transmit varying amounts of each to their progeny. While the phenomenon of heteroplasmy and how it changes from one generation to the next have been described from studies on mammals, genes specifically involved in germline inheritance of mtDNA have not been identified. We have studied the transmission of mitochondria from mother to progeny in Drosophila by asking how mitochondria are segregated into the primordial germ cells (PGCs), the precursors of the female germline stem cells, which by asymmetric division generate eggs throughout adult life. We find that mitochondria within developing oocytes are asymmetrically partitioned to the posterior pole so that upon fertilization they are specifically taken into PGCs. In addition to this early partitioning, nuclei of the future germ cells, like the nuclei of somatic cells, are surrounded by mitochondria providing a second source of mitochondria to the PGCs that is not specific to the germline. We will discuss the mechanism by which mitochondria reach the germ cells and how partitioning of mitochondria to the oocyte posterior ensures the faithful transmission of mtDNA to the next generation and maintains mtDNA variability and diversity.

Program Abstract #4
Development rooted in regulatory networks
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Lastly, we have found that two secreted astacin proteases and a variety of secreted protease inhibitors are necessary for proper ute outgrowth.
To understand the progression from stem cells to differentiated tissues we are exploiting the simplifying aspects of root development. We have profiled mRNA, small RNAs, alternative splicing and DNA methylation at cell-type specific resolution within the Arabidopsis root. We are developing new experimental, analytical and imaging methods to identify networks functioning within different cell types and developmental stages. We are particularly interested in a subnetwork that regulates a key asymmetric cell division of a stem cell and the regulatory networks that control differentiation of the stem cell’s progeny. We have uncovered a clock-like process responsible for the positioning of lateral roots along the root primary axis. Two sets of genes were identified that oscillate in opposite phases at the root tip and are involved in the production of prebranch sites, locations of future lateral roots. Finally, we are analyzing the dynamics of growth of physical root networks using novel non-invasive imaging methods with the goal of identifying the genes regulating root system architecture.

This work is supported by grants from the NIH, NSF, DARPA and the Gordon and Betty Moore Foundation.

Program Abstract #5

The Pou5f1/Oct4 transcriptional network in control of early zebrafish development
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Zebrafish, with the combined advantages of rapid synchronous development, easy experimental manipulation, and extensive genetic tools, have become an excellent model to functionally dissect transcriptional networks in development. We have analyzed developmental roles of the transcription factor Pou5f1 (Pou2), the only zebrafish paralog of mammalian Oct4, a major determinant of pluripotency in ES cells. During blastula and gastrula stages we identified three major functions: (1) During the maternal-to-zygotic transition, Pou5f1 occupies SOX-POU binding sites before onset of zygotic transcription, and activates the earliest zygotic genes. These data position Pou5f1 and SoxB1 at the center of the zygotic gene activation (ZGA) network, and provide a link between ZGA and pluripotency control. (2) We identify two Pou5f1-dependent mechanisms controlling developmental timing during gastrulation. First, several Pou5f1 targets are transcriptional repressors, mediating repression of differentiation genes in distinct embryonic compartments. Second, the dynamics of SoxB1 group gene expression and Pou5f1-SoxB1-dependent gene activation control developmental timing of Pou5f1 target expression during gastrulation. (3) Pou5f1 also controls initiation of productive motile cell behavior during the earliest gastrulation movement, epiboly. Pou5f1 via control of EGF expression regulates endosomal E-cad trafficking through EGFR activation. Dynamic control of E-cad trafficking is essential to effectively generate new adhesion sites when cells move relative to each other. Thus, the Pou5f1-SoxB1 regulatory acts as a "jump-starter" of development by controlling zygotic gene activation as well as initiation of productive cell motility, and orchestrates timing of sequential gene expression into gastrulation.


Program Abstract #6

Role of Shh and Wnt signaling pathways in regulation of postnatal mouse intervertebral disc development and aging
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Intervertebral discs are strong fibrocartilagenous joints between each vertebral body, that allow movements of the spine, and maintain intervertebral distance. Considerable growth and differentiation of the discs occur postnatally, as the body grows. Discs, particularly in the neck and lower back, are major points of weakness in the spine. Their injury or degeneration causes neurological symptoms, including lower back pain, in some 80% of the population. Despite their enormous cost, current treatments are palliative, and prone to failure. The ideal treatment would be to stimulate disc regeneration, using the signals that control their normal postnatal growth and differentiation. We have used the mouse as a model to identify these signals, and the possibility for their usage in disc regeneration. Previously, we showed that the nucleus pulposus cells in the center of each disc secrete Shh which is both necessary and sufficient for postnatal cell proliferation and the expression of differentiation markers in the disc (Dahia et al 2012). In that work, we reported that a consequence of Shh blockade, both in vivo and in vitro, was an up-regulation of canonical Wnt signaling in the disc. Here we show the existence of a signaling loop between Wnt and Shh signaling. Canonical Wnt signaling activates the expression of Shh targets, and Shh signaling causes inhibition of Wnt signaling. At the end of the growth period, both signaling pathways are down-regulated, coinciding with reduced expression of essential matrix components and other differentiation markers in the disc. By treating "old" discs in organ culture with Wnt activators and Shh, we found that these changes were reversed. Expression of matrix components and other differentiation markers in the disc were activated. These results are exciting, because they suggest that biological treatments for disc degeneration may be feasible, using the same signals that control its normal growth and differentiation.

Support by OREF

Program Abstract #7

Dynamics and shaping of the BMP signaling gradient by the BMP antagonists during DV axial patterning
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A gradient of Bone Morphogenetic Protein (BMP) signaling patterns the Dorsal-Ventral (DV) axis of the vertebrate embryo.
Program Abstract #8
Three-dimensional printing technologies to define the architecture of living tissues
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How structure relates to function --- across length-scales from the single molecule to the whole organism --- is a central theme in biology. Bioengineers, however, wrestle with the converse question: will function follow form? That is, we struggle to approximate the architecture of living tissues experimentally, hoping that the structure we create will lead to the function we desire. A new means to explore the relationship between form and function in living tissue has arrived with three-dimensional (3D) printing, but the technology is not without limitations. Here, we present an overview of 3D printing for cell biology and regenerative medicine, and identify key conceptual targets on the horizon.

Program Abstract #9
Specification of differentiated adult progenitors in the Drosophila trachea
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The molecular and cellular mechanisms that keep cells locked in a state of committed differentiation are of interest to the fields of development, tissue homeostasis, wound healing and regeneration. Of equal interest are the mechanisms that enable differentiated cells to reactivate upon injury and replace lost tissues. While several examples of regeneration via differentiated cells are known in the natural world, it is not yet clear how a population of multipotent differentiated cells is singled out within a functional tissue and is capable of reactivation. The remodeling of whole tissues that occurs during Drosophila metamorphosis provides a powerful model for the study of the specification, quiescence and reactivation of differentiated progenitor cells. A population of Drosophila adult tracheal progenitors arises from differentiated cells of the larval tracheal network that retain the ability to reenter the cell cycle and give rise to the multiple cell types of the adult trachea. These progenitors are unique to the second tracheal metamere as homologous cells from other segments express fizzy-related (fzr), the Drosophila homologue of CDH1 protein of the APC complex, and enter endocycle and do not contribute to adult trachea. Here we examine the mechanisms for their quiescence and elucidate the signaling mechanisms that govern their reactivation. Furthermore, we identify a single factor that is both necessary and sufficient to prevent cells of the larval trachea from behaving as adult progenitors. Finally, we elucidate the genetic mechanism responsible for the specification of progenitor identity. These results provide insight into the specification, quiescence and reactivation of a population of differentiated cells with facultative stem cell features. This work was partially funded by the Generalitat de Catalunya, the Spanish Ministerio de Ciencia e Innovacion and the National Science Foundation.

Program Abstract #10
Developmental robustness in the C. elegans embryo
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The 20-cell intestine of juvenile C. elegans nematodes is derived from a single cell, the embryonic blastomere E. The paradigm for E specification is that a cascade of transcription events, started by the maternal factor SKN-1, causes transient activation of the gut specification factors end-1 and end-3 in the early E lineage. These reach a threshold of expression to activate elt-2, which maintains its expression by autoregulation and drives the commitment to gut differentiation. We have created strains in which end-1 and/or end-3 are mutated for particular cis-regulatory sites. In such strains, embryos make variable numbers of apparently normal-sized gut cells, suggesting that specification has become subject to stochastic variation, and that commitment to a gut fate can occur later in the E lineage. Counting of embryonic elt-2 transcripts by single-molecule FISH suggests that activation of elt-2 is more graded in these strains, as opposed to an all-or-none mode as was previously reported for SKN-1-depleted embryos (Raj et al., 2010). As these
effects are confined to the E lineage due to the nature of the strains constructed, we are also able to evaluate adults derived from embryos in which functional guts were made. We find that such adults store lipids at significantly higher levels and display other variable pleiotropic phenotypes, suggestive of primary defects in gut function. Together, these results build a picture in which specification of gut is not an all-or-none event, and that in animals that do make an intestine, the endoderm differentiation network is not fully self-correcting for partially compromised specification.

Program Abstract #11
Wound-induced neoblast specialization in Schmidtea mediterranea
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Planarians are well known for their ability to regenerate entire animals from small tissue fragments. Planarian regeneration requires a population of dividing cells called neoblasts that are distributed throughout the body. Historically, neoblasts have largely been considered a homogeneous population of stem cells capable of differentiating into all cell types. Most studies, however, involved analysis of neoblasts at the population rather than the single cell level, making it difficult to determine how heterogeneous this population is. Here, we combined an RNA sequencing approach with expression screening, and identified 33 new transcription factors transcribed in specific differentiated cells that were also expressed in small fractions of neoblasts during regeneration. Expression of transcription factors associated with distinct differentiated tissues occurred in different neoblasts, whereas transcription factors expressed in the same differentiated tissues were transcribed in the same neoblasts. These results suggest roles for neoblast-expressed transcription factors in the specification of distinct tissues. Furthermore, we found that the transcription factors klf, Pax3/7, and FoxA were required for the differentiation of cintillo-expressing sensory neurons, dopamine-beta-hydroxylase-expressing neurons, and the pharynx, respectively. Our results, together with previous data for regeneration of eyes, protonephridia, and other neurons suggest that specification of cell fate for most, if not all, regenerative lineages occurs within neoblasts, indicating that neoblasts are a heterogeneous population of pluripotent stem cells and lineage-restricted progenitors.

Program Abstract #12
Getting to the root of things: spatiotemporal gene regulatory networks in plant roots
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Roots are of fundamental importance for both plant and human growth and health. Distinct cell type-specific programs within the root perform a variety of functions including defense, nutrient transport, mechanical support and growth. Root spatiotemporal transcriptome, proteome and metabolome profiling in Arabidopsis thaliana roots have given great insight into the diversity of cell type-specific programs. I will highlight how we can use these data and other genome-scale and systems approaches to map gene regulatory networks that regulate secondary cell wall synthesis in root xylem cells and transcriptional regulation of the Polycomb Repressive Complex 2. In addition, I will discuss current progress in mapping cell type and tissue-specific transcriptomes and epigenomes in Solanum lycopersicum, and it’s wild relative, Solanum pennellii.

Program Abstract #13
Rab23 regulates Nodal signaling in vertebrate left-right patterning independently of the Hedgehog pathway
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Asymmetric fluid flow in the node and Nodal signaling in the left lateral plate mesoderm (LPM) drive left-right patterning of the mammalian body plan. However, the mechanisms linking fluid flow to asymmetric gene expression in the LPM remain unclear. Here we show that the small GTPase Rab23, known for its role in Hedgehog signaling, plays a separate role in Nodal signaling and left-right patterning in the mouse embryo. Rab23 is not required for initial symmetry breaking in the node, but it is required for expression of Nodal and Nodal target genes in the LPM. Microinjection of Nodal protein and transfection of Nodal cDNA in the embryo indicate that Rab23 is required for the production of functional Nodal signals, rather than the response to them. Using gain-and-loss-of-function approaches, we show that Rab23 plays a similar role in zebrafish, where it is required in the telost equivalent of the mouse node, Kupffer’s vesicle. Collectively, these data suggest that Rab23 is an essential component of the mechanism that transmits asymmetric patterning information from the node to the LPM. This work was supported by the National Institute of Child Health and Human Development (R03 HD65092).

Program Abstract #14
X-chromosome inactivation: A model to study epigenetic regulation by long noncoding RNA
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X-chromosome inactivation (XCI) equalizes gene expression between male (XY) and female (XX) mammals by silencing one X-chromosome in the female embryo. In this way, genes are expressed from the two female X-chromosomes at the same level as from the single male X-chromosome. XCI is an excellent model by which to study long noncoding RNA (IncRNA) because the epigenetic process is controlled by the "X-inactivation center" (Xic), a region on the X-chromosome that harbors many IncRNA.
Additionally, our combined zebrafish and mouse work has helped to identify new factors involved in fast muscle differentiation. Six1/Six4 (Niro et al., 2010, Dev. Biol. 338:168-182). We have used our zebrafish model to examine the genetic interactions pathways. Funded by NIH/NIAMS.

Our studies are thus revealing how Pbx proteins act with other transcriptional regulators to direct specific cellular differentiation. We have performed RNA-seq analysis on Myf5-Cre;Pbx1flox;Pbx2KO embryos at E11.5, we find that mouse Pbx1 and Pbx2 are required for fast muscle differentiation, with similar requirements to what we observed in zebrafish. The mouse Pbx1/Pbx2-dependent fast muscle genes closely match those that are dependent on mouse muscle-specific differentiation program in zebrafish. We have now examined the requirements for Pbx genes in mammalian skeletal muscle differentiation by directly and sequentially activating sets of muscle differentiation genes. How do factors like Myod activate their transcriptional targets in a temporally and spatially controlled manner to drive differentiation? Our hypothesis is that Pbx and Six proteins together drive fast skeletal muscle differentiation.

These transcripts interact with protein factors to control the initiation, spread, establishment, and maintenance of silencing on a 150 megabase scale. Some examples include:

1. Xist RNA: Coats the X, targets and spreads PRC2 to initiate silencing.
2. Tsix RNA: Antisense transcript that controls Xist.
3. Jpx RNA: Activates Xist by evicting CTCF.
4. Xite: eRNA for Tsix; Xite controls allelic choice & pairing.
5. RepA RNA: Repeat RNA, targets PRC2 to the Xist.

Many aspects of XCI remain poorly understood. We are interested in how X-chromosome pairing is regulated, how pairing is central to allelic choice, how Xist spreads and targets silencing factors on a chromosome-wide scale, and how Tsix prevents this inactivation cascade. We suspect that RNA will be central to these problems. Here we will present our latest work on how RNA regulates the recruitment of Polycomb repressive complex 2 during the initiation of XCI.

**Program Abstract #15**

**Transmitotic persistence of Wnt pathway activity diversifies gene expression in C. elegans embryos**

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Quantitative differences in signaling pathway activity are a powerful potential mechanism for diversifying cell fates during development. However, few examples of this mechanism have been identified in vivo, in part due to the challenges of performing quantitative assays. The Wnt signaling pathway plays a conserved role during animal development, transcriptionally regulating distinct targets in different stages and cell types (i.e. contexts). This dependence of targets on context could reflect not only interactions with differentially expressed transcription factors, but also context-specific differences in the activity of the Wnt pathway itself. We investigated the role of Wnt pathway activity in target expression by using time-lapse microscopy and automated lineage tracing of Caenorhabditis elegans embryos to quantify expression of Wnt ligands and targets and nuclear localization of transcriptional effectors in vivo at single cell resolution throughout development. We measured the Wnt pathway-dependence of candidate targets and identified over twenty important developmental regulators as Wnt targets. Contrary to existing models, most targets require the Wnt-effector transcription factor POP-1/TCF for either activation or repression but not both. These experiments suggest Wnt-mediated transcriptional activation is strongest in cells that received a Wnt signal in two or more consecutive divisions and this effect does not depend on transcription. We found that these repeatedly signaled cells have higher nuclear β-catenin concentrations and are more likely to express targets that require POP-1 for transcriptional activation. Taken together, these results suggest that the persistence of Wnt signaling across mitosis can integrate lineage history and allow Wnt to activate distinct targets in different developmental contexts.

**Program Abstract #16**

**Pbx and Six proteins together drive fast skeletal muscle differentiation**

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The differentiation of muscle precursor cells into contractile skeletal muscle fibers is necessary for normal muscle development and regeneration and, when defective, leads to diseases such as muscular dystrophies. The muscle factor Myod initiates skeletal muscle differentiation by directly and sequentially activating sets of muscle differentiation genes. How do factors like Myod activate their transcriptional targets in a temporally and spatially controlled manner to drive differentiation? Our hypothesis is that Pbx homeodomain proteins direct Myod to a subset of its transcriptional targets, in particular fast muscle differentiation genes, thereby regulating the competence of muscle precursor cells to differentiate. We have shown that Pbx proteins bind with Myod on the promoter of the fast muscle gene mylfp and that Pbx proteins are required for Myod to activate mylfp expression and the fast muscle-specific differentiation program in zebrafish. We have now examined the requirements for Pbx genes in mammalian skeletal muscle differentiation, in collaboration with Licia Selleri’s lab. By performing RNA-seq analysis on Myf5-Cre;Pbx1flox;Pbx2KO embryos at E11.5, we find that mouse Pbx1 and Pbx2 are required for fast muscle differentiation, with similar requirements to what we observed in zebrafish. The mouse Pbx1/Pbx2-dependent fast muscle genes closely match those that are dependent on mouse Six1/Six4 (Niro et al., 2010, Dev. Biol. 338:168-182). We have used our zebrafish model to examine the genetic interactions between Pbx and Six factors, and find that Pbx and Six proteins appear to additively contribute to fast muscle differentiation. Additionally, our combined zebrafish and mouse work has helped to identify new factors involved in fast muscle differentiation. Our studies are thus revealing how Pbx proteins act with other transcriptional regulators to direct specific cellular differentiation pathways. Funded by NIH/NIAMS.

**Program Abstract #17**

**Using a new acoelomorph model to study the evolution of animal regeneration**

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Most animals are able to repair tissue damage by mobilizing cells to proliferate, migrate, and differentiate. However, little is known about how the molecular mechanisms of wound-response and the subsequent regeneration of structures in different animals compare with each other. More models for studying regeneration are needed to compare the process at a molecular level across species to uncover conserved mechanisms. Acoel worms can regenerate, and belong to an evolutionarily informative phyletic lineage (Acoelomorpha). We have developed the three-banded panther worm, Hofstenia miamia, as a new model acoel species with molecular and genomic tools to study gene function during regeneration. Hofstenia regenerate robustly, are easily cultured in the lab, and are amenable to gene expression analysis and RNAi studies of gene function. Studies of candidate genes in Hofstenia suggest that conserved mechanisms mediate stem cell regulation and patterning of new tissue. Unbiased comparisons of regeneration between acoels and other species will reveal how molecular pathways for animal regeneration have evolved.

Program Abstract #18
PIWI-piRNA pathway function in the stem cells of “immortal” Hydra
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Adult stem cells maintain tissue homeostasis and a decline in stem cell function contributes to disease as animals age. Interestingly, Hydra is extraordinarily long-lived and the adult stem cells show no signs of senescence. We aim to understand the molecular mechanisms that support this remarkable stem cell longevity. The three cell lineages in Hydra are each supported by a distinct stem cell type: two strictly somatic and one germline-competent. We find that the PIWI-piRNA pathway is operating in all three stem cell types and we hypothesize that this may contribute to Hydra longevity. PIWI proteins and their bound small RNAs (piRNAs) are known for maintaining genomic stability in the germline, most notably by repressing transposon expression. PIWI has conserved expression in somatic stem cells, but function in these cases has not been well explored. In Hydra we find that the PIWI-piRNA pathway is essential in the somatic lineages. We demonstrate that PIWI proteins in Hydra are strictly cytoplasmic and thus we are focusing on identifying post-transcriptional targets of the pathway. Our data show that transposon targeting is a conserved feature of the pathway in Hydra, but notably this function is most prevalent in the germline-competent stem cells. To determine the function of the pathway that is common to all stem cell types in Hydra we isolated the piRNAs specific to each lineage, mapped these to the transcriptome, and identified putative targets common to all three lines. Interestingly, we found that the pathway may regulate metabolic processes, insulin signaling, and mitochondrial function in stem cells. These processes are well known regulators of lifespan thus we are currently exploring the hypothesis that the PIWI-piRNA pathway functions in Hydra stem cells to maintain longevity through the regulation of insulin signaling and cellular respiration. (Funding Source: NIH 1K01AG044435-01A1)

Program Abstract #19
Patterning and post-patterning modes of evolutionary digit loss in mammals
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For more than 350 million years, the limbs of terrestrial vertebrates have adopted remarkable specializations allowing them to expand into a broad range of niches. One adaptation that arose again and again in tetrapods is the evolutionary loss of digits, particularly in cursorial mammals that travel over deserts and plains. While digit loss has occurred convergently multiple times in the course of vertebrate evolution, the underlying developmental mechanisms remain elusive. We show in a three-toed desert rodent and in three species of hooved ungulates that digit loss mechanisms occur during early limb patterning and during post-patterning stages of chondrogenesis. In the “odd-toed” jerboa and horse and the “even-toed” camel, expansive cell death sculpts the tissue around the remaining toes. In contrast, digit loss in the pig is orchestrated by earlier limb patterning mechanisms including down regulation of Ptc‘h1 expression but no increase in cell death. Together these data demonstrate remarkable plasticity in the mechanisms of vertebrate limb evolution and shed light on the complexity of morphological convergence, particularly within the Artiodactyl lineage.

Program Abstract #20
Binging has its benefits: the genetic basis of the insatiable appetite in the cavefish Astyanax mexicanus.
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Adaptation to new environments requires changes to morphology, behavior, and physiology. While we have started understanding morphological evolution, how behavior and physiology evolve is still largely a mystery. Independently derived populations of Mexican cavefish, Astyanax mexicanus, are a good model to examine population level differences in metabolic adaptation to nutrient poor environments. Many cavefish populations rely on annual flooding for nourishment as caves lack primary producers. Thus to survive, cavefish need to binge when food become available in order to gain weight rapidly. We show that while all cavefish populations tested lose weight slowly during starvation, only some cavefish populations that live in a feast and famine...
cycle consume more food than surface populations. Here we show the genetic basis of the insatiable appetite found in populations of cavefish. We provide evidence that this binging behavior is derived from standing genetic variation present in surface populations. Intriguingly, the same mutated residue has been shown to be linked to obesity in humans. We are in the process of evaluating the differences in functionality between alleles in vitro. Additionally, we are examining the appetite in hybrid populations of surface and cavefish populations. Our results suggest that drastic metabolic and behavioral changes can occur by a single point mutation in natural populations. This work was funded by an NIH genetics and genomics training grant 5t32GM096911-03.

Program Abstract #21
Control of Arabidopsis petal growth
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We are using the Arabidopsis petal as a simple, tractable, system for investigating the molecular mechanisms controlling plant organogenesis. Delimiting the petal primordium depends on defining organ boundaries, and growth of the primordium occurs by a wave of cell divisions followed by cell expansion. We have characterized the role of RABBIT EARS (RBE), encoding a zinc finger transcriptional repressor, which is a master regulator of these processes. Using RNAseq approaches, we have identified a number of genes regulated by RBE. Through these analyses, we have shown that RBE regulates primordium specification through modulating the expression of a miRNA dependent pathway involved in establishing organ boundaries, as well as through controlling the expression of a family of transcription factors that are implicated in growth control through regulating the duration of cell divisions in the developing petal. To identify other regulators of growth, we have utilized natural variation to define the genetic basis for quantitative differences in petal size and shape in different Arabidopsis ecotypes. Together, these analyses are providing us with a better understanding of the molecular mechanisms controlling the growth of this seemingly simple organ type.

Program Abstract #22
The evolution and development of leaves in ferns
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Despite the extraordinary significance that the origin of leaves had for life on earth, the evolution and development of leaves remains vigorously debated. In particular, more than a century of paleobotanical, morphological, and phylogenetic research has not resolved the question of leaf homology in vascular plants. According to the fossil record, leaves evolved independently at least twice from ancestors with naked branching axes bearing sporangia. The leaves (microphylls) of lycophytes are currently hypothesized to have evolved de novo once, while the leaves (megaphylls) of other vascular plants (ferns and seed plants) are thought to have evolved from branching axes once or up to 7 times. Uncertainty over the number of times leaves evolved in megaphyllous species arises from competing interpretations of morphology and phylogeny, specifically within ferns- a group that occupies a key evolutionary position and thus have an important role in this debate. We have investigated the evolution and expression of Class I KNOX and Class III HD-Zip in ferns that fills a crucial gap in our understanding of leaf evolution. We show that there is a conservation in a leaf developmental mechanism between ferns and seed plants and that neofunctionalization of Class III HD-Zips occurred in the ancestor of euphyllophytes. Furthermore, our results indicate that Class III HD-Zips may have an ancestral role in sporangia development. We hypothesize that a sporangium-specific developmental program was co-opted for the development of microphylls and megaphylls and therefore there is a common origin of all vascular plant leaves. Finally, we investigated the expression of Class I KNOX genes in Elaphoglossum section Squamipedia to better understand the genetic basis of leaf diversity in this group. Our research was funded by National Science Foundation grant DEB-1020443.

Program Abstract #23
Genetic origins of divergence and convergence in domestic pigeons
Michael Shapiro
Univ. of Utah, USA
The rock pigeon is among the most phenotypically diverse and widely distributed avian species in the world. Pigeons were domesticated thousands of years ago and breeders have selected for spectacular variation in numerous anatomical and behavioral traits. Following in the footsteps of Charles Darwin, T.H. Morgan, and others, we are using the rock pigeon (Columbia livia) as a powerful model to learn about avian diversity. We are combining traditional laboratory genetics, whole-genome sequencing, and developmental analyses to understand the evolution of diversity among the more than 350 modern breeds of this species. In doing so, we are addressing fundamental questions about the genetic architecture of phenotypic change, including, how many genetic changes are required to yield pronounced anatomical changes? What are the identities of these key genes? What types of mutations underlie phenotypic variation (e.g., coding versus regulatory)? How do different loci interact to produce complex traits? Do similar traits in different breeds evolve via similar or different genetic mechanisms? In particular, our combined approach is revealing a spectrum of mutations that control the orientation, color, and placement of plumage within and among breeds. For example, genome sequencing across breeds revealed both coding and cis-regulatory changes that lead to derived color phenotypes, and through laboratory crosses and mechanistic studies we found explanations for how these mutations interact to produce complex pigment variation. The genomics era is driving new discoveries about the molecular basis of diversity in non-traditional model organisms.
The domestic pigeon is a promising model with which to explore the genetic architecture of derived, constructive phenotypes in a bird that is amenable to genetic, genomic, and developmental investigation.

**Program Abstract #24**
**The evolution and conservation of left-right patterning mechanisms**
Martin Blum, Axel Schweickert
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Morphological asymmetry is a common feature of animal body plans, from shell coiling in snails to organ placement in humans. Molecularly, the growth factor Nodal is key for determining this laterality. Many vertebrates, including humans, use cilia for breaking symmetry during embryonic development: rotating cilia produce a leftward flow of extracellular fluids that induces the asymmetric expression of the signaling protein Nodal. In contrast, Nodal asymmetry can be induced flow-independently in invertebrates. Here, we ask when and why flow evolved. (1) We infer that the Nodal cascade was present already in the last common ancestor of bilateria, the urbilateria. We argue that the gastro-intestinal tract was the first functionally asymmetrical organ, and that the gut tube was asymmetrical in urbilateria. The Nodal cascade itself might have been lost in ecdysozoa, and the elucidation of molecular mechanisms underlying LR asymmetries in these species might lead to the identification of novel homologies between protostomes and deuterostomes. (2) We hypothesize that a flow-based mechanism of symmetry breakage exists in the entire deuterostome lineage. We predict in which tissue and at what embryonic stage LR cilia and leftward flow should be present in sea urchin and amphioxus embryos. (3) We propose that vertebrate evolution depended on maintaining LR organ asymmetries on the background of a now perfectly bilaterally symmetrical axial skeleton. The absence of leftward flow in chick and pig, perhaps in all birds and also in other mammals, is puzzling, and we will present and discuss a solution to this riddle. Our thoughts provide a coherent hypothesis on evolution and conservation of LR patterning mechanisms, which is testable and - we hope - will provoke investigations in different model organisms throughout the animal kingdom.

**Program Abstract #25**
**Germ cell development and regeneration in planarians: implications for understanding parasitic flatworms**
Phillip Newmark1,2, Bo Wang1,2, Jim Collins1,2
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Planarians are free-living flatworms with amazing regenerative abilities. Their regenerative prowess is based upon a population of adult stem cells, called neoblasts, that serve as the source of new tissue during regeneration and tissue homeostasis. Using the functional genomics tools available for studying planarians, we have been investigating how these stem cells give rise to the germ cell lineage and how reproductive system development and regeneration are controlled systemically. I will discuss how our work on planarian germ cell development has led us to study the biology of schistosomes, parasitic flatworms with great significance for global health. We have shown that, like planarians, schistosomes have neoblast-like stem cells in the adult stage of the life cycle, providing one potential explanation for their longevity. Extending this work to the intramolluscan stage of the schistosome life cycle, we find that the so-called germinal cells in the sporocysts, resemble neoblasts morphologically and express similar genes that are required for germinal cell proliferation and maintenance. Thus, applying the lessons learned from studying planarians will help inform the biology of their parasitic cousins.

**Program Abstract #26**
**A bioengineering approach to study Wnt-mediated asymmetric stem cell division**
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Developmental signals, such as Wnts, are often presented to cells in a local manner. Wnts can induce different types of cellular responses, and these may depend on how cells read both the level and direction of the signal. To ask whether a local Wnt signal can influence asymmetric divisions of stem cells, I immobilized Wnt proteins and Wnt inhibitors on beads and applied these to embryonic stem (ES) cells. At the single cell level, the Wnt-bead induces asymmetric distribution of Wnt/ß-catenin signaling components, orients the plane of mitotic division and directs asymmetric inheritance of centrosomes. Significantly, before cytokinesis is completed, the Wnt-proximal daughter cell expresses high levels of nuclear ß-catenin and pluripotency genes, whereas the distal daughter cell acquires hallmarks of differentiation. Blocking Wnt signaling locally produces asymmetric cell fates in the opposite manner. We suggest that a spatially restricted Wnt signal can induce an oriented cell division that generates distinct cell fates at predictable positions relative to the Wnt source.

**Program Abstract #27**
**Amyloid precursor proteins as regulators of G protein-dependent neuronal guidance**
Philip Copenhaver, Jonathan Zweig, Tracey Swanson, Jenna Ramaker
Oregon Health & Science Univ., USA
The amyloid precursor protein (APP) is best known as the source of amyloid peptides that have been postulated to cause
Alzheimer’s disease. In addition, APP has also been shown to regulate neuronal growth in the developing nervous system, albeit via mechanisms that are poorly understood. In vitro studies have shown that APP can affect cellular responses via the heterotrimeric G protein Gia, suggesting that APP might function as an unconventional G protein-coupled receptor; however, compensatory interactions by other APP-related proteins have hindered an analysis of this process in the mammalian brain. Using Manduca (hawkmoth) and Drosophila as simpler models, we have shown that the sole insect ortholog of APP (APPL) co-localizes with Gia in the leading processes of developing neurons, and that the two proteins directly interact. APP-Gia interactions are also regulated by Gia activation. In embryo culture, both APPL and Gia function to prevent neuronal migration and outgrowth into inappropriate regions. Likewise in cultured murine hippocampal neurons, stimulating APP activation causes growth cone collapse in a Gia-dependent manner, indicating that this pathway is evolutionarily conserved. Using human brain samples, we have found that reduced APP-Gia interactions correlate with the severity of Alzheimer’s pathology, suggesting that disruption of this pathway might provoke neurodegenerative responses. Based on evidence that mammalian Contactins can bind APP, we recently found that insect Contactin is expressed within embryonic regions that are inhibitory to neuronal outgrowth, while treating migrating neurons with contactin-Fc fusion proteins inhibits their motility. We are now testing the model that Contactins function as endogenous APP ligands that can activate APP-Gia signaling in a context-dependent manner, providing a mechanism for regulating the behavior of developing neurons during embryogenesis. Funding: NIH AG025525, NS080036 (PFC); OPAR grant (JMR).

Program Abstract #28

Hardwired and activity-dependent regulation of fru+ olfactory circuit development underlying sex-specific behaviors
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In Drosophila, sex-specific behaviors are innate, yet adaptable and are tightly regulated by the function of a single behavioral switch gene fruitless (fru), which encodes a putative transcriptional regulator. Sex-specific alternative splicing of fru is required for the execution of gender-specific courtship behaviors. Fru expression labels approximately 2000 inter-connected neurons in the nervous system, which define neuronal circuits underlying sex-specific behaviors. Very little is known about transcriptional regulation of fru, and how fru-positive neuronal circuits are assembled during development. We found that fru expression in the adult Or47b olfactory receptor neurons (ORNs) is controlled by Or47b function and neuronal activity. Despite the temporal correlation of the onset of fru and O47b expression during development, ORN activity is not required for the initiation of fru expression during development, but rather its maintenance in adult flies. This suggests other hardwired molecular mechanisms that coordinate fru and Or47b expression during development. In agreement with this, in a genetic screen we identified a chromatin modulatory protein, Alhambra/AF10 (Alh), in sensory and functional specification of fru-positive olfactory receptor neurons (ORNs). In alh mutants the expression of both Or47b and fru is expanded to developmentally related neurons without altering the connectivity programs. The expansion of fru expression in alh mutants during development does not require Or47b function in ORNs, however Or47b is required for the maintenance of fru expression in alh mutant adults. Our results suggest that alh and olfactory receptor activity constitute hardwired and activity-dependent components of a molecular circuitry that sets up and maintains fru expression in ORN circuits.

Program Abstract #29

Programming and reprogramming neuronal diversity in the cerebral cortex
Paola Arlotta, Giulio Srubek Tomassy, Simona Lodato, Caroline Rouaux
Dept. of Stem Cell and Regenerative Biology, Harvard Univ., USA

The neocortex contains an unparalleled diversity of neuronal subtypes, each defined by distinct traits that are developmentally acquired under the control of several neuron subtype-specific and pan-neuronal genes. The regulatory logic that orchestrates the coordinated expression of these unique combinations of genes is not known for any class of cortical neurons. Here, we report on the identification of novel transcriptional dynamics underlying developmental generation of excitatory pyramidal neuron diversity in the cerebral cortex, and define some of the governing principles that shape the identity of one class of neurons, corticospinal motor neurons. In addition, we show that pyramidal neuron diversity impacts the behavior of other cell types during cortical development and discuss the critical effect on oligodendrocytes to guarantee generation of normal patterns of myelin distribution in different cortical layers. Once development is complete, it is well known that pyramidal neurons become permanently postmitotic and do not change their class-specific identity for the life span of the organism. We demonstrate that during a defined window of postmitotic development (“critical window of nuclear plasticity”) pyramidal neurons can change their identity in vivo, acquiring critical features of alternate neuronal lineages. Collectively, we discuss the principles that govern development and reprogramming of neuronal diversity in the neocortex.

Program Abstract #30

Identification of a potent regulator of retrograde mitochondrial transport in axons
Catherine Drerup, Alex Nechiporuk
Oregon Health & Science Univ., USA

The formation and maintenance of neural circuits requires the active transport of proteins and organelles in neural processes. While anterograde movement (away from the cell body) utilizes the kinesin family of motor proteins, retrograde transport (towards the cell body) is accomplished by one motor complex, cytoplasmic dynein. The diversity of cargos moved by dynein and the regulation of
Program Abstract #31
Regulatory programs controlling serotonin neuron identity
Evan Deneris, Steven Wyler, William Spencer, Lauren Donovan
Case Western Reserve Univ., U.S.A.

The development of diverse neuron identities is an essential step in the formation of neural circuits. Recent studies have demonstrated that neuron-type identity must be maintained throughout life to preserve circuitry. However, the gene regulatory programs controlling the acquisition and life long maintenance of neuron-type identity are poorly understood. We are investigating the regulatory programs controlling the acquisition and maintenance of serotonin (5HT) neuron identity. 5HT neurons provide critical modulatory input into neural circuits that shape emotional behaviors in response to environmental stressors. Altered 5HT signaling and gene expression, particularly in early life, have been implicated in adult mood and anxiety disorders suggesting an early critical period for 5HT modulation of emotional circuits. A 5HT neuron-specific transcriptional network controls the acquisition of 5HT identity through direct activation of a 5HT-type gene battery, Tph2, AADC, Sert, Vmat2, required for 5HT synthesis, reuptake and vesicular transport. The Pet-1 ETS protein is an essential factor in the network as it is required to initiate expression of the 5HT gene battery in postmitotic precursors. Whole genome expression profiling has revealed that Pet-1 activates hundreds of other genes encoding diverse features of 5HT neurons. Moreover, Pet-1 appears to repress hundreds of additional genes suggesting that 5HT identity is generated not only through gene activation but also through repression. Pet-1 is a terminal selector as hundreds of other genes encoding diverse features of 5HT neurons. Moreover, Pet-1 appears to repress hundreds of additional genes suggesting that 5HT identity is generated not only through gene activation but also through repression. Pet-1 is a terminal selector as continuous Pet-1 expression is sustained through direct autoregulation and is required to maintain 5HT identity. Pet-1 ensures long maintenance of some 5HT identity features while other features need Pet-1 only up to early postnatal life. These findings suggest a fetal through early postnatal sensitive period for transcriptional maintenance of key 5HT identity features. This research is supported by NIH grants P50 MH096972 and RO1 MH062723.

Program Abstract #32
VEGFR1: a developmental link between neuronal migration and vasculogenesis
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Neuronal migration is essential for appropriate circuitry to form. Disruption of migration causes neurological disease states, leading to devastating cognitive and reproductive problems. As such, it is critical to understand the mechanisms underlying normal neuronal migration to begin to address the event(s) associated with a disease. In vertebrates, migration of gonadotropin-releasing hormone-1 (GnRH) neurons to their final location within the brain is necessary for sexual maturation and reproductive function. Failure of this process leads to hypogonadotropic hypogonadism (HH), resulting in delayed puberty and infertility. To discover ligand/receptor signaling impacting this migration, we compared transcriptomes of migrating vs. post-migrating GnRH neurons. Microarray analysis revealed that vascular endothelial growth factor receptor-1 (VEGFR1), a receptor tyrosine kinase, was upregulated in migrating neurons. VEGFR1 is commonly associated with developing vasculature. Notably, VEGFR1 ligands, VEGFA and VEGFB, were expressed along the GnRH migratory route and in GnRH cells, respectively, lending credence to a potential role in regulating GnRH neuronal migration. VEGFR1 protein was found in migrating GnRH neurons. Functional assays revealed chronic treatment with a blocking antibody specific for VEGFR1 decreased the distance GnRH cells migrated. In addition, acute in situ assays blocking VEGFR1 resulted in an immediate decrease in cell migration rate. Unexpectedly, blocking VEGFA significantly increased GnRH migration rate, suggesting a dynamic balance between VEGFA and VEGFB in modulating VEGFR1-mediated GnRH migration. These data demonstrate a novel role for VEGFR1 in neuronal migration and may provide new candidates for genetic screening in HH patients. Furthermore, this work sheds light on the complex, yet well-known players in vasculogenesis and may provide an unexplored link between two critical developmental processes—vasculogenesis and neuronal migration.

Program Abstract #33
Measuring cellular forces within living embryonic tissues
Otger Campas
Cell-generated mechanical forces play a critical role during tissue morphogenesis and organ formation in the embryo. Despite their relevance in sculpting functional embryonic structures, very little is known about the mechanisms by which cellular forces affect/control developmental processes, mainly because it has not been possible to measure cellular forces within developing tissues in vivo. We have developed a new technique that permits direct quantification of cellular mechanical stresses in situ within living tissues and developing organs. Using this novel technique, we quantify the stresses generated by mammary epithelial cells cultured within 3D aggregates and confirm that these stresses are dependent on myosin II activity and more than two-fold larger than the stresses generated by cells of embryonic tooth mesenchyme when analyzed within similar cultured aggregates or in developing whole mouse mandibles. A detailed version of this work can be found in Campas et al., Nature Methods 11 183-189 (2014). Using the same technique we measure the cellular forces involved in different morphogenetic processes in living Zebrafish embryos.

**Program Abstract #34**

* C. elegans FBN-1, a fibrillin-like protein, promotes resistance to biomechanical force during development

Melissa Kelley¹, John Yochem¹, Michael Krieg², Miriam B. Goodman³, Martin Chalfie², Andrea Calixto², Alison Frand³, Melissa Kelley¹, John Yochem¹, Michael Krieg², Miriam B. Goodman³, Martin Chalfie², Andrea Calixto², Alison Frand³

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During development, biomechanical forces contour the body and provide shape to internal organs. Using genetic analysis in combination with a FRET-based tension sensor system, we have identified an uncharacterized biomechanical force that is exerted by the developing foregut (pharynx) on the anterior epidermis of the *C. elegans* embryo. We have also discovered a regulatory network that is required to maintain epidermal integrity in response to embryonic biomechanical forces. Most notably, this network includes a C. elegans fibrillin-like protein, FBN-1, which is likely secreted at the apical surface of epidermal cells as a component of the embryonic sheath. Human fibrillins have been implicated in disorders of connective tissues and mutations in fibrillin1 lead to Marfan syndrome. Using a microarray approach, we have discovered that expression of *C. elegans* *fbn-1* is regulated by a conserved splicing factor, MEC-8/CRA b, which is required for the correct processing of *fbn-1* mRNA. Acting in a parallel pathway to MEC-8 are two conserved proteins, SYM-3/FAM102A and SYM-4/WDR44, which localize to vesicles at or near the plasma membrane. Our data indicate that SYM-3 and SYM-4 function with the RAB-11 GTPase, a known regulator of endocytic recycling and exocytosis. Thus, SYM-3–SYM-4–RAB-11 may promote the correct targeting of FBN-1 or other extracellular matrix proteins to the embryonic sheath. In addition to providing resistance to a pharyngeal pulling force, FBN-1 stabilizes the epidermis during embryonic elongation, when circumferential actinomyosin bundles arrayed along the body axis undergo contraction. Finally, FBN-1 promotes stable adhesion between neuronal support cells, which experience mechanical strain in response to pulling forces associated with the process of retrograde dendritic extension. Taken together, we have identified a novel network that is required to maintain epidermal architecture in response to a variety of biomechanical forces during embryogenesis.

**Program Abstract #35**

*Anisotropic stress orients remodelling of mammalian limb bud ectoderm*

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How forces are integrated with cell rearrangements to shape embryonic tissue is not well understood in vivo, especially among later vertebrates. In the early limb bud, dorsal and ventral ectoderm converge to form the apical ectodermal ridge (AER), though underlying mechanisms are unclear. By combining finite element modelling with measurement and manipulation of physical parameters in the mouse embryo using laser ablation and atomic force microscopy, we show that mesodermal growth and ectodermal tension together generate a stress pattern that is dorsosvertically biased. Live imaging reveals that ectoderm remodels by concomitant cell division and neighbour exchange along the predominant axis of tissue stress. Initial growth of mesoderm anisotropically tenses overlying ectoderm to polarise cortical actin among AER progenitors, a process that requires canonical Wnt and Fgf signalling shown using conditional mutants. AER progenitors intercalate at the dorsosventral boundary where they generate a tensile gradient that reorients resolution of multicellular rosettes on adjacent surfaces, a process facilitated by ?-catenin-dependent attachment of cortex to membrane. Therefore, feedback between tissue stress pattern and cell intercalations remodels mammalian ectoderm.

**Program Abstract #36**

*FGF signaling establishes a contractile gradient to drive polarized endoderm movements underlying morphogenesis of the tailbud*
Evolution of ectoderm-mesoderm communication during skeletal patterning in echinoid larvae
Deirdre Lyons, Megan Martik, Julian Kimura, David McClay
Duke Univ., USA
The life cycle of indirect-developing sea urchins and sand dollars (echinoids) includes a ciliated pluteus stage supported by a calcium carbonate endoskeleton. The skeleton is made by primary mesenchyme cells (PMCs), while the pattern of the skeleton is determined by local signals from overlying ectoderm. The endoskeleton is also a model for studying patterning evolution, since specific parts of the skeleton can vary between species, and these differences have adaptive significance. Two of the most variable elements are the recurrent and posterior connecting rods that protect the larval body, but the developmental basis of their variation is not well understood. Comparing the sea urchin *Lytechinus variegatus*, which forms a small recurrent rod and no posterior connecting rod, with the sand dollar *Mellita quinquiesperforata*, which forms both, we found that the differences in their skeletons are already obvious in the arrangement of primary mesenchyme cells (PMCs) at gastrula stages. *Mellita* gastrulae possess a novel antero-dorsal chain of PMCs, which contributes to the recurrent and posterior connecting rods, and appears to form as a result of a heterochronic shift in ectodermal patterning, relative to *Lytechinus*. We investigated differences in ectodermal patterning between these species using gene expression analysis, immunohistochemistry, pharmacological inhibition, and inter-species chimeras. We found that Nodal signaling controls the position of the antero-dorsal chain in the oral/aboral axis, demonstrating that ectodermal inputs to spatiotemporal patterning of underlying PMCs have diverged between these two echinoids. These data provide the framework for studies that address the details of these two patterning systems at the molecular level, and cast light on which signals are likely responsible for skeletal evolution in echinoids. Funding from NIH

Specification, positioning and patterning of the anterior neuroectoderm in sea urchin embryos
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Studies in several deuterostome developmental model systems, including the sea urchin embryo, suggest that an early, broad regulatory state initiates specification of the presumptive anterior neuroectoderm (ANE). During development, a posterior-to-anterior wave of inductive signaling progressively restricts this broad ANE potential to the anterior pole, where it is subsequently patterned to form various neural tissues. However, the molecular mechanisms used to position and pattern the ANE along the anterior-posterior (AP) axis are incompletely understood. Our recent studies in the sea urchin show that confining the ANE to the anterior pole involves the integration of information from the Wnt/β-catenin, Wnt/JNK, and Wnt/PKC pathways. We have also found that secreted Wnt signaling modulators synthesized at the anterior pole act as a signaling center that establishes the ANE boundary and subsequently patterns the ANE territory. Using a combination of molecular manipulations, high-throughput genome-wide assays, gene regulatory network analysis and classical experimental embryology approaches, we are attempting to produce a systems-level model of these fundamental developmental processes. Moreover, functional and expression data comparisons from invertebrate and vertebrate deuterostomes strongly suggest that aspects of the early Wnt network and the anterior signaling center may be shared by all deuterostomes. The phylogenetic position of the sea urchin at the base of the deuterostome lineage makes it an attractive model for studying the evolution of the ANE positioning and patterning mechanisms in deuterostomes.
Program Abstract #39
Evolution of miRNA signaling in development: Insights from the hemichordate *Saccoglossus kowalevskii*
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miRNAs act as post-transcriptional regulators of gene expression networks, and multiple evolutionary expansions of miRNAs are associated with increasing complexity. However, despite growing data for the importance of miRNAs in the development of model organisms, and genome-wide small RNA studies in numerous species, the functional roles of miRNAs in the development of a wide range of organisms are unknown. The question remains whether the evolution of miRNA targets and functions have driven the evolution of developmental pathways or if they are instead uniquely regulated in different lineages. We are investigating the developmental expression and function of miRNAs in the direct-developing hemichordate *Saccoglossus kowalevskii*. Hemichordates and vertebrates share a common ancestor and many developmental signaling pathways, making it an ideal model for uncovering how ancestral miRNAs may have contributed to the evolution of development in deuterostome lineage. Small RNA sequencing shows that *Saccoglossus* miRNAs are dynamically expressed throughout development, suggesting potential roles in a number of developmental processes. In order to identify the function of these miRNAs, spatiotemporal expression data and target predictions are being combined with functional perturbations in the developing embryo. An initial functional screen has confirmed a conserved role for miR-1 in muscle development and ongoing investigations are focused on the regulation of developmental signaling pathways by the neural miRNAs miR-7 and miR-124 in *Saccoglossus*. The targets and functions of *Saccoglossus* miRNAs are being compared with their homologs and functional counterparts in vertebrates and protostomes in order to gain insight into the evolving role of miRNAs in development. Our data provide a first exploration of miRNA function in hemichordate development and will contribute to understanding how the role of miRNA regulation in development has changed through evolution.

Program Abstract #40
Shaping the *Ciona* notochord
Michael Veeman, Maia Carlson, Wendy Reeves
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As in many chordate embryos, the *Ciona* notochord converges and extends to form a tapered rod. In *Ciona*, notochord taper depends both on the timing of notochord cell intercalation and on asymmetric cell divisions within the notochord lineage. An analysis of notochord cell volumes before and after intercalation argues that notochord intercalation may be considerably more stereotyped than suggested by earlier blastomere labeling studies. Here we use genetic labeling methods to fate map the *Ciona* notochord with both high resolution and large sample sizes. We find that patterns of cell intercalation in the notochord are somewhat stochastic but far more stereotyped than previously believed. Cell behaviors vary by lineage, with the secondary notochord lineage being much more constrained than the primary lineage. Within the primary lineage, patterns of intercalation reflect the geometry of the intercalating tissue. We identify the latest point at which notochord morphogenesis is completely stereotyped, which is shortly before the onset of mediolateral intercalation and immediately after the final cell divisions in the primary lineage. These divisions are consistently oriented along the AP axis. We are also able to use this strategy to quantify the asymmetry of all of the late divisions in the notochord, which vary remarkably in different regions/sublineages. Our results indicate that the interplay between stereotyped and stochastic cell behaviors in morphogenesis can only be assessed by fate mapping experiments that have cellular resolution and examine large numbers of embryos. We will also present data as to the molecular basis of asymmetric cell division in the notochord.

Program Abstract #41
General approach for *in vivo* recovery of cell type specific effector gene sets
Julius Barsi
CALTECH, USA
Differentially expressed, cell type specific effector gene sets hold the key to multiple important problems in biology, from theoretical aspects of developmental gene regulatory networks (devGRNs) to various practical applications. Although individual cell types of interest have been recovered by various methods and analyzed, systematic recovery of multiple cell type specific gene sets from whole developing organisms has remained problematical. My presentation will delineate general methodology aimed at obtaining this biological information and is applicable to all model organisms for which transgenic tools are available. It has been devised using the sea urchin embryo, material of choice because of the large-scale devGRNs already solved for this model system. The method utilizes the regulatory states expressed by given cells of the embryo to define cell type, and includes a Fluorescence Activated Cell Sorting (FACS) procedure that results in no perturbation of transcript representation. I have extensively validated the method by spatial and qualitative analyses of the transcriptome expressed in isolated embryonic skeletogenic cells and as a consequence, generated a prototypical cell type specific transcriptome database: http://www.spbase.org:3838/cellspecific/ (Research supported by NIH grant HD067454).

Program Abstract #42
Genetics of morphologic variation between dog breeds
Elaine A. Ostrander
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Skull shape, leg length, ear and tail position, and body size are just a few of the many breed-defining features that distinguish the
175 domestic dog breeds that exist in the US today. Differences between breeds are large and striking, resulting from selective breeding that isolated and propagated heritable traits to create breeds of purpose and fancy. In the case of variance between breed-associated skull shape, previous studies suggest that cranioskeletal modularity exists among dogs, particularly between bones that articulate the face and neurocranium. The result is a repertoire of forms, including those superficially reminiscent to human brachycephaly (face-shortening), dolichocephaly (face-lengthening), and hydrocephalus (neurocranium enlargement). While these cephalic disorders are associated with human morbidity, their canine analogs appear benign, suggesting that novel and relatively benign developmental mechanisms are responsible for canine cranioskeletal shapes. Identification of the underlying genes is likely to impact our understanding of mammalian skull development. Towards that end, we have used museum skulls to perform a principal components analysis, thus allowing us to determine sets of measurements that are coordinately regulated. Genome-wide, association studies have allowed us to identify regions of the genome that contain contributing genes, and next generation sequencing has led us to the underlying mutations. Similar approaches have been used by us to find genes important in variance associated with leg length, ear and tail position, and body size, where variant forms of at least six genes play major roles in determining the gradient of skeletal size observed between giant and small domestic dog breeds. A subset of these examples are reviewed.

Program Abstract #43
Phenotypic variation in individuals
Ben Lehner\textsuperscript{1,2}
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We study the causes of phenotypic variation amongst individuals, including the distribution and effects of genetic variation, somatic mutations and epigenetic differences (stochastic/environmental effects). I will present some our recent work on how inherited genetic variation influences dynamic processes, on somatic mutations in cancer, and on the importance of early inter-individual variation in gene expression during embryonic development.

Program Abstract #44
Genome evolution and development
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With complete genome sequences in hand from over a dozen diverse animal clades, we can broadly compare and contrast the content and organization of metazoan genomes, looking for patterns of evolutionary conservation and change. For some genomic features like gene content, exon-intron structure, and even gross chromosomal organization, there is remarkably deep conservation. Some phylogenetic groups, represented by important model systems, are exceptional, and have experienced extensive remodeling of their genomes relative to the prototypical metazoan. Other genomic features are much more evolutionarily labile across the entire metazoan tree. Broad trends, including the importance of gene and genome duplication, are evident. We will discuss how the dynamics of genomes over half a billion years have constrained and enabled the evolution of metazoan development.

Program Abstract #45
Contact-mediated cell polarization during embryogenesis
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In many species, early embryonic blastomeres develop a contact-induced polarity called radial polarity, which is important for subsequent morphogenetic or specification events. We are investigating the mechanisms of radial polarization in the C. elegans embryo. During the four-cell stage, blastomeres polarize radially when the RhoGAP protein PAC-1 localizes to cell contacts and locally inactivates the RhoGTPase CDC-42. CDC-42 remains active at contact-free surfaces, where PAC-1 is absent, and recruits PAR polarity proteins to polarize the cell. In C. elegans, radial polarity is important for the directed cell movements of gastrulation. We have found that the symmetry-breaking cue PAC-1 localizes to cell contacts through recruitment by the adhesion protein E-cadherin and its associated catenins. We identify a novel linker protein that connects PAC-1 to E-cadherin, and show that E-cadherin mislocalization is sufficient to relocalize PAC-1 and depolarize cells. Our results show that the adhesive and polarity functions of E-cadherin can be separated, and that E-cadherin performs an instructive role in cell polarization by directly recruiting a symmetry-breaking polarity regulator to cell contacts.

Program Abstract #46
Surrounding tissues canalize the motility of cardiopharyngeal progenitors towards collective polarity and directed migration
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During directed collective migration cells must maintain group polarity and interpret external cues in order to properly reach their destination. Combining cellular simplicity and abundance of molecular tools, Ciona intestinalis is a unique chordate model to study migration at cellular resolution. During Ciona embryogenesis, bilateral pairs of cardiogenic progenitors (trunk ventral cells, TVCs) undergo a stereotyped, polarized migration away from the tail towards the ventral trunk. To identify tissues contributing to TVC polarity and migration we quantified the contact between TVCs and surrounding tissues and blocked secretion in a tissue specific
manner using targeted expression of a dominant negative form of Sar1, which is required for transport of proteins from the endoplasmic reticulum to the Golgi apparatus. Our data indicate that a combinatorial input from diverse tissues is required for proper TVC migration and polarity. When signaling was inhibited in one of the TVCs, the other was capable of migrating on its own, even though they normally migrate as a polarized pair where leader and trailer cells are invariably determined. The mesenchyme makes early contact with the TVCs and confers robust specification of the trailer but appears dispensable for directed migration. The notochord, which does not contact the TVCs, appeared to secrete a chemorepulsive signal. During early TVC migration the endoderm touches the leader and then "encases" both TVCs as they complete their migration. The endoderm appears to provide a ventral attracting cue as well as the relevant inputs to establish and maintain leader-trailer polarity. The epidermis touches the TVCs from their birth onwards and appears to be required for their ventro-lateral migration, adhesion to each other and flattening onto the trunk epidermis. These studies reveal that the TVCs have a range of migratory capabilities, which are channeled into stereotyped behavior by signaling from multiple tissues.

Program Abstract #47
The receptor DCC mediates oscillatory, self-organizing polarity to orient invasion towards netrin
David Sherwood, Zheng Wang, Lara Linden, Kaleb Naegeli, Quiyi Chi
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The receptor DCC directs dynamic polarizing activities in animals towards its extracellular ligand netrin. How DCC polarizes towards netrin is poorly understood. By performing live-cell imaging of the DCC ortholog UNC-40 during anchor cell invasion in C. elegans, we find that UNC-40 clusters, recruits F-actin effectors and generates F-actin in the absence of UNC-6 (netrin). Time-lapse analysis revealed that polarized UNC-40 clusters assemble, disassemble and reform again at periodic intervals in random regions of the cell membrane. This oscillatory behavior indicates that UNC-40 polarizes through a self-organizing mechanism involving interlinked positive (formation) and negative (disassembly) feedback. Though not required for UNC-40 self-organizing behavior, we show that endogenous UNC-6 and ectopically provided UNC-6 orients and stabilizes UNC-40 clustering. Furthermore, the UNC-40 binding protein MADD-2/Mid1 promotes self-organized clustering and is required for UNC-40 polarization towards UNC-6. Together, our data suggests netrin directs polarized responses by stabilizing the localization of randomly directed self-organized DCC clustering to regions of the cell in contact with netrin.
This work was supported by a Pew Scholars Award, and National Institutes of Health Grants GM100083 to D.R.S.

Program Abstract #48
The neural crest invasive front has a unique molecular signature
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Cell migration is a key phenomenon of many important processes during embryonic development. Although in vivo imaging has provided exciting insights into the richness of cell behaviors, molecular information that underlies local cell migration dynamics is critically lacking. Therefore, efforts that correlate molecular interrogation of single and small cell numbers with quantitative cell behavior analysis should yield important insights into individual and group decision making processes during embryonic cell migration events. Here, we addressed the question whether there is a unique molecular signature of the invasive front during progressive stages of neural crest cell migration that would correlate with our observations of regional differences in cell behaviors. Using FACs, laser microdissection and microfluidic RT-qPCR, we analyzed single cells and small subpopulations within typical multicellular cranial neural crest cell streams during migration and invasion of head targets in chick. We discovered there is a unique gene expression profile within a small subset of lead neural crest cells that is consistent during progressive phases of migration. Expression analysis by multiplexed fluorescent in-situ hybridization (HCR) confirmed the spatial localization of several candidate genes. To test their functional role, we knocked down or over-expressed (within the trailing neural crest subpopulation) genes of interest and measured the affects to cell dynamics and patterning. Our results offer intriguing molecular insights into how cell decisions at the invasive front affect multicellular stream group behaviors during migration of the cranial neural crest. This work was funded by NICHD and the Stowers Institute for Medical Research.

Program Abstract #49
Lattice light sheet imaging of live vertebrate embryos reveals novel processes during olfactory morphogenesis
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The cranial ganglia and sense organs arise from two cell types: neural crest and ectodermal placodes. Both undergo cell migration and/or dynamic cell rearrangements. Most cranial peripheral neurons are derived from the placodes, with glia coming from the neural crest. In the olfactory system, the classical view has been that the olfactory placode forms all olfactory sensory neurons. In contrast, we recently showed that cranial neural crest cells are the primary source of microvillus sensory neurons in zebrafish (Saxena et al., 2013). Now, we expand upon those findings by utilizing the novel imaging technique lattice light sheet (LLS), a new derivative of Bessel plane SR-SIM (Gao et al., 2012). We have applied LLS to image zebrafish embryos for the first time and in doing so have revealed new details of olfactory organ formation at the highest spatiotemporal resolution to date in live vertebrates. Cell tracking analysis has produced a detailed timeline of olfactory organogenesis and traced the dual origins of migrating and
differentiating neurons in real time. We have compiled both putatively stochastic and directed cell migration movements to build a four-dimensional ‘blueprint’ for assembly of a major sensory organ and created a new experimental system in which to study genetic perturbation at high-resolution in live vertebrates. In sum, LLS imaging allows us to answer cell migration/differentiation and cell-cell interaction questions that previously could not be addressed and to build a comprehensive developmental overview of the olfactory circuit and surrounding nasal cavity. [Funding Sources: National Institutes of Health; HHMI Janelia Farm Visiting Scientist Program]

Program Abstract #50
On the verge of neuronal replacement—cellular pliancy and rethinking age-old dogma
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My lab is interested in understanding how proliferation and differentiation are coordinated in the developing retina and how those processes become uncoupled in retinoblastoma. Recently, we made a discovery that has fundamentally altered our understanding of the molecular and cellular mechanisms of retinal development and may also have a major impact on efforts to restore vision in some patients with retinal degeneration. We discovered that individual retinoblastoma tumor cells express multiple developmental programs simultaneously. This occurs through deregulation of the epigenetic programs that are directly or indirectly regulated by the RB1 protein. To explore this finding further, we developed a novel experimental system to quantify the epigenetic reprogramming of individual retinal tumor cells by using 4 factors (Oct4, Klf4, Sox2, and Myc) and somatic cell nuclear transfer. We discovered that the epigenetic barriers to reprogramming dramatically differ across retinal cell types, and they are developmental stage-specific. Moreover, we have used a 3-dimensional culture system to show for the first time that mouse iPSCs can form the optic cup and differentiated retinas. One of the most exciting results from these experiments is that our iPSC lines derived from retinal neurons are more efficient at differentiating into laminated retinas than fibroblast derived iPSCs or embryonic stem cells. Our current research is focused on elucidating the underlying molecular mechanisms that contribute to this epigenetic memory of retinal derived iPSCs. It may also provide crucial preclinical data on the use of retinal-derived iPSCs for future clinical trials of photoreceptor-replacement therapy to treat retinal degeneration.

Program Abstract #51
Investigating the role of the Hippo pathway member Nf2 in inner cell mass specification.
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The first two lineages to be specified during mouse development are the trophectoderm (TE), which will go on to form the placenta, and the inner cell mass (ICM), which will generate the fetal body itself. Proper segregation of these two cell types requires precise spatial control of the Hippo signaling pathway and its downstream transcriptional activator, Yap. In outside cells of the embryo, Yap is nuclear-localized and can induce expression of the TE-specifying transcription factor Cdx2. In contrast, in inside cells, the Hippo pathway kinases Lats1/2 and the adaptor protein Amot induce phosphorylation and cytoplasmic retention of Yap, preventing Cdx2 expression. The factors acting upstream of Lats1/2, Amot and Yap in this context are not well understood. Here we investigate the role of the upstream Hippo pathway member Nf2 in TE/ICM segregation. We show that injection of dominant negative Nf2 mRNA causes Yap mislocalization and ectopic Cdx2 expression in inside cells of the embryo, effects that can be rescued by overexpression of Lats2 kinase. Moreover, zygotic Nf2 mutant blastocysts have mild defects in Yap localization and Cdx2 expression, but these become much more severe upon removal of both maternal and zygotic Nf2. In addition to Cdx2 misexpression, maternal/zygotic Nf2 mutants fail to establish a pluripotent ICM and form excess TE, resulting in peri-implantation lethality. Finally, to better understand the relationship between Nf2, Lats1/2 and Amot in inside cells of the embryo, we test the ability of various Nf2 mutants defective in Lats1/2 or Amot binding to rescue maternal/zygotic mutant embryos. Together these data demonstrate that Nf2 is absolutely required for Hippo signaling and ICM specification in the preimplantation embryo and provide insights into its molecular role in an in vivo context.

Program Abstract #52
The sinus venosus contributes extensively to the coronary blood vasculature through VEGF-C stimulated angiogenesis
Kristy Red-Horse
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Identifying coronary artery progenitors and understanding their developmental pathways could inspire new regenerative treatments for coronary artery disease. Multiple sources of coronary vessels have been proposed—the sinus venosus, endocardium, and proepicardium—but the relative contributions of each and how they populate the heart is not known. We created an ApjCreER mouse line as a lineage tracing tool and used it to map sinus venosus-derived coronary vessels onto the entire heart. The results showed a striking regionalization to coronary development. ApjCreER traced vessels contributed to a large number of coronary arteries, capillaries, and veins on the dorsal and lateral sides of the heart while untraced vessels, likely derived from the endocardium, predominated the ventral face and ventricular septum. VEGF-C was expressed in the epicardium where sinus venosus-derived coronary vessel sprouts first emerge, and their growth was dramatically inhibited in VEGF-C-deficient hearts. Thus, complementary migration routes unite to give rise to the coronary vasculature with sinus venous sprouting requiring VEGF-C.
Program Abstract #53

The bHLH transcription factor Twist1a functions to limit cardiomyocyte production
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The production of the correct number of cardiomyocytes is crucial for the proper formation of the heart, as too few or too many cells can lead to a dysfunctional organ. Elucidating the network of transcription factors that dictate how many cardiomyocytes are produced is therefore a high priority for understanding the mechanisms underlying cardiac development and disease. Here, we show that the bHLH transcription factor gene twist1a limits cardiomyocyte production in the zebrafish embryo. Reducing the function of twist1a with anti-twist1a morpholinos (MOs) results in an increased number of cells expressing myocardial markers. This phenotype is a striking contrast to the severe reduction in cardiomyocyte production observed in mutant embryos lacking the bHLH transcription factor gene hand2. This opposition is reminiscent of the genetic antagonism observed between Twist1 and Hand2 in the mouse limb, leading us to consider whether genetic interaction between twist1a and hand2 influences cardiomyocyte production. Interestingly, twist1a knockdown does not increase the production of differentiated cardiomyocytes in hand2 null mutants, suggesting that Twist1a activity depends upon the presence of Hand2. To evaluate the cellular mechanism of Twist1a function, we investigated the origins of the excess cardiomyocytes found in twist1a morphants – do these cells result from increased specification or increased proliferation? EdU incorporation assays suggest that twist1a limits the proliferative capacity of cardiac progenitors, and twist1a morphants exhibit an expanded progenitor population, marked by nkx2.5 and hand2. Together, our data indicate a previously unrecognized role for twist1a in inhibiting the production of cardiomyocytes from the cardiac progenitor pool and suggest that this function is dependent on, and potentially upstream of, hand2 function.

Program Abstract #54

Molecular control of blood vessel morphogenesis
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Cardiovascular function depends on patent, continuous blood vessel formation by endothelial cells (ECs). Blood vessel development initiates during ‘vasculogenesis’ via the aggregation of ECs into linear aggregates, which then organize into tubes with central lumens allowing blood flow. However, the mechanisms underlying vascular ‘tubulogenesis’ in vivo are only beginning to be unraveled. We recently showed that a novel GTPase-interacting protein called Rasip1 is required for the formation of continuous blood vessel lumens and for blood vessel development. Rasip1 null embryos exhibit disrupted localization of junctional complexes, loss of proper cell polarity, and loss of adhesion of ECs to extracellular matrix (ECM). In vitro studies also showed that depletion of either Rasip1 in cultured ECs lead to failed tubulogenesis. Here, we demonstrate that Rasip1 is also required for postnatal angiogenesis in models of angiogenic blood vessel growth, including retinal blood vessel growth and subcutaneous blood vessel angiogenesis (Directed In Vivo Angiogenesis Assay). We find, however, it is dispensable for adult blood vessel lumen maintenance, making it a promising therapeutic target. Current studies are aimed at identifying the cellular and molecular processes regulated by Rasip1, in addition to its control of other GTPases including Cdc42, Rac1, RhoA and Rap1, that together coordinate to control functional blood vessel formation in the early embryo.

Program Abstract #55

Co-repressor degradation dynamics set the pace for lateral root development
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Development requires cells to send and receive information, often in the form of small molecules or peptides. An emerging theme in biology is that information can be encoded in the dynamics of signaling, not just in the presence or absence of signaling molecules. In plants, response to the hormone auxin is a useful tool for studying the role that signal dynamics play in development. Auxin triggers degradation of an Aux/IAA repressor protein, which activates transcription of a large number of genes. Auxin facilitates Aux/IAA degradation by mediating interaction between them and auxin receptors called TIR1/AFBs. Each component in the auxin signaling pathway belongs to a gene family, and different family members play distinct roles throughout development. Our previous work revealed that Aux/IAAs exhibit a range of degradation rates. However, it remains an open question whether this range of dynamics contributes to the specificity of auxin responses in plants. We hypothesized that these IAA degradation rates act as biological pacemakers to coordinate developmental processes. To test this, we characterized the effect of altered rates of Aux/IAA degradation on lateral root initiation. Our data demonstrate that Aux/IAA degradation dynamics determine the rate of progression through lateral root development in Arabidopsis.

Program Abstract #56

New models for studying skeletal development and repair
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Although the rib cage is a highly conserved feature of the vertebrate skeleton and the enclosed rib cage (bone and cartilage portions
analytics with a sternum) is a defining feature of birds and tetrapods, little is known regarding the developmental mechanisms responsible for rib outgrowth or proximal-distal specification. Using genetically modified mice in the Sonic hedgehog (Shh) and programmed cell death pathway, we have been able to determine that rib development is critically influenced by the number of progenitors present at very early somite stages. Similar to other skeletal structures that extend from the axis (i.e. the limb), we propose a model whereby the proximal-distal compartments of the rib skeleton is specified early and full development involves a lateral expansion of these progenitors. To determine if development of the skeleton is recapitulated during repair, we have investigated repair in both the distal (cartilage) and proximal (bone) compartments after a large-scale segmental injury in adult mice. We find that ribs can repair completely, that repair is dependent on the presence of surrounding connective tissues, and that while some aspects of repair recapitulate development, some events may be unique to an injury situation.

Program Abstract #57
Ttc26 is essential for cilia microtubule architecture, developmental patterning and is mutated in the classic hop-sterile mouse
Scott Weatherbee, Daisy Xin, Lewie Zeng, Kasey Christopher, Yong Kong
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The primary cilium is critical for a variety of developmental processes, including left-right specification, organogenesis, and tissue patterning. Several studies suggest that hundreds of proteins are found in the cilium and thus could be necessary for cilia function, however many of these proteins have not been studied in model systems. We took a forward genetics approach to identify a new model for studying cilia defects. hop-sterile (hop) is a recessive mouse mutant that spontaneously arose at the MRC Radiobiology Unit at Harwell, UK in 1967. hop homozygotes are viable but display preaxial polydactyly on all four limbs, and move their hindlimbs with a hopping gait. In males, most sperm lack tails, resulting in sterility, but in the few flagella that form, the axonemal microtubule organization is disrupted [1]. This constellation of defects suggested that the hop mutation affected a key cilia gene. Using exome capture and high-throughput sequencing, we identified a nonsense mutation in the Tetratricopeptide repeat protein 26 (Ttc26) gene, which produces a prematurely truncated protein in hop mutants. Ttc26 regulates cilia length and morphology in C. elegans, trypanosomes and zebrafish. In hop mice, Ttc26 is no longer found in mutant cilia despite normal localization of other cilia markers. And, unlike many cilia mutants, cilia numbers are not reduced in hop tissues. However, we observed abnormal microtubule architecture in hop primary cilia suggesting impaired ciliogenesis. Ttc26 normally interacts with IFT88, a protein involved in ciliary transport, and we found that this interaction is lost in hop. This result likely explains why Ttc26 is absent from mutant cilia, and suggests that Ttc26 must be moved into cilia to regulate normal microtubule organization. Altogether, the hop mutant is a unique mouse model that provides insight into how Ttc26 affects cilia formation and function.

Program Abstract #58
Facial neuron migration mechanisms
Vicky Prince, Sarah Wanner, Crystal Love, Anastasia Beiriger
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We seek to understand the migration of neurons from initial birth-place to final destination. All CNS neurons undergo radial migration, but subsets of neurons also migrate tangentially within the neuroepithelial plane. To better understand tangential migration we study facial branchiomotor neurons (FBMNs), a distinct and identifiable population of motor neurons that undergoes a tangential migration conserved from mammals to fishes. We use zebrafish embryos for these studies as they offer experimental advantages including powerful imaging. Our studies have demonstrated a critical role for axon tracts in zebrafish FBMN migration. Laser ablation experiments established that the first FBMN to migrate out of hindbrain rhombomere (r) 4, the “pioneer” FBMN, and importantly its trailing axon, are necessary for proper migration of FBMNs. Ongoing imaging experiments are investigating both the early origins and the ultimate fate of pioneer neurons. A second axon tract, the medial longitudinal fasciculus (MLF) also plays an important role in FBMN migration. Although initial FBMN migration precedes extension of the MLF into r5, surgical manipulation demonstrated that subsequent FBMN migration is MLF dependent. The FBMN/MLF interaction involves adhesion molecule Cdh2: morpholino depletion of Cdh2 stalls FBMN migration in r4/r5, with neurons coalescing at the midline. Intriguingly, disrupting chemokine or semaphorin signaling partially blocks FBMN migration, but the inappropriate midline location of FBMNs in Cdh2-deficient hindbrains is “rescued” by disrupting chemokine or semaphorin signaling. Finally, we are exploring the role of transcriptional repressor Rest in FBMN migration. Our data indicate that Rest functions to suppress FBMN maturation, which in turn is necessary for neuronal migration to occur. In the absence of Rest, or its nuclear translocator Prickle1b, FBMNs mature precociously at both morphological and molecular levels, leading to impaired neuronal migration.

Program Abstract #59
A Rho-GAP regulates two GTPases during C. elegans embryonic morphogenesis.
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C. elegans embryos undergo very dynamic movements during morphogenesis. Like all the steps of morphogenesis, ventral epidermal enclosure involves rearrangement of the actin cytoskeleton. Recent work in our lab has shown that embryonic epidermal enclosure is regulated by axonal guidance signals known to control neuronal development. In C. elegans, these signals, UNC-
cdc25a times the cell cycle to facilitate mesodermal cell differentiation during posterior body formation in zebrafish

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During the early stages of vertebrate body formation, an embryo grows from the head to the tail to generate the anterior-posterior (AP) axis. Throughout embryo elongation, the hind end of the embryo maintains a population of undifferentiated cells that provides the raw cellular material for posterior body formation and a population of differentiating cells that will contribute directly to the growing AP axis. As elongation is completed, the numbers of undifferentiated cells are winnowed to depletion. To prevent premature depletion of the undifferentiated cells, both the numbers and the rate of release must be kept in balance until the AP axis is finished. At the onset of this study, we hypothesized that careful control of proliferation in the undifferentiated cells is critical for the completion of the vertebrate body. Through single cell fate-mapping experiments, we have found that proliferation in the undifferentiated cells can be divided into an early-rapid phase followed by a late-quiescent phase. The two phases of proliferation are matched by expression of the mitotic phosphatase, cdc25a. In undifferentiated cells, cdc25a is expressed during the early-rapid phase followed by depletion of cdc25a during the late-quiescent phase. Further by misexpressing cdc25a during the late-quiescent phase, we have found that if expression of cdc25a and proliferation are not restricted the ability of undifferentiated cells to proceed through mesodermal differentiation and contribute to the AP axis is blocked. CMB is supported by an F32 from the NIGMS.
Movement of tasiR-ARF creates a gradient of accumulation across the leaf that dissipates towards the ventral/bottom side. This gradient is interpreted into a sharp on-off domain of expression of the target and ventral cell fate determinant ARF3. Likewise, miR166 traffics from its site of biogenesis in the bottom epidermal layer to restrict expression of dorsal determinants, the HD-ZIPIII transcription factors, to the upper domain of the leaf. Mathematical modelling predicts that the opposing small RNA gradients generated through mobility are uniquely suited to create sharply defined and robust patterning boundaries. This model is supported by our recent observations, which will be presented. Our observations indicate that leaves are partitioned into dorsal and ventral domains via a novel patterning mechanism involving opposing gradients of small RNAs that act as mobile morphogen-like instructive signals.

Program Abstract #63
Interplay between cell proliferation, recruitment, and the control of organ size
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Much of the evidence that organs "know" the size they need to attain comes from developmental studies in the Drosophila wing disc. The wing disc contains different cell types, but only cells that express the selector gene vestigial (vg) contribute to the adult wing blade. Thus, the size of the adult wing is determined by the size of the vg-expressing population, rather than by the size of the entire wing disc. In addition to acquiring vg expression by descent, cells can be "recruited" to express vg through a propagation signal received from Vg-expressing neighbors. How the interplay between cell proliferation and recruitment affects disc growth and wing size has not been investigated. We quantified in vivo the expansion of the Vg pattern to determine the relative contributions of cell proliferation and recruitment to organ size. Interestingly, we observed that proliferation and recruitment are temporally decoupled: cell proliferation is the dominant process of growth during most of larval life, while recruitment only becomes dominant during the last day of larval development. In addition, when cells that already express Vg are inhibited from proliferating, cells in the recruiting pool (i.e., cells that neighbor the vg domain) proliferate faster than they normally do. This compensatory growth approximately rescues the normal size of the wing disc by increasing the number of "potentially recruitable" cells, but it cannot rescue normal size of the Vg domain or the adult wing. Using mathematical modeling, we develop the idea that, if the rate of recruitment is proportional to the rate of cell proliferation, then the timescale of the period when proliferation dominates disc growth is self-limiting: the faster the rate of cell proliferation, the shorter the time when proliferation is dominant. This model might explain why wing size is widely robust to changes in rates of cell proliferation, and provides insights into the developmental mechanisms that control organ size.

Program Abstract #64
NanoTube-mediated communication between stem cells and the niche cells in Drosophila testis
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It is well established that many adult stem cells reside in a specialized microenvironment, or niche. Whereas the stem cell niche provides essential signals for stem cell identity and proliferation, it is not well known how the niche signal is limited to stem cells, while other cells in proximity are not misspecified by the niche signaling. Here we show that in Drosophila testis, germline stem cells (GSCs) extend microtubule-based nanotube into the hub cells, the major component of the stem cell niche. We further demonstrate that the nanotube mediates Dpp signaling between the hub and GSCs. We propose that confinement of the signaling to a spatially-limited surface area of the nanotube allows privileged perception of niche-derived signals by stem cells.

Program Abstract #65
Quantitative proteomics screen identifies aldh1l1 as a potential regulator of positional memory
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The zebrafish caudal fin has the remarkable ability to regenerate following amputation. Interestingly, amputations of varying length all complete regeneration at the same time. This ability relies on the remaining wounded tissue recalling its precise location relative to the original fin and adjusting regenerative outgrowth accordingly, an attribute known as positional memory. To identify potential novel regulators of positional memory in the caudal fin, we performed label-free protein quantification using nano liquid chromatography – tandem mass spectrometry (LC-MS/MS) on protein isolated from dorsal-proximal and ventral-distal regions of 20 AB WT fish (1:1 gender ratio). We quantified 1708 individual proteins, with only 16 genes showing at least 4-fold expression level differences in proximal vs. distal regions. Aldehyde dehydrogenase 1 family, member L1 (aldh1l1), an enzyme involved in folate metabolism, is one hit that showed increased proximal expression. Alterations in expression have been validated at the transcript level using qRT-PCR and at the protein level by Western blot. If aldh1l1 is involved in positional memory, knocking protein levels down in a proximal amputation (to mimic distal levels) should result in decreased regenerative proliferation and decreased outgrowth. To test this, we injected antisense morpholino oligonucleotides targeting aldh1l1 into the dorsal half of a proximally amputated fin and found ~50% inhibition in regenerative growth, as compared to the uninjected ventral side. These findings suggest aldh1l1 may help regulate positional memory in the caudal fin. Furthermore, our screen has identified several additional proteins differentially expressed in proximal and distal regions of the fin, which are also strong candidates for genes regulating positional memory.
Program Abstract #66

**MicroRNA regulation of neural precursor maintenance and specification**

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During development neural precursors first divide symmetrically to produce new precursors, thereby expanding the precursor population. Subsequently, neural precursors begin to divide asymmetrically, generating first neurons and later glia while at the same time maintaining new precursor production. Near the end of development most precursors stop dividing and terminally differentiate. Changes in the balance of symmetric proliferative divisions, asymmetric self-renewing divisions and terminal divisions have important consequences for brain size and developmental disorders. Vertebrate neural precursor cells have apicobasal polarity and how this polarity is modulated influences whether precursors undergo symmetric or asymmetric divisions. For example, precursors that maintain high levels of Par proteins associated with apical membrane persist in a self-renewing state whereas loss of apical Par proteins correlates with differentiation. Distribution of apical Par proteins during symmetric proliferative and asymmetric divisions has been extensively investigated but the mechanisms that downregulate Par proteins to promote terminal differentiation are not known. Using zebrafish as a model system, we found that microRNA *miR-219* negatively regulates *pard3* and *prkci* mRNAs, which encode apical Par proteins, via single target sites within their 3’ UTRs. Blocking the ability of *miR-219* to bind these target sites prevented downregulation of apical proteins in the developing spinal cord, maintained precursors in a proliferative state and interfered with production of late-born neurons and glia. Notably, loss of *miR-219* function and maintenance of apical Par proteins increases the duration and level of Sonic Hedgehog signaling in the spinal cord. Therefore, modulation of apical Par protein levels by *miR-219* may tune the responsiveness of neural precursors to Sonic Hedgehog, determining whether they proliferate or differentiate.

Program Abstract #67

**Hippo signaling activation in apolar blastomeres occurs prior to inner positioning in the mouse embryo**

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In the mouse embryo, asymmetric divisions during the 8-16 cell division generate two cell types, polar and apolar cells, which are allocated to outer and inner positions, respectively. This outer/inner configuration is the first sign of formation of the first two cell lineages, trophectoderm (TE) and inner cell mass (ICM). Outer polar cells become TE and give rise to the placenta, while inner apolar cells become ICM and give rise to the embryo proper and yolk sac. Here, we analyzed the frequency of asymmetric divisions during the 8-16 cell division and assessed the relationships between cell polarity, cell and nuclear position, and Hippo signaling activation, the pathway that initiates lineage-specific gene expression in 16-cell embryos. While the frequency of asymmetric divisions varied in each embryo, we found that more than six blastomeres divided asymmetrically in most embryos. Interestingly, many apolar cells in 16-cell embryos were located at outer positions while only one or two apolar cells were located at inner positions. Live imaging analysis showed that outer apolar cells were internalized by surrounding polar cells. Using isolated 8-cell blastomeres, we carefully analyzed the internalization process of apolar cells and found evidence of higher cortical tension in apolar cells than in polar cells. Lastly, we found that apolar cells activate Hippo signaling prior to taking inner positions. Our results suggest that polar and apolar cells have intrinsic differences that establish outer/inner configuration and differentially regulate Hippo signaling to activate lineage-specific gene expression programs.

Program Abstract #68

**Engineering proteins for visualization and control of signaling networks in vivo**

Klaus Hahn  
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The spatio-temporal dynamics of signaling determines how specific proteins can produce a host of different, sometimes opposing responses. This talk will describe approaches to visualize and manipulate signaling networks in living cells and animals, including broadly applicable methods to control proteins with light. The role of Rho family GTPase ‘circuits’ in regulating motility will be probed using engineered allosteric switches to control the activity and interactions of kinases, and new biosensors for Rho family molecules, some based on novel designs, will be described as part of these studies.

Program Abstract #69

**CHOOSE DEVELOPMENT: An undergraduate research fellowship program bringing SDB members together to diversify the population of young developmental biologists**

Graciela Unguez1, Karen Bennett1, Ida Chow2  
1New Mexico State Univ., USA; 2Society for Developmental Biology, USA; 3Univ. of Missouri, Columbia/John Hopkins, USA

*Choose Development* is a 3-year (2013-2016) NSF-funded project through which the Society for Developmental Biology (SDB) is working to increase the number of undergraduate students from underrepresented groups going into academic fields related to developmental biology. This pioneering program emphasizes professional development and research training under long-term and continuing supervision by expert developmental biology research faculty. In 2013, 11 SDB Fellows were selected from a national applicant pool and matched to research laboratories of SDB faculty members across the country
active learning sessions. Utilizing pre-course and post-course tests as an instrument with which to measure learning during the 
prerecorded by the professor. The lectures and the modules covered identical content. All students were required to participate in the 
session. Students were offered the options of attending live, traditional lectures by the professor and/or viewing online modules 
suggest that pairing active learning sessions with traditional lectures may lead to superior performance compared to pairing with 
traditional lectures. While active learning has been well established to improve student performance in natural science courses, we 
with decreased learning. A multivariable analysis confirmed a statistically significant increase in learning with attendance of 
attendance of the traditional lectures was associated with increased learning, whereas greater use of online lectures was associated 
course, we observed a significant increase in scores from a mean of 30% to a mean of 72%. We additionally found that greater 
into my introductory level Genetics course during Spring 2013 and 2014 semesters. Here the challenge is that not everyone can 
make it to office hours; moreover, 3 lecture hours per week is not enough time to pursue interesting discussions related to course 
content. Students used the online forum to post and answer questions following lecture, continue classroom discussions, generate 

Program Abstract #70
PULSE: Partnership for Undergraduate Life Science Education
Jo Anne Powell-Coffman
Iowa State Univ., U.S.A.
Years of teaching and learning scholarship describe the great need for large-scale changes in college-level science instruction, as 
summarized in the Vision and Change (V&C) report. However, there are recognized barriers to implementing the Vision and 
Change recommendations. The Partnership for Undergraduate Life Science Education (PULSE) program is a collaborative effort 
that was initiated by NSF, NIH, and HHMI, and the PULSE community includes ~40 Leadership Fellows, with records of 
administrative experience at diverse institutions, including community colleges, liberal arts colleges, regional comprehensive, and 
research-intensive universities. The mission of PULSE is to catalyze Dept.-level changes that enable transformation of 
undergraduate life science instruction and learning into scholarly, student-centered, outcome-focused programs that emphasize critical 
and the processes of science. Towards this, PULSE builds structures for peer support and review that promote Dept.-level 
pedagogical change and innovation in undergraduate life science education. PULSE also seeks to support the local scholars that 
drive change in their home Dept.s. These structures and products include the PULSE website (www.pulsecommunity.org), the 
PULSE Vision and Change Rubrics (1), regional networks and workshops, and the PULSE Ambassadors program.
(1) Aguirre KM, Balsier TC, Jack T, Marley KE, Miller KG, Osgood MP, Pape-Lindstrom PA, and Romano SL. PULSE Vision and 
Change Rubrics. 2013. CBE Life Sciences 12:579

Program Abstract #71
Flipping back the "flipped classroom": improved learning with traditional lectures?
Kiran Musunuru, Zarin Machanda, William Anderson
Harvard Univ., USA
The study of biochemistry and metabolism is achieving a renewed prominence in life sciences education in light of a recent joint 
initiative of the American Association of Medical Colleges (AAMC) and the Howard Hughes Medical Institute (HHMI) to define 
the natural science competencies needed by future physicians. To fill a gap in the introductory life sciences curriculum at our 
college, we launched a new semester-long course covering the principles of biochemistry and metabolism and attuned to the 
AAMC-HHMI recommendations. Our course included both a weekly lecture component and a weekly case-based active learning 
session. Students were offered the options of attending live, traditional lectures by the professor and/or viewing online modules 
prerecorded by the professor. The lectures and the modules covered identical content. All students were required to participate in the 
active learning sessions. Utilizing pre-course and post-course tests as an instrument with which to measure learning during the 
course, we observed a significant increase in scores from a mean of 30% to a mean of 72%. We additionally found that greater 
attendance of the traditional lectures was associated with increased learning, whereas greater use of online lectures was associated 
with decreased learning. A multivariable analysis confirmed a statistically significant increase in learning with attendance of 
traditional lectures. While active learning has been well established to improve student performance in natural science courses, we 
suggest that pairing active learning sessions with traditional lectures may lead to superior performance compared to pairing with 
online modules, i.e., the "flipped classroom" model.

Program Abstract #72
Sharing is caring: Use of online community forums to extend classroom and laboratory discussions
Mary K. Montgomery
Macalester College, USA
Labs that accompany undergraduate developmental biology courses invariably involve students coming back after hours to make 
observations and follow up on experiments. In Fall 2012 I began utilizing the online discussion forum available through Moodle (an 
open-source learning platform) so that (1) I could verify that students were returning to the lab, and (2) they could share their 
observations and results with each other, in addition to simply writing them up in a lab notebook. The real-time postings reflected 
the enthusiasm and excitement of working with live embryos and served to prompt other students to return to the lab, but to space 
their visits so that they collectively provided a more consistent and comprehensive timeline of observations. I employed the same 
approach in Fall 2013 to same effect. Encouraged by the positive experience with Moodle I began incorporating the use of Piazza 
into my introductory level Genetics course during Spring 2013 and 2014 semesters. Here the challenge is that not everyone can 
make it to office hours; moreover, 3 lecture hours per week is not enough time to pursue interesting discussions related to course 
content. Students used the online forum to post and answer questions following lecture, continue classroom discussions, generate
new discussions, and share links to helpful educational resources they found on their own. Advantages of Piazza over Moodle include the ability to post anonymously and to collectively edit answers to posted questions. Students can answer each other’s questions and the teaching assistants and myself endorse or edit the responses. Response times averaged 3 hrs. Not surprisingly, there was a correlation between the quantity and quality of postings with overall course performance. The use of such online platforms can support the learning that goes on during official class and lab time, encourage peer learning and a sense of intellectual community, and make more efficient use of our time as instructors.

Program Abstract #73
Teaching developmental biology content in non-developmental biology courses
Edward Freeman
St. John Fisher College, USA

Biology curricula vary based on the volume of information that encompasses modern Biology, as well as, faculty expertise and teaching responsibilities versus research requirements. To ensure students are exposed to Developmental Biology (DB) themes I have threaded DB content through courses I teach in a Biology program at a liberal arts college. Freshman level Zoology provides a platform to introduce gametogenesis, fertilization, cleavage, cellular determination & differentiation and early embryonic development. This coverage provides a foundation for more detailed DB content consideration in advanced, elective courses such as Endocrinology, Reproductive Biology and Comparative Anatomy. Endocrinology and Reproductive Biology students in my courses consider organ development prior to organ function. For example, Endocrinology students study pituitary gland development and cellular differentiation in the anterior pituitary gland. Reproductive Biology students consider primordial germ cell migration, the chemical cues that target early germ cells, and the somatic cells of the genital ridge in the context of building a functional gonad. This inclusion of DB content in advanced courses allows students to fully appreciate adult form which is essential as they begin to understand organ regulation and function. In Comparative Anatomy a major theme is the influence of the environment on genotype to produce adult structure. Students are exposed to DB content with lecture coverage of organ development, in-lecture student led discussions on DB papers that consider the impact of the environment, and the completion of an assignment to read the primary literature and write a short review paper.

Data concerning student (freshman - senior) perceptions of the coverage of DB in non-Developmental Biology courses will be presented. In addition, examples of lecture content, in-class paper review topics, exam questions, and student generated review article topics will be provided.

Program Abstract #74
Developing a reverse genetic screen in Caenorhabditis elegans as a model for collaborative, authentic research in college science courses
Jennifer Tenlen¹, Daihong Chen¹, Jennifer Heppert², Andrew Lumpe¹, Derek Wood¹
¹Seattle Pacific Univ., USA; ²Univ. of North Carolina-Chapel Hill, USA

Student participation in authentic, original research has been demonstrated to enhance their engagement in biology courses, and to improve their processing and critical thinking skills. Integration of faculty research into lab courses allows many more students the opportunity to engage in authentic research than could be accommodated by individual faculty labs. The Genomics Education National Initiative (GENI; http://www.geni-science.org/) was created to provide tools for collaborative, authentic research in the classroom. We have implemented a quarter-long research project in which students use RNA interference (RNAi) in a sensitized genetic background to identify novel genes involved in endoderm specification in the nematode Caenorhabditis elegans. The long-term goal of this project is to develop a web-based platform to disseminate protocols and reagents that would allow interested instructors to incorporate this research project into their curriculum. Students’ findings would be shared with other collaborators, ultimately leading toward publication of the research in peer-reviewed journals. To assess the impact of this research on students’ feelings of efficacy in research, and engagement in the course, we administered pre- and post-research surveys. At the end of the quarter, students reported a statistically significant improvement in their ability to design experiments and collect data to address a specific question. Based on students’ feedback, we are further refining the protocols and developing follow-up projects to allow students to continue functional analysis of promising candidates genes that arise from the screen. This research was supported by NSF TUES DUE-1322848.

Program Abstract #75
Use of heart rate monitors to assess student engagement in lecture
Diana Darnell, Paul Krieg
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Some years ago, Bligh carried out a series of classroom lecture studies showing that student heart rates decreased over the course of a 80-minute lecture (Bligh 1972, 1998, 2000). The decline in heart rate was interpreted as a measure of decreasing arousal, which is one component of engagement. In addition, Bligh reported a single event where a question from a student resulted in elevation of heart rate in observers. It appears that these studies are unique in using a physiological readout as a measure of student engagement or attention in the classroom. We plan to repeat and extend this study using current technology in interactive lecture, allowing more students to participate, more data to be collected, and additional analysis of the impact of interruptions and activities.
We propose that lightweight heart rate monitors with wireless connectivity (Mio Alpha; resembling fat wrist watches), may be used to measure student engagement in the classroom setting. In preliminary studies using 14 monitors worn by medical student volunteers, we have replicated Bligh's observations in classes with varying degrees of interactivity. We observed, 1) a decrease in average heart rate across a 50-minute lecture class for some lectures, but an increase in average heart rate for others, and 2) a temporary increase in heart rate in response to some student questions. In addition, we have observed that pair-share sessions (an interactive learning method) resulted in elevated average heart rate. Results will be presented from analysis of heart rate data from more than 20 lecture classes. The long-term objective is to determine if heart rate can be used to assess the relative effectiveness of different interactive and non-interactive teaching methods to increase student engagement.


Program Abstract #76
Teaching Evo-Devo using case studies
Eric P. Ingersoll
Penn State Abington, USA

Teaching an upper division course in Evo-Devo can be challenging since no comprehensive text is available that contains sufficient content for a semester-long course. Rather than relying on a single text, with contributions and assistance from colleagues, I have developed an Evo-Devo course that uses journal articles as the main source of information for the course. This allows students to not only see the effects of the developmental and genetic alterations that result in evolutionary change, but also the processes by which these changes were discovered. The course begins with several weeks of background on developmental biology and laboratory techniques necessary for understanding the papers. Following this, the case study portion of the class begins. Each week students are assigned one review article and one primary research article. Part of the week is spent learning the background for the paper, such as information on the specific developmental process being studied and the state of the field prior to the study. At least one class is spent as a class discussion where students are asked to provide information from the paper, draw conclusions from the experimental data, and critically evaluate the study. Students are graded both on examinations and class participation. Student feedback on the course has been generally positive and most students seem to enjoy the case study and discussion-based format.

Program Abstract #77
Evo-Devo of the Pharyngeal Apparatus of Bluegill and Pumpkinseed Sunfish
Kelly Grant, Greg Andraso, Nikhil Kanthala
Gannon Univ., US

We are interested in the evolutionary divergence of the pharyngeal apparatus in two species of sunfish, bluegills (Lepomis macrochirus) and pumpkinseeds (Lepomis gibbosus). Specifically we investigated growth patterns and are beginning to explore differences in gene expression, cell migration and proliferation.

The pharyngeal apparatus is formed by the pharyngeal arches and functions as a second set of jaws to process prey. The prey-processing occurs when the dorsally-located pharyngobranchials 2, 3, 4 (pb) press down on ceratobranchial 5 (cb5). In adult sunfish, the morphologies of cb5 and pb3 are quite different between the two species. Bluegills have a delicate bone structure with sharp, cardiform teeth; pumpkinseeds have larger, robust bones with molariform teeth used for crushing prey.

We began investigating these bones in young fish; we noticed that the shapes of the bones and the teeth are quite similar in fish less than 30mm; this had not been previously reported. The shape of the bones starts to diverge around 45mm, suggesting a target size for investigating differences in proliferation and gene expression.

While cb5 has a similar shape in the young of both species, it is larger in the smallest pumpkinseed that we have recorded. We plan to investigate the size of the initial cartilages in embryos. If we observe a difference in cartilage size, we would like to address whether the difference is autonomous to the neural crest cells that form cb5 or whether the species-specific patterning comes from the pharyngeal endoderm.

This is a good project for undergrads. The fish are readily found in Lake Erie, students get to collect the fish which eliminates the need for prolonged husbandry, and it is a noncompetitive niche.

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

Program Abstract #78
Frog wrangling in Woods Hole at the NXR
Cristy Salanga1, Esther Pearl1, Robert Grainger1,2, Marko Horb1
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The National Xenopus Resource (NXR) is a recently established stock center located at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts. The goals of the NXR are to produce, acquire, maintain, and distribute mutant, inbred, and transgenic X. laevis and X. tropicalis lines and to provide research training as well as promote intellectual interchange through hosting mini-courses and workshops. Current workshops include Bioinformatics and Advanced Imaging, with future courses aimed to teach other diverse skills including TALEN and CRISPR/Cas genome editing and transgenesis techniques, among others. The NXR also offers a research hotel service, where visiting researchers can come to the MBL and perform short-term projects using NXR resources. Last, the NXR offers custom genome editing and transgenesis resource services; the NXR will clone, inject, and breed TALEN, CRISPR, or transgenic animals for individual researchers. The NXR is working to foster cooperation and collaboration within the
Stem Cell regulation and signaling pathways in the planarian

Program Abstract #81
Identification of HECT E3 ubiquitin ligases involved in regulating regeneration in planarians
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E3 ubiquitin ligases constitute a large family of enzymes that modify specific proteins by catalyzing the attachment of ubiquitin. Ubiquitylation serves as a vital regulatory mechanism for protein function, subcellular localization, or longevity. In spite of their importance in cell physiology, the exact roles and targets of most E3 ligases are poorly understood. We are utilizing the abundant stem cell population and regenerative capacity of the planarian Schmidtea mediterranea as a tool to analyze the function of E3 ubiquitin ligases and identify potential target proteins in vivo. E3s are classified into two major groups: HECT and RING E3s. As a first step, we have examined the function of HECT E3s, which directly bind and ubiquitylate their targets. Using bioinformatic searches we identified 17 HECT E3s in the planarian genome. Whole-mount in situ hybridization experiments showed that HECT genes were expressed in diverse tissues. Interestingly, 11/17 HECT genes were enriched in the stem cell population (neoblasts) or their progeny. To investigate the function of all HECT E3s, we inhibited their expression using RNAi and found that orthologs of herc (sherc-1), hual1, wwp1, and trip12 had roles in neoblast regulation. Specifically, sherc-1 was enriched in neoblast progeny and played a modest role in regulating stem cell division. By contrast, hual1 RNAi led to a significant expansion of the neoblast population and death by lysis. Furthermore, we observed that hual1, wwp1, and trip12 were required for tissue regeneration. Our experiments showed that wwp1 was necessary for intestinal tissue homeostasis and uncovered an unexpected role of trip12 in posterior tissue specification. Our data provide insights into HECT E3 ligase roles in tissue regeneration and suggest that planarians might be a useful model to identify protein targets of E3 ubiquitin ligases involved in stem cell-based tissue regeneration.
[Supported by CIRM RN2-00940-1 and NSF IOS-1350302 to R.M.Z.]
Program Abstract #82
SUMO Proteins Regulate Cell Fate Decisions During Tissue Maintenance and Regeneration in Planarians
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Small ubiquitin-like modifier (SUMO) proteins can alter functions of other proteins through post-translational modification. SUMOylation has been implicated in a wide range of processes associated with the regulation of cellular fate decisions during cell cycle progression and apoptosis. However, it remains poorly understood as to how SUMO proteins regulate physiological events such as tissue renewal and regeneration that require a fine balance between cell division and cell death. The planarian flatworm Schmidtea mediterranea is an emerging model organism that allows analysis of stem cell-mediated tissue turnover and regeneration in the context of the whole body. The genome of the S. mediterranea displays evolutionary conservation of SUMO proteins, which enable us to study critical aspects of SUMOylation during adult tissue maintenance and repair. Downregulation through RNA-interference of three genes from the canonical SUMO pathway, Ubc9, SUMO1 and SUMO protease, resulted in regional defects characterized by loss of posterior tissue and abnormal regeneration of the tail. These localized abnormalities were associated with a severe reduction in stem cell proliferation and unbalanced cell death in specific areas of the body, indicating that SUMO proteins have regional functions that regulate tissue maintenance and regeneration. We are further investigating epigenetic and transcriptomic changes to reveal mechanisms and cells involved in this novel cellular regulatory process. Together, our data reveal intriguing roles for SUMO proteins in the control of cell fate decisions during homeostasis and regeneration.
We acknowledge support from the Univ. of California, Merced; Jane Villas funds, Hellman Fellows and UC Cancer Research Coordinating Committee.

Program Abstract #83
A SoxB1 gene is required for nervous system regeneration and function in the planarian Schmidtea mediterranea
Kelly G. Ross, Katrina L. Cable, Kerilyn C. Omuro, Carlo G. Quintanilla, Ricardo M. Zayas
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SoxB1 transcription factors play important roles in maintaining pluripotency and nervous system development. In the vertebrate CNS, SoxB1 group genes are key determinants in neural stem cell self-renewal and differentiation. In mice, knockout of a SoxB1 member (Sox1) causes epileptic seizures. Additionally, human genome-wide association studies show a correlation between Sox1 mutations and epilepsy. However, the downstream targets of Sox1, especially in the post-embryonic CNS, are largely unknown. The planarian Schmidtea mediterranea is an excellent model organism to study transcription factors in the adult CNS due to their amazing ability to replace tissues from a pluripotent stem cell population. To identify SoxB1 homologs, we performed BLAST searches using human SoxB1 protein sequences and found two SoxB1 genes in the planarian genome. In situ hybridization to a SoxB1 group member (SoxB1b) showed expression in the nervous system and stem cell progeny. Remarkably, when we investigated the function of SoxB1b using RNAi we observed that both uninjured or regenerating SoxB1b(RNAi) animals exhibited seizure-like behaviors. Furthermore, regenerate developed smaller brains and reduced sensory structures compared to controls. We hypothesized that SoxB1b is the ortholog of Sox1 in planarians and that it controls transcription of genes required for differentiation of neurons involved in sensory and neuromuscular functions. To test our hypothesis, we isolated RNA samples from control and SoxB1b RNAi planarians and performed mRNA sequencing. Our analysis revealed a significant downregulation of genes essential for CNS biology, such as ion channels and neurotransmitter receptors. We are currently validating candidate target genes of SoxB1b and investigating their role in CNS regeneration and planarian seizure-like behaviors, which should provide insights into how abnormal SoxB1b function causes seizures in humans. Supported by CIRM RN2-00940-1 to R.M.Z.

Program Abstract #84
Acute serotonergic signaling regulates planarian anterior - posterior patterning
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The planarian flatworm Dugesia japonica rapidly regenerates a complete body plan from small excised fragments. During the regenerative process, patterning is instructed by “positional control genes” expressed in planarian muscle [1]. Given the role of Ca2+ signaling in both regeneration [2] and muscle function, we were interested in studying the role of a planarian voltage-operated Ca2+ channel isoform (Dj-Ca1B), RNAi of which uniquely causes both motility (impairment) and anterior-posterior patterning defects (creating an environment permissive for anteriorization). Interestingly, recent data show knockdown of tryptophan hydroxylase (TPH), the rate limiting enzyme in serotonin (5-HT) synthesis, also increases anteriorization outcomes [3], yielding a hypothesis that defects in Ca1B cause serotonergic impairments. Consistent with this hypothesis, application of exogenous 5-HT rapidly rescued the Ca1B RNAi motility defect, which returned upon washout of 5-HT from the media. Like Ca1B RNAi, various serotonergic antagonists also rescued regenerative polarity to yield two-headed regenerants. Application of exogenous 5HT, or serotonergic agonists, to regenerating worms resulted in the opposite effect. Such treatments inhibited head regeneration (producing no-headed or cycloptic worms). As Ca1B RNAi did not decrease TPH mRNA levels, these data suggest serotonergic neurons are present in Ca1B RNAi worms, but their functionality is impaired causing anterior-posterior patterning defects.
Program Abstract #85
Stem cell survival and activation in the *C. elegans* gonadal primordium

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One of the most conserved aspects of stem cell biology is the close association of stem cells with niche cells – supporting cells that regulate stem cell activity and protect these cells from external influences. To understand how a niche functions, we have studied the germ line stem cell niche in the nematode worm, *C. elegans*. In this simple genetic system, two germ line stem cells and two somatic gonadal precursor cells (necells) interact predictably during embryogenesis to form the gonadal primordium. Niche formation is critical for the survival of the germ line stem cells, and ultimately controls these cells’ switch between quiescent and activated states. We have taken two approaches to investigate how this process occurs. First, using laser ablations we are examining the fate of germ cells that do not make contact with somatic gonadal precursor cells. Germ cell survival depends on early contact with the necells and we are working to uncover the molecular mechanisms underlying this phenotype. Second, previous research from our lab has implicated adhesion molecules including E-cadherin in gonadal primordium formation. We are taking advantage of the recent advances in genome editing technology to conditionally disrupt E-cadherin function in the niche and assay the role it plays in stem cell activation. The results will show if these molecules have signaling as well as adhesive roles in the germ line stem cell niche. Niche function bears directly on many aspects of human health, including regenerative medicine and cancer biology, and the high degree of conservation previously observed between worms and mammals indicates our results will be applicable to problems in human stem cell biology.

Program Abstract #86
Taranis buffers regenerating tissue against aberrant cell fate changes caused by the endogenous wound response

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During regeneration, proper patterning and cell fate must be re-established to generate a functional organ or appendage. How a regenerating organ restores proper cell fate and pattern after tissue damage is still an open question. We use a genetic ablation system in wing imaginal discs of *Drosophila melanogaster* to screen for and characterize dominant modifiers of regeneration. We found that alleles of *taranis* (*tara*), which encodes a putative Trithorax Group protein and a member of the TRIP-Br/SERTAD family, resulted in regenerated wings with posterior-to-anterior fate transformations when heterozygous. This regulation of cell fate was regeneration-specific, because *tara* function was dispensable for posterior cell fate during normal wing development. We will present evidence that *tara* regulates the expression of *engrailed* (*en*) during regeneration to ensure maintenance of posterior identity. When *Tara* levels were reduced, *en* expression was initially elevated then subsequently silenced in a *polyhomeotic*-dependent manner. We hypothesized that the endogenous wound response, which requires JNK signaling, may play a role in these fate transformations. Indeed, we found that elevation of JNK signaling above levels normally experienced during regeneration resulted in similar posterior-to-anterior fate transformations caused by mis-regulation of *en* expression. Therefore, we propose that *tara* functions as a buffer against JNK-mediated changes in cell fate during regeneration to ensure that the regenerated imaginal disc can produce a properly formed and patterned adult wing. With this work, we present evidence for a novel regeneration-specific mechanism that buffers regenerating tissue against cell fate changes induced by the wound response.

Funding for this work was provided by the Roy J. Carver Charitable Trust

Program Abstract #87
Growth coordination during regeneration in *Drosophila* occurs through nitric oxide synthase regulation of steroid hormone signaling

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The proportional growth of distinct organs cannot solely be explained through tissue-autonomous or systemic mechanisms of regulation. Experimental observations suggest that inter-organ communication is necessary for coordinating growth between different tissues. Here we demonstrate the first description of an inter-organ growth coordination pathway. In *Drosophila* larvae, imaginal disc damage activates a regenerative checkpoint that both extends the regenerative period of development and slows the growth of undamaged imaginal discs. This reduction of growth may function to coordinate regenerative growth with the growth of undamaged tissues. While *Drosophila* insulin-like peptide 8 (Dilp8) has been identified as an important regulator of the regenerative checkpoint and growth coordination, the pathways through which *Dilp8* mediates growth control are still unknown. We show *dilp8* to be necessary and sufficient for reducing growth of undamaged imaginal discs during regeneration. Further, we demonstrate that the steroid hormone ecdysone is necessary for imaginal disc growth, and show that *Dilp8* reduces growth through inhibiting production of the ecdysone.

Nitric oxide synthase (NOS) has been shown to regulate developmental growth of the imaginal discs; however, the mechanism of growth regulation by NOS has not been characterized. We determine that NOS function in the endocrine organ is necessary for growth coordination during regeneration, and show that increasing NOS in the endocrine organ reduces expression of critical ecdysone biosynthesis genes, and that NOS in the prothoracic gland is necessary for growth coordination during the regenerative

checkpoints. Based on this work, we propose that Dilp8 produced in regenerating tissues signals through NOS in the endocrine organ to decrease ec dysone signaling, thus coordinating regenerative growth with developmental growth. Funded by March of Dimes 5-FY12-60, NIGMS RO1-GM099803 to A.H. and NIH T32 GM008136 to J.J.

Program Abstract #88
Morphological Mechanisms of CNS Regeneration in Hemichordates
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Solitary hemichordates, also known as acorn worms, are marine invertebrate deuterostomes and sister group to the echinoderms. Some species of hemichordates have been shown to have a remarkable capacity to regenerate all body structures, including their Central Nervous System (CNS). All acorn worms have a tripartite body system, with an anterior proboscis, middle collar region, and a posterior trunk. The collar region houses a dorsal neural tube that has been shown to develop in a similar fashion to the vertebrate neural tube. Ectoderm invaginates and rolls up to form a tube-like structure that is positioned dorsal to the dorsal vessel. While studies have detailed the development of the neural tube, it is not yet known how and when the neural tube regenerates. In order to gain a clear understanding of when internal structures are elaborated, we have made serial sections of sequential stages of collar regeneration in the hemichordate, Ptychodera flava. The time series will show when the neural tube regenerates and whether the dorsal vessel is present prior to CNS regeneration. In vertebrates, signaling molecules emanate from the notochord and dorsal vessel to partially pattern the neural tube and peripheral nervous system, respectively. It is not yet known if signaling molecules from the dorsal vessel induce formation of the hemichordate neural tube. Once the timing of neural tube regeneration is known, future studies can be aimed at identifying and inhibiting expression of potential signaling molecules that may be directing CNS regeneration in hemichordates. I would like to thank the National Science Foundation Graduate Research Fellowship and BEACON, A Center for the Study of Evolution in Action, for funding.

Program Abstract #89
Role of Abcg2 During Mouse Embryonic Stem Cell Differentiation.
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1U.S. EPA, Office of Research and Development, NHEERL, ISTD, SBB, USA; 2NIEHS, Office of Policy Planning and Evaluation, USA

Abcg2 is a multidrug resistance ATP-binding cassette (ABC) transporter whose activity may be considered a hallmark of stem cell plasticity. The role of Abcg2 during early embryogenesis, however, is unclear. Studies done with mouse embryonic stem cells (mESCs) suggest that inhibition of Abcg2 by either chemical means or RNAi may reduce colony expansion of pluripotent cells and promote differentiation. Our group also observed a correlation between xenobiotics that alter mESC differentiation and chemicals that induce expression of ABCGg2 in primary human hepatocytes (ToxCast assay). On the other hand, knockout mice display a normal phenotype unless challenged by certain toxicants. Chemical inhibitors of Abcg2, such as K0143 and fumitremorgin C (FTC) were utilized to evaluate the role of Abcg2 in cultured J1 cells. MK571 and verapamil, inhibitors of Abcc1 and Abcb1, respectively, were utilized as well. Based on cellular accumulation of the Abcg2 substrate, pheophorbide A, maximum inhibition of Abcg2 was observed at approximately 1µm K0143 or FTC. Neither verapamil nor MK571 had a notable effect on Abcg2 function. While growth and differentiation of mESC could be affected by FTC, K0143, and verapamil at concentrations greater than 10 µm, lower concentrations did not influence cell proliferation or alter the expression of selected pluripotency/lineage markers on the appropriate days of culture (culture days 2, 4, 6, 9). These transcript markers included Pou5f1, Nanog, T, Gsc, Bmp4, Nes, Ncam1, Des, Ttr, Gata4, Abcg2, Myf4, and Myl7. Hence, Abcg2 does not appear to play a fundamental role in mESC differentiation based on observations in an adherent culture system. The possibility that Abcg2 plays a role in protecting embryonic cells from damage caused by xenobiotics, or that regulation of this efflux transporter by exogenous compounds may affect early differentiation is being considered. (Funded by U.S. EPA. This research does not reflect EPA policy.)

Program Abstract #90
TRIP6 regulates neural stem cell maintenance in the postnatal mammalian subventricular zone
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Postnatal neurogenesis persists throughout life in the subventricular zone (SVZ)-olfactory bulb pathway in mammals. Extrinsic or intrinsic factors have been revealed to regulate neural stem cell (NSC) properties and neurogenesis. Thyroid hormone receptor interacting protein 6 (TRIP6) belongs to zyxin family of LIM proteins, which have been shown to interact with various proteins to mediate cellular functions. However, the role of TRIP6 in NSCs is still unknown. By performing double immunofluorescence staining, we found that TRIP6 was expressed by Sox2-positive NSCs in postnatal mouse forebrains. To study the function of TRIP6 in NSCs, we performed overexpression and knockdown experiments with neurospheres derived from postnatal day 7 SVZ. We found that TRIP6 increased the sphere size and proliferation of NSCs, but inhibited their differentiation. To further investigate the mechanism of TRIP6 in NSCs, we performed Luciferase reporter assay and found that TRIP6 activated Notch signaling, a pathway required for NSC self-renewal. Our data suggest that TRIP6 regulates NSC maintenance and it may be a new marker for NSCs.
Program Abstract #91
Role of Wnt signaling in the adult mouse spinal cord
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The spinal cord functions to connect the brain and the peripheral nervous system as a conduit for motor and sensory information. Although development of the mammalian spinal cord has been well studied, much less is known about the signals that regulate the proliferation and maintenance of the various cell types in the adult spinal cord. Wnt signaling pathway has been shown to regulate the development and maintenance of the central nervous system at the level of neural stem cells. As a self-renewal and proliferation signal in various stem and progenitor cell compartments, Wnt signaling is a good candidate for regulating cell proliferation and maintenance in the adult spinal cord.

In the adult spinal cord, ependymal cells, astrocytes, and oligodendrocyte lineage cells together account for the vast majority of the proliferating cells. Compared to the oligodendrocyte lineage cells, signals that control ependymal cells and astrocytes turnover are less well understood. Even though both populations proliferate slowly, ependymal cells are the main cell population in the intact spinal cord with in vitro neural stem cell properties, while astrocytes are essential for normal neurological function and are the main source of new cells after spinal cord injury. We found that Wnt signaling is active in the ependymal cells surrounding the central canal, as well as white matter astrocytes. Lineage tracing experiments using a common Wnt pathway target gene, Axin2, suggest that a subset of the Axin2-expressing ependymal cells and astrocytes proliferate and generate more of themselves over a period of at least six months. These results suggest that Wnt signaling is involved in regulating turnover of these two cell populations. Future experiments will offer exciting insights into how cell proliferation is regulated in the adult spinal cord in homeostasis, and may facilitate the development of therapeutic strategies for spinal cord injury and neurodegenerative diseases.

Program Abstract #92
In vivo generation and survival of supernumerary hair cells by proliferation and transdifferentiation of Lgr5+ supporting cells by β-catenin and Atoh1 expression in postnatal mouse cochleae
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During the development of the inner ear, Wnt signaling plays a role in the formation of the otic placode and Wnt-responsive cells remain present in the postnatal quiescent cochlea and vestibular sensory organs. Lgr5, a Wnt target gene, has been proposed to be a stem cell marker in various tissues, and is expressed in the inner ear during the first week of postnatal development. We hypothesize that these Lgr5+ supporting cells might represent the progenitor/stem cells of the postnatal mouse inner ear with potential for hair cell regeneration, and that Wnt/β-catenin overexpression in these cells might result in transdifferentiation of these supporting cells to hair cells for regenerative purposes. We previously showed that Lgr5+ supporting cells expressing a stable form of β-catenin proliferated and formed many isolated replication foci throughout the cochlea that persisted up until P21, but failed to transdifferentiate into hair cells. Therefore, we hypothesized that transdifferentiation of the β-catenin-overexpressing proliferating Lgr5+ supporting cells may require the ectopic expression of the hair cell-specific transcription factor, Atoh1. We found that co-induction of β-catenin and Atoh1 had a synergistic effect on proliferation and transdifferentiation. Numerous ectopic hair cells were found surrounding the endogenous inner hair cells, ~1,400 cells per cochlea, nearly 10-fold higher than in previous in vivo models. These new ectopic hair cells underwent incomplete maturation, and were innervated despite incomplete alignment of pre- and postsynaptic markers. Surprisingly, we found that only a subset of Lgr5+ supporting cells medial to the inner hair cells were responsible for the observed synergy. Thus, we concluded that β-catenin and Atoh1 have synergistic effects on proliferation and transdifferentiation of a subset of neonatal cochlear Lgr5+ supporting cells. These results provide a basis for combinatorial therapeutics for hearing restoration.

Program Abstract #93
CDX2 controls cell differentiation and stem cells in the mouse intestinal epithelium
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Intestinal stem cells (ISCs) renew the adult gut epithelium and are a useful model to study stem cell biology and control of cell differentiation. Self-renewal of ISCs and their differentiation requires control of genes by transcription factors (TFs) and chromatin through mechanisms that are not well understood. One pool of ISCs, marked by Lgr5, resides at the base of the crypts, cycles rapidly, and can be isolated using Lgr5-GFP knock-in mice. To gauge the scope of gene expression changes during cell differentiation, we performed global RNA-seq analysis of Lgr5+ ISCs and purified enterocytes, the most abundant differentiated cell type. This analysis identified genes enriched in ISCs or enterocytes and others expressed equally in both populations. One gene highly expressed in both, Cdx2, encodes a homeodomain TF that maintains active chromatin at thousands of enterocyte genes. Abundant Cdx2 expression in ISCs suggested a previously unappreciated role in this cell. We observed replication defects of Cdx2-/- mouse ISCs and an inability to produce mature cell types. These findings reveal functions for CDX2 in ISCs distinct from its requirement to activate enterocyte genes for nutrient digestion and absorption. To identify direct CDX2 targets, we performed ChIP-seq, and RNA-seq of wild-type and Cdx2-/- ISCs. Underscoring the role for CDX2 in cell proliferation, analysis of genes dysregulated in Cdx2-/- ISCs revealed significant aberration in genes related to the cell cycle. We detected CDX2 binding near 10% of dysregulated genes, including genes involved in cell signaling, which may account for the observed defects. 80% of genes bound
by CDX2 in ISCs are also bound in differentiated intestinal cells, suggesting that CDX2 binding in ISCs may anticipate functions in mature cells. Our study reveals a broad role for CDX2 in maintaining stem cell properties and functions and identifies specific transcriptional targets that may mediate canonical stem cell behaviors.

Program Abstract #94
Identification of Clonal Skeletal Progenitor Cells
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The process hematopoiesis occurs in a complex system within the bone marrow known as the Hematopoietic Stem Cell niche. Identifying the skeletal progenitor cells that give rise to the various cellular components of the niche is important towards understanding the maintenance of homeostasis within the bone and the hematopoietic system. Here, we test our hypothesis that clonal skeletal progenitors (CSPs) can be identified by the presence of cell surface markers CD105, AlphaV, and absence of markers CD45, Thy1, 6C3, and Tie2. Cells were harvested from GFP+ adult C57BL mouse bones, sorted by FACS and their capacity to form ectopic bone was assayed by transplanting the cells under the kidney capsule of GFP- mice. We observed by pentachrome staining that only a CD45- CD105+Tie2-AlphaV+Thy1-6C3- population formed ectopic bones with robust marrow. In comparison, CD105+CD45-Tie2-AlphaV+Thy1+6C3- and CD45-Tie2-AlphaV+CD105+Thy1-6C3+ populations only formed ectopic bones without bone marrow. This data suggests that CSPs are characterized by cell surface marker profile CD45- CD105+Tie2-AlphaV+Thy1-6C3-, are multipotent, and capable of reconstituting the HSC niche. Identification of the multipotent CSP will allow for the characterization of poorly understood cell types within the niche, that can give insight into treating various disorders linked to niche dysregulation and bone disorders.

Program Abstract #95
Wnt activation in nail epithelium couples nail growth to digit regeneration
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The digit tip of mammalian, including humans and rodents, can regenerate after amputation, like those of amphibians. Digit tip regeneration occurs in association with nail regrowth, and neither the nail nor the digit regenerate when the amputation is proximal to the nail. It is unknown why regeneration of nail and digit always coupled and why regeneration capacity is limited to the area associated with the nail. We found that nail stem cells (NSCs) reside in the proximal nail matrix and that the mechanisms governing NSC differentiation are coupled directly with their ability to orchestrate digit regeneration. Early nail progenitors undergo Wnt-dependent differentiation into the nail. After amputation, this Wnt activation is required for nail regeneration and also for attracting nerves that promote mesenchymal blastema growth, leading to the regeneration of the digit. Amputations proximal to the Wnt-active nail progenitors result in failure to regenerate the nail or digit. Nevertheless, β-catenin stabilization in the NSC region induced their regeneration. These results establish a link between NSC differentiation and digit regeneration, and suggest that NSCs may have the potential to contribute to the development of novel treatments for amputees.

Program Abstract #96
Quantification and cell-type identification of miR-133b expression after spinal cord injury in Xenopus laevis
Minus Helton, Mackenzie Hamilton, Kurt M. Gibbs
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Unlike mammals, Xenopus laevis tadpoles can regenerate their spinal cords after injury, but are unable to do so as adults. The factors underlying this developmental loss of regenerative capacity have yet to be uncovered. MicroRNAs (miRNAs) are small (approximately 19-22 nucleotides), non-coding RNA molecules that play vital roles in cellular growth, development, and maintenance in embryos and adult organisms. miRNAs simultaneously regulate the expression of many genes, and have shown functional conservation from round worms to humans. In our previous work, we have identified hindbrain neurons that are capable of regenerating their axons after spinal cord injury in tadpoles, but fail to regenerate in the adult frog. As miR-133b has been implicated in axon regeneration in other organisms, we questioned if it was also involved with axon regeneration in X. laevis as well. Using quantitative real-time polymerase chain reaction (qRT-PCR), we assayed the expression of miR-133b in the hindbrain of tadpoles and adult frogs, after spinal cord injury. Our results show that tadpoles increase miR-133b expression for at least one week after injury, whereas adults show a decreased expression. In situ hybridization revealed that mir-133b expression is neuron specific and expressed by regeneration competent neurons in the hindbrain. These data suggest that the developmental decline in axon regeneration could be mediated by miRNAs, and that restoring specific miRNA expression in adults might improve functional recovery after spinal cord injury. This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 5P20GM103436-13.

Program Abstract #97
A Role for Leptin as a Modulator of Nutrition-Dependent Limb Regeneration Rate in Xenopus Laevis
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Regeneration of structures is locally controlled by injury activated gene networks, but the rate and quality of regeneration can
Program Abstract #98

DNA methylation dynamics regulate the formation of a regenerative wound epithelium during Axolotl limb regeneration
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The formation of a blastema during regeneration in the axolotl limb involves drastic changes in the behavior and function of cells at the site of injury. The earliest events, such as the formation of the wound epithelium and subsequent apical epidermal cap, involve in vivo dedifferentiation and are controlled by signaling from the nerve. As a possible mechanism for the significant changes in gene expression patterns of cells involved in regeneration, we have investigated epigenetic modifications to the genome. We report a modulation of the expression DNMT3a, a de novo DNA methyltransferase, within the first 72 hours post injury that is nerve-signaling dependent. Treatment of simple lateral wounds with decitabine, a DNMT inhibitor, results in a shift in cellular behavior towards a regenerative response. Furthermore, decitabine treated wounds are able to participate in regeneration while untreated wounds inhibit the regenerative response. Elucidation of the specific epigenetic modifications that mediate cellular dedifferentiation may lead to insights for initiating a regenerative response in organisms that lack this ability. Research funded by a Defense Advanced Research Projects Agency (DARPA) subcontract from Tulane Univ. (TUL 519-05/06), a US Army Multidisciplinary Univ. Research Initiative (MURI) subcontract from Tulane Univ. (TUL 589-09/10), and the National Science Foundation through its support of the Ambystoma Genetic Stock Center at the Univ. of Kentucky, Lexington.

Program Abstract #99

Molecular regulation of spinal cord injury in axolotl
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Salamanders are among the few organisms fortunate in their ability to regenerate many body parts in response to major injury, including re-growing a fully functional spinal cord following injury or tail amputation. Despite the obvious medical potential, our detailed understanding of the underlying molecular pathways remains largely in its infancy. We have developed a spinal cord injury model in axolotls and have used in vivo labeling and imaging of glial cells and neurons to begin to characterize the response of radial glial cells and axons to injury. In addition we have carried out transcriptional profiling using axolotl microarrays to identify key molecular pathways that are differentially regulated after injury at different time-points during the regeneration of this complex tissue. We have identified the early response genes c-Fos and c-Jun; that can heterodimerize and form the AP1 transcription factor to play essential roles in the early response to injury. Here we will present data from axolotl in vivo studies where the normal dynamics of expression of these early response genes are altered after injury. This data establishes that these genes are essential for initiating a proliferative response in the neural stem cells of the spinal cord after injury and that in addition that specific temporal and spatial control of expression of these genes is necessary for promoting functional axonal regeneration.

Program Abstract #100

Nanog expression is required to initiate lineage specification in axolotl embryos
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In mammalian embryos pluripotency arises in the inner cell mass (ICM), and is dependent on the transcription factor Nanog. All vertebrate tissue is derived from one of three somatic germ layers, ectoderm, endoderm, or mesoderm. Recently we reported that the mechanisms governing mesoderm specification and pluripotency are conserved from axolotls to mammals, and we showed that the master regulator of mammalian pluripotency, Nanog, is conserved in axolotls. We also showed that primordial germ cells can be induced directly from the animal cap of axolotl embryos by combined FGF and BMP-4 signalling. Together, these results indicate that the pluripotent ground state from which mammalian embryos initiate development is conserved in axolotls, and that axolotl embryos can be used as an experimental model to understand the lineage specification of pluripotent cells. The first fate choice
made by pluripotent cells in axolotl animal caps is the decision to commit to either the germ line or somatic tissues. This binary decision is governed by a molecular switch involving Nanog and extracellular signals. Nodal signals act through Nanog to promote generalized somatic development, inducing a state from which specification to the individual somatic lineages proceeds; FGF/BMP signalling, on the other hand, acts through Nanog to specify the germ lineage.

**Program Abstract #101**

**Influence of Schwann cells on neuromast regeneration in zebrafish larvae.**

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The zebrafish posterior lateral line (PLL) is a mechanosensory system that develops embryonically as 7-8 neuromasts distributed along the trunk and tail connected by a continuous stripe of interneuromastic cells (INC). Neuromasts are formed by hair cells, support cells, and mantle cells. The INCs remain in a dormant state due to the presence of underlying Schwann cells and become activated during larval development to form intercalary neuromasts. However, there are still no studies that describe if these cells participate in regeneration after the total elimination of a neuromast. To eliminate a single neuromast we used electroablation (2 locally applied current pulses of 8uA/2s) in transgenic larvae expressing fluorescent proteins in PLL components. The injury results in total disconnection of the INCs, Schwann cells and PLL nerve. By in vivo imaging we observed that the INC cells fill the gap between 0-13 hours post damage (hpd). Until 36hpd INC cells are still accumulating at the injury site. At 48-72hpd the regenerated neuromast recovers all cell types, a process that is completed in 58.25%±4.4% of the larvae. Importantly, when we inhibit Schwann cell survival by blocking ErbB signaling, we obtain 100% regeneration. Finally, using genetic mosaics, we show that a single interneuromastic cell can form all cell types in a regenerated neuromast and that, during this process, it transiently expresses the sox2 gene. We provide new data aimed at understanding the nature of the INCs in terms of their developmental and regenerative potential and show that this potential is controlled by a factor produced by Schwann cells.

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**Program Abstract #102**

**Determination of Cell Death in Polypterus senegalus Limb Regeneration**

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The Senegal bichir (*Polypterus senegalus*) possesses the particularity of being an amphibious fish (this means, it has lungs instead of gills, and in order to obtain oxygen, it needs to come to the surface and breathe air) with the ability to regenerate missing fins with a remarkable accuracy. Fin regeneration in the Senegal bichir begins with the development of a blastema, and the tissue differentiation process in the fin after being lost, takes approximately 9 days to start being noticeable, while full regeneration of the fin takes around five weeks to be completed. Through studies performed on catsharks (*Scyliorhinus canicula*) it has been observed that, during the embryonic stage of such organism, the development of the pectoral and pelvic fins involves the manifestation of cell death during the tissue differentiation stage. Likewise, this process has been determined during the embryonic development of tetrapods. This was discovered by fluorescent staining (Acridine Orange under Abram's method) on the catshark, and by performing the TUNEL assay on the tetrapods. The aim of this paper is to carry out Abram's method staining using Acridine Orange and the TUNEL assay during the regeneration stage of the Senegal bichir fins. Bearing in mind the evolutionary similarities between the catshark and the Senegale bichir, we have motives to suggest the manifestation of cell death during the process of cellular differentiation in the regeneration of the Senegal bichir fins.

**KEYWORDS:** apoptosis, regeneration, differentiation, development, fins.

**Program Abstract #103**

**Spatial and temporal processes involved in the naturally occurring sarcomere disassembly during electric organ regeneration in the teleost Sternopygus macrurus**

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**New Mexico State Univ., USA**

Exciting new findings are increasing our understanding of the protein composition of sarcomeres—the highly ordered contractile units of striated muscle—and the common pathways of sarcomere assembly in both heart and skeletal muscle. Although disruptions in these pathways are implicated in muscle diseases, events involved in the disassembly of sarcomeres remain largely unknown. The electric fish *S. macrurus* has the unique ability to regenerate all tail tissues after amputation including skeletal muscle and the muscle-derived cells of the electric organ, i.e., electrocytes. During regeneration, some muscle fibers fuse and disassemble their sarcomeres to convert into electrocytes. To study changes in the spatial and temporal expression of protein components of distinct sarcomeric regions during the muscle-to-electrocyte conversion, we have begun to use immunofluorescence and ultrastructural approaches. Specifically, we are using markers of the Z-disk (EA53 antibody), inter-Z-disk region (E398P antibody), A-bands (MF20 antibody), and I-bands (phalloidin stain) to describe this naturally occurring process of sarcomere disassembly during electrocyte differentiation. Our preliminary data suggest a pattern of sarcomere breakdown characterized by dissociation of the I-band followed by loss of A-band then Z-disks structures while the inter-Z-disk components are conserved. This pattern of sarcomere disassembly differs from a dedifferentiation process predicted by a current model of sarcomere formation during muscle differentiation (Ehler and Gautel, *Adv. Exp. Med. Biol.*, 2008).

32
Program Abstract #104

Conditional gene trap mutant reveals a critical role of tbx5a in cardiac regeneration
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For testing the roles of critical developmental regulators during regeneration, tools allowing for spatial and temporal control of the mutations are required. Our laboratory is carrying out an insertional mutagenesis screen using conditional gene traps in zebrafish. One of the mutants, t 18, has an insertional mutation in the tbx5a gene essential for heart and upper limb development. Tbx5a protein in the tbx5a<sup>lox58</sup> mutant is truncated after first 48 amino acids. Homozygotes display a fully penetrant linear heart phenotype. To enable for conditional manipulations, a mutagenic cassette (MC) in the gene trap is flanked by oppositely oriented modified site-specific recombinase sites, FRT-10, FRT+10, lox71 and lox66, recognized by their corresponding recombinases, Flp and Cre.

Microinjection of Flp<sup>+</sup> mRNA into 1-cell stage zebrafish embryos mediated an intramolecular recombination between modified FRT sites resulting in a stable inversion of the MC and turning an insertional mutation off. The established Flp-reverted allele, tbx5a<sup>pos58</sup>, is homozygous viable and does not display overt phenotypes. For the heart-specific control of the tbx5a in a spatial and temporal manner, we generated a cardiomyocyte-specific CreER<sup>+</sup> driver, mnt2a:CreER<sup>+</sup>. The tbx5a<sup>pos58</sup>;mnt2a:CreER<sup>+</sup> and tbx5a<sup>pos58</sup>;mnt2a:CreER<sup>+</sup> double transgenic lines were established. An administration of 4-HT resulted in a cardiac-specific re-mutation of the tbx5a locus in both embryonic and adult hearts. We have found that tbx5a is essential for cardiac regeneration. At 30 days post amputation, 5/7 of tbx5a<sup>pos58</sup>;mnt2a:CreER<sup>+</sup> adults raised from embryos treated with 4-HT at 2dpf showed impaired heart regeneration. When tbx5a<sup>pos58</sup>;mnt2a:CreER<sup>+</sup> adults were treated with 4-HT, they displayed severe cardiac regenerative defects. Thus, tbx5a is a key regulator of cardiac regeneration in zebrafish. We acknowledge funding provided by the NIH R01 grant HD061749.

Program Abstract #105

Cells and signals coordinating bone regeneration in zebrafish
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In contrast to mammals, zebrafish bone can completely regenerate. Investigating this remarkable innate ability could provide insights towards developing regenerative therapies to treat bone injuries. Bone regeneration following zebrafish fin amputation is mediated by de-differentiated, lineage-restricted osteoblasts that subsequently produce replacement bone. However, the molecular mechanisms that control the osteoblast regenerative program from its initiation through re-ossification are poorly understood. We find that, upon fin amputation, a pool of proliferative twist2+/Runx2+ pre-osteoblasts is generated by an epithelial to mesenchymal transformation (EMT) of mature osteoblasts. Localized Wnt/b-catenin signaling maintains these Runx2+ progenitors near the distal tip of the regenerating fin mesenchyme. Bone Morphogenetic Protein (BMP) signaling promotes osteoblast re-differentiation by activating sp7 expression. Further, BMP constrains Wnt activity by inducing Dickkopf-related Wnt antagonists. As such, opposing activities of Wnt and BMP coordinate the simultaneous demand for growth and differentiation during bone regeneration. While BMP-dependent osteoblast maturation is lineage intrinsic, the Wnt ligands needed to sustain pre-osteoblasts are produced by neighboring non-osteoblast cells that comprise a “stem cell” niche. These studies provide a conceptual framework to understand innate bone repair and regeneration mechanisms and rationally design therapies to enhance osteogenesis and fracture healing.

Program Abstract #106

The novel gene lix1 plays a role in zebrafish development and regeneration
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Limb Expression 1 (lix1) is a functionally novel gene linked to Retinoic Acid (RA) signaling and the Fat Hippo Warts (FHW) tumor suppression pathway. The FHW pathway is known to play an important role in normal development as well as tumor suppression. Though the FHW pathway has been increasingly studied, little is known about the pathway’s upstream regulation. Recent research with the lix1 homolog lowfat (lft) showed Lft is able to bind Fat in the pathway to affect wing growth and leg regeneration. In vertebrates, the novel gene lix1 may be an upstream regulator of the FHW pathway, and thus control normal growth, development, and regeneration.

lix1 was first discovered in a differential display screen for genes up regulated by RA. Retinoic acid signaling is involved in many developmental pathways as well as regeneration, and can up-regulate lix1 expression in developing chick limb buds. This suggests lix1 may be a downstream target of RA. We have begun to explore the role of lix1 during vertebrate development and regeneration using zebrafish.

Here I show that zebrafish embryos express lix1 early in development, especially in midline structures. These include the fore- and midbrain as well as the floorplate, notochord, and neural tube. Fat is also expressed in the midline in developing embryos, suggesting an interaction between Lix1 and Fat. lix1 knockdown results in improper development, especially in regard to tissue organization and growth, reminiscent of the role of lft in FHW growth control. Changes in RA levels also affect lix1 expression, with increased RA levels leading to lix1 up-regulation. RA is most likely upstream of lix1, and may partially regulate the FHW pathway. In addition to being expressed during development, lix1 is expressed in regenerating fins almost immediately after...
amputation. I am further exploring the interactions of lix1 with FHW and RA signaling using mutant and transgen fish, and knockdown of FHW genes for epistasis assays.

Program Abstract #107
Glucagon signaling is essential for beta cell neogenesis and transdifferentiation from alpha cells in zebrafish
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The inter-conversion of cell lineages via transdifferentiation is an adaptive mode of tissue regeneration, and an appealing therapeutic target. However, its clinical exploitation is contingent upon discovery of contextual regulators of cell fate acquisition and maintenance. In murine models of diabetes, glucagon-secreting alpha cells transdifferentiate into insulin-expressing beta cells following targeted beta cell depletion, regenerating the form and function of the pancreatic islet. However, the molecular mediators of this mode of regeneration are unknown. Here, using lineage-tracing assays in a transgenic zebrafish model of beta cell ablation, we demonstrate conserved plasticity of alpha cells during islet regeneration. In addition, we show that glucagon expression is upregulated after injury, and through gene knockdown and rescue approaches, that it is necessary for alpha to beta cell fate switching. Importantly, while beta cell neogenesis was stimulated by glucose, alpha to beta cell conversion was not, suggesting that transdifferentiation is not mediated by glucagon control of hepatic glucose production. Overall, this study supports the hypothesis that alpha cells are a deep endogenous reservoir of potential new beta cells. It further reveals that glucagon plays an important role in maintaining endocrine cell homeostasis through feedback mechanisms that govern cell fate stability. This work was supported by grants to Ryan M. Anderson from Juvenile Diabetes Research Foundation (JDRF) and the Grace W. Showalter Foundation.

Program Abstract #108
Radial glia are required for specific cell lineages and the maintenance of axonal anatomy during spinal cord development in zebrafish.
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Development of the nervous system rests on the precise regulation of neural stem cell proliferation and progenitor cell patterning. Radial glia are embryonic neural stem cells that not only give rise to both neuronal and glial progeny, but also serve to mediate axon pathfinding and neural regeneration in vertebrates. In a previous mutagenesis study, we identified a class of zebrafish genes required for radial glial development in the spinal cord. Mutation of kif11, a kinesin motor, resulted in mitotically arrested radial glial cells, reduced cell survival, and reductions in oligodendroglia, secondary interneurons, and secondary motorneuron populations. To determine if loss of radial glial division and reductions in neuronal and glial populations are related, we have generated two novel genetic tools to directly test the requirement of radial glia for the generation of these specific populations. Using a new transgenic radial glial labeling line, gfap:nls-mCherry, we demonstrate that subsets of later born neurons are radial glial derived. In addition, we generated a gfap:nfsb-mCherry transgenic line to specifically eliminate Gfap+ radial glia through the use of the Nitroreductase ablation system. We are currently characterizing the effects of temporal radial glial ablation on cell type differentiation and overall maintenance of neuronal and axonal patterning in the spinal cord. These diverse approaches aim to delineate the potential contributions of radial glia towards development and regeneration of the nervous system. This work was funded by NIH HD060023, NSF 1054168 and NSF RUI 0615594 grants awarded to MJBarresi.

Program Abstract #109
Use of the zebrafish lateral line to model glutamate excitotoxicity of hair cell afferent neurons
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Noise overexposure, which can cause hearing loss, damages the mechanosensory hair cells as well as the postsynaptic afferent neurons that relay information from the hair cells to the auditory brainstem. The effects of noise damage in the mammalian cochlea, which include a swelling of the afferent terminals and a reduction in neuronal firing, are recapitulated by prolonged application of glutamate receptor agonists. These deleterious effects of excessive glutamate receptor activation are referred to as glutamate excitotoxicity. Despite the importance of afferent neurons in relaying sensory information to the brain, the inaccessibility of afferent neurons within the cochlea has made it difficult to visualize neuronal responses to both normal levels of hair cell mechanotransduction and excessive glutamate receptor activation. The objective of these studies is to determine the extent to which the effects of glutamate excitotoxicity and recovery that have been observed in the mammalian cochlea can be modeled in the afferent neurons of the zebrafish lateral line. We used a combination of electrophysiological recordings and in vivo time lapse calcium imaging of 6-7 day post fertilization transgenic HuC:GCaMP5 zebrafish. Loose patch recordings of afferent neurons demonstrate that AMPA application initially increases then diminishes afferent neuron firing. Afferent terminals monitored in...
HuC:GCaMP5 larvae exhibit calcium transients in ambient conditions and prolonged increases in calcium during AMPA exposure followed by terminal swelling. These data support the use of zebrafish as a model for glutamate excitotoxicity of hair cell afferent neurons. Future work will determine whether afferent terminals regenerate and recover function following glutamate excitotoxicity.

Program Abstract #110
Taurine induced rod photoreceptor differentiation in the zebrafish retina
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From embryo to adult, taurine plays an integral role in maintaining a healthy retina. In mammals, taurine depletion results in photoreceptor cell death and blindness. In contrast, addition of taurine to iPS, ESC, or developing mouse retinal explants induces rod photoreceptor differentiation. The pathway by which taurine produces rods is largely unknown; thus, we began to characterize the role of taurine in rod cell generation in the developing and adult zebrafish retina. Taurine transporter (TatU) expression (24 hpf) in the developing zebrafish retina precedes taurine synthesis (60 hpf). Thus, we tested if exogenous taurine application earlier in development disrupted the retin. Taurine-treated embryos displayed normal eye size and retinal lamination with increased proliferation, assayed by phospho-histone 3 (PH3), with no change in cell death. To examine differentiation at 72 hpf, taurine-treated Tg[rho:Eco.NfsB-EGFP]nt19 (expressing in rods), Tg[Tg(gnat2:GAL 4-V16) X Tg(UAS:NfsB-mCherry)] (expressing in cones), and Tg[glap:EGFP]nt11 (expressing in Müller glia) larvae displayed significantly more rods at the expense of cones and Müller glia. In the developing and adult retina, tauT (sle6a6b) mRNA was localized in the circumferential marginal zone which houses neuronal progenitors for retinal growth, the inner nuclear layer that contains Müller glia which produce rod progenitors to generate rod photoreceptors, and photoreceptors. During development, morpholino-mediated knockdown and pharmacological blockade of TatU expression and activity, respectively, significantly reduced the number of rods at 60&72 hpf, with significantly more PH3-positive cells. Similarly, TatU blockade in the undamaged adult retina induced a significant increase in the number of PCNA-positive cells in the outer nuclear layer, likely proliferating rod precursors. Thus, taurine signaling is involved in photoreceptor differentiation in both the adult and developing zebrafish retina.

Program Abstract #111
Gli-1 modulates RPE transdifferentiation
Karla Barbosa-Sabanero, Chelsey Judge, Sara Tirell, Brant Center, Trisha Patel, Agustin Luz-Madrigal, Katia Del Rio-Tsonis
Miami Univ., USA
The retinal pigmented epithelium (RPE) of the embryonic chick can be induced to transdifferentiate to retina if fibroblast growth factor 2 (FGF2) is present after retina removal. During transdifferentiation, the RPE cells lose their phenotype, proliferate and eventually become retina cells. Previous studies in our lab have shown that over-expression of Sonic Hedgehog (Shh) inhibits this process. Therefore, the aim of this study is to determine the mechanisms by which Shh inhibits FGF-induced transdifferentiation. In this study we analyzed if Shh acts through its canonical pathway using its downstream target Gli-1. Immunohistochemical analysis indicated that Gli-1 is present in the embryonic chick RPE. Analysis by RT-qPCR demonstrated that Gli-1 is down-regulated 24h post-retinectomy in the absence of FGF2. Over-expression of Gli-1 resulted in an up-regulation of RPE markers such as MiFT and Otx-2, and a down-regulation of the retinal marker Pax-6 when analyzed by immunofluorescence. Moreover, RPE transdifferentiation was efficiently blocked in RCAS Gli-1-infected RPE as shown by histology. Finally, loss of function experiments by electroporating morpholinos against Gli-1, showed that the inhibition of Gli-1 promotes transdifferentiation and up-regulates Pax-6. In conclusion, our results suggest that Shh can inhibit FGF-induced RPE transdifferentiation acting through its canonical pathway via Gli-1 maintaining the identity of the RPE. Furthermore, Gli-1 inhibition is sufficient to promote transdifferentiation of the RPE. Our results provide a novel mechanistic insight of RPE transdifferentiation and cell fate. Supported by NEI EY17319 to KDRT, SIGMA XI G20110315157252 to KYBS, and CONACYT to KYBS and ALM.

Program Abstract #112
Utilizing oocyte biology to understand nuclear reprogramming
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Introduction of human nuclei into a Xenopus oocyte results in reprogrammed pluripotent nuclei with high efficiency, yet little is known of this mechanism of reprogramming. To gain greater understanding of in vivo reprogramming, we examined the oocyte and egg by RNA-Seq and mass spectrometry. We also examined oocyte protein expression upon introduction of human nuclei. To determine how the oocyte/egg are structurally organized to drive reprogramming, we sequenced total RNA from oocytes and eggs. We find 61 RNAs preferentially expressed in the oocyte and 25 RNAs preferentially expressed in the egg. RNA-Seq of animal and vegetal halves of oocytes reveal 170 vegetally and 69 animaly localized RNAs. Proteomics analysis of the oocyte identified over 900 highly expressed proteins in the oocyte germinal vesicle (GV), including proteins involved in DNA replication/repair, chromatin structure and remodeling, and metabolism. Injection of HeLa nuclei into the GV results in expression of OCT4, NANO8 and SOX2 within 24-48 hours, suggesting that we can identify not only Xenopus reprogramming factors, but also the somatic response to reprogramming. We used TMT tag/tandem mass spectrometry to identify Xenopus proteins that increase in the GV at specific time points during reprogramming. We find 44 proteins increase during reprogramming, an additional 14 proteins increase 2 hours post transfer and another 40 proteins increase 6 hours post transfer. Proteins identified will be tested functionally with drug
inhibition during reprogramming. Current work is focused on profiling somatic responses to reprogramming, including RNA-Seq for human transcript expression changes and ATAC-Seq to investigate changes in chromatin state. Utilizing the unique biology within the oocyte to tease apart mechanisms of reprogramming provides valuable insights into plasticity during differentiation, and provides avenues for identification of novel factors involved in reprogramming.

Program Abstract #113
FGF2 and noggin promote neural crest induction from hESC-derived embryoid bodies
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Neural crest cells are fascinating as they can produce a wide array of cell types throughout the body, including craniofacial bone and cartilage, melanocytes, blood vessels around the heart and neurons and glia of the peripheral nervous system. Unraveling how these various cell types arise from neural crest precursors, could help to better understand the mechanisms involved with their abnormal development and alternatively, provide a foundation for cell-based therapies. Here we assess the early requirements for the production of neural crest precursors from human embryonic stem cell (hESC) -derived embryoid bodies. We identify presumptive human neural crest precursors using markers associated with earlier premigratory and migratory, rather than later postmigratory, events, and demonstrate their rapid induction in defined feeder-free culture, with the paracrine factors FGF2 and noggin. With or without the prospective isolation of p75+ cells, these neural crest precursors can be differentiated to form multiple neural crest derivatives, such as peripheral neurons, glia, myofibroblasts and pigmented cells. Our study provides insights regarding the signaling events that can rapidly induce neural crest precursors from hESC-derived embryoid bodies.

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Program Abstract #114
Function human genetics using human embryonic stem cells
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Next generation sequencing and genome-wide association studies have transformed human genetics by revealing numerous disease-associated sequence variants at unprecedented speed. Model organisms such as the mouse have greatly facilitated the functional validation of disease-associated genes. However, the ever-increasing number of sequence variants identified by human geneticists challenges us to develop more efficient experimental platforms with the speed and scale that surpasses conventional mouse genetics. An ideal platform should also allow the study of human-specific disease mechanisms.

We combine directed differentiation of human embryonic stem cells (hESCs) with powerful genome-editing technology to study human pancreas development. Through the use of TALENs (Transcription Activator Like Effector Nucleases) and CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats), we have generated hESC knockout mutants for 7 of the 10 neonatal diabetes-associate transcription factors identified to date (GATA6, MNX1, PDX1, PTF1A, NGN3, RFX6 and GLIS3). Mutations in these genes may also contribute to juvenile and adult onset diabetes. Through directed differentiation to recapitulate pancreatic development, our studies have not only revealed essential roles of these genes in the specification of insulin-secreting beta cells, but also helped define developmental mechanisms underlying disease phenotypes. Notably, we have identified human-specific requirements for GATA6 and NGN3 in pancreatic development, and we are investigating the molecular mechanisms that could account for these species differences.

Our findings are beginning to shed light on both conserved and human-specific mechanisms of pancreatic development and neonatal diabetes. Our experimental approach provides a framework for using hESCs as a model system for genetic studies.

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Program Abstract #115
Prolonged Culture Yields hiPSC-CMs with Enhanced Structural and Contractile Properties
Marita Rodriguez, Charles Murry, Nathan Sniadecki
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Cardiomyocytes derived from human stem cells (hSC-CMs) are round, poorly spread, have highly unorganized cytoskeletons, and produce weak, unaligned contractile forces. In contrast, adult cardiomyocytes are elongated, brick-shaped cells, which have a high level of cytoskeletal organization and produce strong, unidirectional contractile forces. Therefore, cardiomyocytes derived from hSC-CMs cells would serve as a more effective model of adult cardiomyocytes if they were first matured to a more adult-like state. It has previously been shown that prolonged culture can lead to enhancements in the structural and contractile properties of hSC-CMs. However, the effect of prolonged culture on the maturation of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) has only been partially examined in previous works, especially with regards to the quantification of their contractile properties. In this work, hiPSC-CMs that were 20, 70, and 100 days post differentiation were seeded onto arrays of microposts in order to assess differences their structural and contractile maturation. Upon analyzing these properties, we found that older cells demonstrated enhanced contractile function and improved sarcomeric organization when compared to younger cells. However, even after 100 days of growth in culture, the hiPSC-CMs are significantly less mature than their adult counterparts. Therefore, these results suggest that prolonged culture is not a practical means to achieve mature hiPSC-CMs, and that additional culture stimuli are required in order to accelerate this process. Past studies with cardiomyocytes have shown that exposing them to mechanical cues
such as substrate stiffness, cell alignment, and cell-cell contact can lead to improvements in their maturation and contractile performance. Therefore, this same technique should be investigated as a means to enhance iPSC-CM maturation in future studies. This work was funded by the NSF Graduate Research Fellowship Program.

Program Abstract #116
The function of microRNA-182 in the generation of pluripotent stem cell
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MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression and have critical functions in various biological processes. Induced pluripotent stem cells (iPSCs) can be generated by overexpression of the transcription factors Oct4, Sox2, Klf4 and c-Myc (OSKM). Recent studies have demonstrated miRNAs critically regulate cell reprogramming. MiR-182 is highly expressed in mature oocyte, one-cell zygote and embryonic stem cells which means it may have some functions in the reprogramming process. Here, we found that knockdown of miR-182 facilitates the efficiency of reprogramming by OSKM, whereas overexpression of miR-182 blocks reprogramming. Further, we will elucidate which molecular pathways does miRNA-182 involves in the induction of pluripotency.

Program Abstract #117
Atypical PKC-iota controls stem cell self-renewal via the regulation of asymmetric cell division
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Proper tissue homeostasis and injury response requires the precise modulation of stem and progenitor cell number which, depending on context, may require regulating symmetric vs. asymmetric cell division. Atypical PKCs (Prkci and Prkcz) are key mediators of asymmetric cell division among diverse species. Here, we hypothesize that precise control of atypical PKC activity results in either stem/progenitor cell expansion or stem cell depletion depending on whether symmetric or asymmetric cell division is favored. In support, we find that loss of Prkci leads to an enhanced generation of cells with pluripotent characteristics. Absence of Prkci also boosts the generation of specific tissue progenitors including neural stem cells, cardiac, and erythroid progenitors without compromising the ability of these populations to undergo differentiation. We believe that the reason for these increased progenitor populations in Prkci null cells is due to a favoring of symmetric Numb localization and downstream activation of Notch1 and Hes5. Additional inhibition of PKCs including Prkcz results in the generation of an even higher percentage of cells that express Oct4, SSEA1, and interestingly also Dppa3 (Stella), and Ddx4 (VASA). These studies suggest that the precise control of symmetric vs. asymmetric cell division via atypical PKCs influences the generation of pluripotent, multipotent, and possibly even totipotent populations. Furthermore, inhibition of Prkci and/or Prkcz may be useful for developing regenerative therapies.

Program Abstract #118
Early identification of hemogenic and non-hemogenic endothelium in the placental model system permits novel investigation of hematopoietic stem cell development.
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Hematopoietic stem cells (HSCs) originate in the endothelium of hemogenic (HSC-forming) embryonic vessels, which is otherwise indistinguishable from non-hemogenic endothelium. The inability to isolate hemogenic endothelium prior to hematopoietic gene expression has precluded investigation into the factors controlling HSC development. The placenta and its precursor, the allantois, are rich sources of HSCs. Using the placental model system, we have discovered that the hemogenic endothelium is defined by lineage differences originating early in development. We used a Tbx4-cre allele and Cre reporter to trace the fates of cells that have expressed Tbx4, which is expressed broadly in the allantois during vasculogenesis. We show that the allantois endothelium forms from two lineages: descendants of cells that have expressed Tbx4 (the Tbx4 lineage), and descendants of a small population within the allantois that have never expressed Tbx4 (non-Tbx4 lineage). Non-Tbx4 lineage endothelium segregates to the umbilical vessels and proximal placenta, regions thought to be hemogenic, while Tbx4 lineage endothelium localizes to the distal placenta.

When we examined the hematopoietic potential of these endothelial lineages in normal development, hematopoietic cell culture, or transplantations, we found that the Tbx4 lineage endothelium does not contribute to HSCs or circulating blood in the embryo or adult, while the non-Tbx4 lineage is a rich source of hematopoietic cells. Furthermore, cells expressing HSC markers are found in both lineages, but only acquire hematopoietic fate when arising from the hemogenic lineage, demonstrating that current HSC markers are relevant only in the context of predefined lineages. Thus, Tbx4 tracing reveals multiple lineages within the endothelium and permits early identification of future hemogenic endothelium and investigation of factors controlling HSC creation.

This work was supported by the National Cancer Institute intramural program.

Program Abstract #119
Hematopoietic stem cell specification is regulated by Calmodulin-dependent Kinase II
Wilson K. Clements, Sarah C. Rothschild
Retinoic acid regulates ureteric bud patterning and branching independent of Raldh2
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Retinoic acid (RA) is a potent morphogen synthesised by retinaldehyde dehydrogenase type 2 (Raldh2), which is expressed in metanephric mesenchyme. RA regulates a variety of crucial events during early embryonic development, playing important roles in cell differentiation, and organogenesis. It has long been recognized that overexposure to retinoic acid causes teratogenesis in rodents as well as humans. Although kidney defects that result from disruption of RA signaling have been widely documented, the mechanism by which exogenous RA regulates ureteric bud is unknown. Here, we provide novel evidence that a major role for RA is to regulate ureteric bud patterning and branching. For teratogenic RA insult, 30 mg/kg b.w. of all-trans RA suspended in peanut oil with 10% (vol/vol) absolute ethanol was injected intraperitoneally into pregnant mice at E9.0. We found that an excess of retinoic acid results in different reported malformations in the mice kidney. The results showed that duplex ureteric bud, bilateral fused kidney and reduced ureteric bud branching. In order to examine whether excess retinoic acid is teratogenic by inducing local Raldh2 deficiency, but RA exposure was not followed by decreased levels of Raldh2, the retinoic acid-synthesizing enzyme in metanephric mesenchyme during kidney development. To determine whether RA supplementation could affect apoptosis in the metanephros of RA-overexposed embryos, Capase-3 assay was performed on E15.0 embryos. In the mouse model of RA-induced kidney defect, no significant apoptosis was observed in the metanephros of RA-overexposed embryos. In the present study we demonstrate that exogenous RA can cause bilateral fused kidney and reduced ureteric bud branching, showed different concentration effect, which is independent of suppressing Raldh2. The RA signaling is critical for ureteric bud patterning and branching. This work was supported by the National Basic Research Program of China (No. 2011CB944000)
Program Abstract #122
Morphogenesis of the developing mouse lung
Princeton Univ., USA
Foundation, and the Henry & Camille Dreyfus Foundation.
Supported by the NIH (GM083997, HL110335, and HL118532), the David & Lucille Packard Foundation, the Alfred P. Sloan Foundation, and the Henry & Camille Dreyfus Foundation.
The ureteric bud (UB) is an epithelial tube that undergoes branching morphogenesis, giving rise to the renal collecting ducts. Although the genes that regulate UB development and branching are well studied, the cellular behaviors involved remain obscure. Using time-lapse microscopy combined with fluorescent-labeling techniques, we study morphogenesis at the single-cell level, within the tips of the branching UB. We find that, contrary to mitotic cells in many epithelia, whose daughter cells remain in contact, epithelial cells of the UB tip disperse immediately following mitosis. In a process we term “mitosis-associated cell dispersal” (Developmental Cell. 2013; 27(3): 319-30), we observe that pre-mitotic cells round up and move apically; mitosis occurs within the lumen – while a thin process maintains connection to the basal surface. One daughter cell inherits the basal process, allowing reinsertion at the origin of, while the other daughter reinserts one-to-three cell-diameters away. Apical contact is maintained between daughter cells until the untethered cell reestablishes basal surface contact, likely limiting the initial distance of dispersal. Since mitosis occurs at a high rate in the UB tips, this behavior causes extensive cell rearrangements that may contribute to branching morphogenesis; and we are currently exploring other developing epithelia for similar behavior.

Program Abstract #123
Mammary ducts are elongated by MAPK induced high motility cell clusters
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Proliferation and cellular rearrangements are fundamental mechanisms that drive epithelial tissue remodeling. We sought to identify the relative contributions of these fundamental cell behaviors during mammary duct elongation. Using organotypic culture and time-lapse imaging we visualized duct elongation in real-time with cellular resolution. Proliferation accounted for approximately 25% of new cells in elongating ducts but proliferation was not required for duct initiation. We showed that branches were primarily formed by cell migration and probed for differences in the motility of individual cells within the epithelium. We found that cells at the elongation front had double the mean speed of cells within the body of the tissue. This data indicated that increased cell speed correlated with branch elongation but left the question why do branches form in some locations but not others? Growth factor signaling was sufficient to drive mammary duct elongation so we interrogated signaling downstream of growth factors. We found that the elongation front of ducts was enriched for activated MAPK signaling. Inhibition of MAPK signaling resulted in collapse of mammary branches demonstrating a requirement for the MAPK pathway. To determine if MAPK signaling was sufficient to induce elongation we mosaically expressed a constitutive active mutant of the kinase MEK in the absence of outside stimulation. Activated MEK was sufficient to drive duct elongation and clusters of mutant cells were at the elongation front of forming ducts. Interestingly, expression of mutant MEK in too few or too many cells abrogated elongation, indicating a specificity for the number of motile cells required to elongate a duct. These data lead us to conclude that ducts initiate at sites that are locally enriched for MAPK signaling and that the mechanism of branch elongation is collective cell migration of high motility cell clusters.

Program Abstract #124
Mesenchyme-free airway branching driven by a growth-induced mechanical instability
Victor Varner, Jason Gleghorn, Celeste Nelson
Princeton Univ., USA
Space-filling, branched networks form the basic architecture of numerous organs in the body. During development these complex structures originate as simple epithelial tubes, which are then sculpted by a series of branching events. In most cases, branching involves reciprocal signaling between an epithelium and its surrounding layer of 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 neighboring mesenchyme. This FGF template is thought to specify the locations of new branches. Interestingly, however, when the mesenchyme is removed, and reciprocal signaling is disrupted, isolated epithelial explants still branch in culture. In the absence of an FGF pre-pattern, it is unclear how the locations of these branches are determined.

Here, using a combination of experiments and modeling, we suggest that mesenchyme-free branching of the airway epithelium is driven by a growth-induced mechanical instability. We removed the mesenchyme from embryonic day E12.5 mouse lungs and embedded the isolated epithelial explants in 3D gels of reconstituted basement membrane protein. Time-lapse imaging of cultured explants was used to quantify branching dynamics. New epithelial branches formed simultaneously, with the airway epithelium cast into a folded geometry with characteristic wavelength λ. To investigate the mechanics of this process, we constructed a computational model of the growing airway epithelium embedded within the surrounding gel. Taken together, our results suggest that, in the absence of a biochemical pre-pattern, purely physical interactions can pattern branch locations within the developing airway epithelium.

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Program Abstract #125
Grhl2 as a suppressor of EMT during murine neural tube closure
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Embryonic neural tube (NT) formation is a complex morphogenic event that is essential to establish the central nervous system. In the final step of NT fusion, opposing neural folds seal at the midline to generate the closed NT overlaid with a sheet of ectoderm. Prior to fusion, cells specified as neural ectoderm (NE), non-neural ectoderm (NNE), and neural crest (NCC) lie within a single contiguous ectodermal sheet. During fusion, rapid remodeling events occur that are tightly coordinated in time and space such that the NE and NNE separate from each other but remain as individual tightly associated epithelia while NCC undergo an epithelial-to-mesenchymal transition (EMT) and migrate to distal sites. Many studies have focused on mechanisms underlying NCC EMT, but little is known about how the NNE is regulated to remain epithelial. The transcription factor Grainyhead-like 2 (Grhl2) is expressed in the NNE and is essential for NT fusion, as loss of Grhl2 results in 100% cranial NTDs. Grhl2 regulates expression of key epithelial genes, but it is unknown how loss of Grhl2 leads to NTD and if Grhl2 regulates other processes in the NNE. Histology and immunofluorescence results show that in the absence of Grhl2, NNE cells gain mesenchymal characteristics. RNA-seq and bioinformatic analysis of NNE reveals several genes that may act as EMT suppressors and be direct targets of Grhl2. These genes are down-regulated in Grhl2 mutant embryos and individual gene knockdowns in vitro result in EMT phenotypes. Live imaging of NT closure shows that mutant NNE cells exhibit increased movement and leave the epithelial sheet. These results indicate that Grhl2 regulates the NNE not only through up-regulation of epithelial genes, but also by directly suppressing EMT. This disruption in the proper balance of epithelial and mesenchymal-like characteristics in cells of the neural folds could prevent proper cranial NT fusion. Funding: The Howard Hughes Medical Institute, NCI #1F31CA180438-01

Program Abstract #126
Mechanisms of folate action during neural tube formation
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Folate deficiency increases the incidence of neural tube defects (NTDs), one of the most common birth defects, which result from a failure of neural tube closure during embryogenesis. The elucidation of the mechanisms underlying folate action has been challenging. This study introduces Xenopus laevis as a model to determine the cellular and molecular mechanisms involved in folate action during neural tube formation.
Folate receptor is readily expressed in Xenopus eggs and persists throughout neurulation. In the closed neural tube folate receptor localizes to differentiated neurons. We show that molecular knockdown of the folate uptake system, folate receptor-a (FR-a) impairs neural tube formation and leads to NTDs. FR-a knockdown in neural plate cells only is necessary and sufficient to induce NTDs. FRa-deficient neural plate cells fail to constrict and elongate, widening the neural plate ventral midline and preventing closure of the neural tube.
Pharmacological inhibition of folate action by methotrexate during neurulation induces NTDs by impairing narrowing of the neural plate and elongation of the neural tube. In contrast, methotrexate-ester and pyrimethamine, lipophilic inhibitors of dihydrofolate reductase, do not induce NTDs, suggesting that folate interaction with its uptake systems, rather than folate metabolism, is important for the formation of the neural tube. Inhibiting folate action impairs neuronal differentiation without affecting neural induction. Folate appears to promote neural tube formation and to prevent neural tube defects by enabling changes in cell shape and progression of neuronal differentiation during neurulation. Further studies in this organism may unveil novel cellular and molecular events mediated by folate and lead to new means for preventing neural tube defects.
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Program Abstract #127
Mechanisms of glutamate action during neural tube formation
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Failure of neural tube formation leads to one of the most common birth defects known as neural tube defects (NTDs). Use of antiepileptic drugs (AEDs) during pregnancy increases the incidence of NTDs by unclear mechanisms. We hypothesize that AEDs induce NTDs by impairing neurotransmitter signaling during neurulation. In this study we investigate the mechanisms by which glutamate signaling participates in neural tube formation. Transcripts for components of neurotransmitter vesicular release, synaptobrevin, syntaxin, SNAP25, synaptotagmin and the vesicular glutamate transporter, VGluT1, are present in Xenopus laevis neural plate. We also detect mRNAs for the connexins Cx43, Cx38, Cx46, Cx32 and Cx26, which may mediate neurotransmitter release through hemichannels. In addition, transcripts of glutamate receptor subunits, GluR1, GluR7 and NR1 are present in the early neural plate. To determine the spatiotemporal profile of glutamate release, we expressed a membrane-anchored extracellular glutamate-sensing fluorescent reporter, iGluSnFR. We find strong iGluSnFr signal in the neural plate. Time-lapse imaging shows localized transients in iGluSnFr signal at cell junctions, which increase in frequency in the absence of divalent cations, suggesting hemichannel-mediated glutamate release from neural plate cells. To evaluate the potential role of vesicular glutamate release during neurulation, we used a translation-blocking morpholino against VGluT1. Knocking down VGluT1 induces NTDs in a dose-
dependent manner and decreases iGluSnFr signal. We examined calcium dynamics using the genetically encoded calcium sensor, GCamp6s. Results show that spontaneous calcium transients are apparent in neurulating embryos and addition of exogenous glutamate induces calcium transients. Altogether, these findings suggest that an active glutamate signaling is present during neurulation, which may trigger calcium dynamics that in turn are important for the formation of the neural tube.

Program Abstract #128
Claudins are required for convergent extension and apical constriction during neural tube closure
Amanda Baumholtz, Annie Simard, Aimee Ryan
McGill Univ., Canada
Neurulation is a critical developmental process that converts the flat neural plate into a closed neural tube. Throughout this process the neural plate epithelium undergoes extensive remodeling including cell intercalation and apical constriction. These events require the maintenance of cell interactions within the neural epithelium through intercellular junctions. The most apical of these junctions are tight junctions (TJs), where members of the claudin family regulate apical-basal cell polarity and cell adhesion, and link the TJ to the actin cytoskeleton. We hypothesize that claudins are required for coordinating the morphogenetic events that drive neural tube closure. To test our hypothesis, we used the C-terminal domain of Clostridium perfringens enterotoxin (cCPE) to remove a subset of claudins from the neural plate of chick embryos. 100% of treated embryos had open neural tube defects (NTDs) that could not be rescued by folic acid supplementation. We confirmed that cCPE specifically removed Claudin-3, -4, and -8 but not Claudin-1 from TJs in chick embryos. In situ hybridization analysis of gene expression in the ectoderm and along anterior-posterior boundaries of the neural tube suggested that NTDs were not due to defects in ectoderm differentiation or patterning. cCPE-treated embryos displayed defects consistent with a disruption of convergent extension: a shortened anterior-posterior axis, a reduced length-to-width ratio, misshapen somites, and a broadened notochord. Transmission electron microscopy analysis of cCPE-treated embryos showed that midline cells failed to undergo apical constriction. These results suggest that claudins are required for two morphogenetic events that drive neural tube closure – convergent extension and apical constriction.

Program Abstract #129
Functions of p120-Catenin in the establishment of the Antero-Posterior Axis of the Mouse Embryo
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p120-catenin is a member of family of armadillo proteins involved in intercellular junctions. Biochemical and cell-based experiments indicate that p120-catenin promotes the stability of cadherins on the cell surface by inhibiting their endocytosis. In addition, p120-catenin also functionally interacts with some members of the Rho-GTPases family such as RhoA, Cdc42 and Rac1, proteins that are important regulators of cytoskeletal dynamics and cell migration. It was previously known that deletion of p120-catenin causes early embryonic lethality in mice, but the basis of that lethality was not determined. We have generated mouse embryos that lack p120-catenin in all cells, or specifically in the epiblast or in the visceral endoderm using the Sox2-Cre and Ttr-Cre transgenes, respectively. We find that embryos lacking of p120-catenin in all cells show duplications of the anterior-posterior (A-P) body axis detected by the duplicated expression of mesoderm markers like Wnt3, T and Mesp1. In addition p120-catenin null embryos fail to segment somites and to form a proper neural tube. The correct positioning of the primitive streak in the epiblast, which gives rise to the mesoderm and definitive endoderm, depends on earlier movement of extraembryonic Anterior Visceral Endoderm (AVE) cells. Our preliminary observations suggest that p120-catenin has two distinct roles in the AP axis specification: regulation of AVE cell migration and regulation of the pools of β-catenin at the plasma membrane in the mouse epiblast. Currently we are analyzing possible genetic interactions of p120-catenin with Wnt3 and β-catenin genes in the establishment of the AP axis. These studies help clarify the role of Wnt signaling in the establishment of the antero-posterior polarity of the mouse embryo. This work is has been supported by Pew Latin American Fellows Program in the Biomedical Sciences, USA., and CONACYT, México.

Program Abstract #130
Tubulogenesis using Wolffian duct as a model: Tubule elongation and cell epithelialization are coordinated by FGF signals
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Kyoto Univ., Japan
In many organs of the body, epithelial tubulogenesis is a fundamental process required to maintain physiological functions, including a transportation of nutrients, gases and wastes, and barrier functions between the body and its environment. Tubule formation during development involves a variety of morphological events such as tissue elongation and cell epithelialization/luminal formation. Although these steps must proceed in a coordinated manner in the embryo, the mechanisms underlying such coordination remain poorly understood. To address this question, we use the Wolffian duct of chicken embryos (WD) as an experimental model. WD is also called nephric duct since its formation is one of the earliest events in kidney development of vertebrates. Time-lapse analyses combined with the WD-specific gene manipulation technique (Atsuta et al., DGD, 2013) revealed that cells located in the elongating-front (front cells) are motile with numerous pseudopodia, whereas cells residing in the rear region (rear cells) are epithelial in shape with less motility. We have found that FGF8, expressed in tissues that surround the front cells, attract the front cells by enhancing their motility through Ras/MAPK signaling. FGFs appear to switch between motile front cells and quiescent epithelial cells (rear cells) through Cdc42 activation. We proposed a model that during normal development, the front cells are
attracted to FGFs emanating from surrounding tissues, and maintain their motility (non-epithelial) with high Cdc42 activity. The rear cells, in contrast, which receive progressively less FGFs, achieve cell epithelialization through inactivation of Cdc42. Thus, FGFs provided from frontal environment coordinate the balance between tubule elongation and cell epithelialization during the WD formation.

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**Program Abstract #131**

**Imaginal disc growth factors regulate tube morphogenesis in the fly ovary**

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Morphogenetic cues from distinctly different cell-types give rise to respiratory organs called dorsal appendages (DAs) on the eggshell of *Drosophila melanogaster*. During oogenesis, two patches of somatic follicle cells that lie dorsal to the oocyte form the DAs by reorganizing into tubes and crawling over the adjacent, squamous, “stretch” follicle cells. The Sox transcription factor, Bullwinkle (BWK), functions in the germline nurse cells to regulate DA formation; it acts through the tyrosine kinases SHARK and SRC42A in the overlying stretch cells. Mutations in *bwk* or *shark* lead to DA-adhesion defects, aberrant cell migration, open tubes, and moose-antler-like DAs. To discover how the BWK-SHARK-SRC42A pathway regulates DA formation on the protein level via the stretch cells, we adapted an established magnetic-bead cell-separation protocol for a novel application: mass spectrometry. We purified stretch cells from wild-type vs. *bwk* egg chambers, compared relative protein expression, and identified differentially expressed proteins, including Imaginal disc growth factors (IDGFs), which have vertebrate orthologs. Stretch-cell-specific RNAi in vivo and mRNA localization suggest that precise levels of IDGFs non-autonomously regulate DA formation. The receptor is unknown but cell-culture studies suggest the *Drosophila* insulin receptor is involved. Other questions remain: Do the six IDGFs function together? Are they necessary and sufficient to guide tubes and ensure tube closure? Using RNAi, mutant alleles, misexpression constructs, and mosaic analysis, our goal is to determine the role of these newly identified factors in DA morphogenesis. Our results demonstrate the power of using magnetic-bead cell separation and mass spectrometry to target differential protein expression and identify morphogenetic factors in a small subpopulation of cells that non-autonomously regulate tube morphogenesis.

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**Program Abstract #132**

**TGF-β signaling in *Ciona* notochord morphogenesis**

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*Ciona intestinalis* is a marine invertebrate chordate with a small, simple genome. The *Ciona* notochord consists of only 40 cells that intercalate into a single-file column. We have recently identified a TGF-β ligand as being expressed in a striking posterior to anterior gradient in the intercalating notochord. Ectopic expression of the notochord master regulatory gene brachyury causes ectopic expression of TGF-β, arguing for a downstream role. We have been coupling specific perturbations of TGF-β signaling with in vivo confocal imaging to determine potential developmental roles for this gradient. TGF-β receptor inhibiting drugs and notochord specific expression of dominant negative versions of several TGF-β signal transduction components are both found to interfere with notochord cell intercalation. Specific phenotypes seen include rounded cells that fail to mediolaterally elongate, cells that elongate and align with their neighbors but nevertheless fail to intercalate, and cells that are excluded from the bulk of the notochord and distort the otherwise smooth notochord boundary. Ongoing experiments using morpholino oligos to specifically knock down this gene will be presented. Our results thus far argue for an unexpectedly early role of notochord-specific TGF-β expression in the earliest stages of notochord morphogenesis.

**Program Abstract #133**

**Cellular dynamics of tissue fusion in the mammalian secondary palate development**

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Tissue fusion is a process common to the formation of multiple organs and often involves the removal of an intervening epithelial seam between two separate embryonic prominences. The fusion of the mouse secondary palate, in which the epithelium between the two palatal shelves (medial edge seam, MES) must be removed for confluence of the structure, is an excellent model system for understanding the cellular dynamics of tissue fusion. Although apoptosis is known to be involved in the removal of MEE, it is still not clear whether other cell behaviors contribute to this process. Confocal live imaging in explant culture revealed that cell intercalation between two epithelial layers is correlated with directional actin contractility. Changes in actin cytoskeleton and cell morphology allow epithelial cells to intercalate into single layer of MES by rearranging cell-cell junctions. Further, migration of the MES in the oral and posterior directions occurs along lateral actin cables and may play a role in removal of the seam. To test the functional significance of these observations, we generated mice lacking epithelial non-muscle myosin heavy chain IIA (NMHCIIA). NMHCIIA mutant mice failed to undergo appropriate removal of the MES and exhibited defects in cell intercalation. Further, by utilizing ex vivo palate explant culture, we have identified myosin regulatory light chain 9 (MYL9), Rho kinase (ROCK) and Myosin light chain kinase (MLCK) as upstream regulators of the cellular behaviors mediated by NMIIA. Together, these data
In WT embryos, these gaps between Svp+ CBs are observed, but only transiently during the final stages of DV closure, and resolve. Furthermore, in fixed embryos, we observe gaps in the DV between contralateral CBs. These gaps occur most often between a downstream proteins that allow for remodeling of the cytoskeleton. In this study, we investigate the role of the actin regulator protein Enabled (Ena) in DV morphogenesis. Loss of Ena in the DV results in cell shape and alignment defects. Live imaging and receptor and ligands that are important for mediating changes in CB shape have been identified, we know very little about the adhesion. Furthermore, Netrin and its receptor Frazzled/DCC also play a role in CB outgrowth and alignment. While several receptors and ligands acting between CBs is critical for the assembly of the cardiac tube. The ventricular myocardium emerges from the cardiac crescent, a region of cardiomyocyte proliferation and differentiation. This process involves cell proliferation, survival, and differentiation. Cardiac progenitors have been shown to express the platelet-derived growth factor (PDGF) receptor, which is known to play a role in cardiac fusion. The PDGF signaling pathway is essential for heart tube assembly, in all vertebrates, begins with the movement of cardiac cells from bilateral positions toward the embryonic midline, a process called cardiac fusion. Despite studies revealing a role for the adjacent endoderm in regulating cardiac fusion, the central question of which signals control cardiac cell movement toward the midline remains relatively unanswered. Using a combination of forward genetics and high-resolution cell tracking in zebrafish, we have revealed a novel role for the platelet-derived growth factor (PDGF) signaling pathway during cardiac fusion. We identified an ENU-induced splice-site mutation in pdgfra, named refuse-to-fuse (ref), that results in a premature truncation of the Pdgfra receptor before its catalytic domain. Although Pdgfra has been shown to be expressed in cardiac progenitors and to be important for outflow tract development, it has not been previously implicated in cardiac fusion. Cardiac cells in ref mutants move aberrantly from their initial bilateral positions in the anterior lateral plate mesoderm (ALPM), failing to fuse properly at the midline. This work was supported by grant R03DE022818 from the NIH/NIDCR.
Program Abstract #137
Formation of the cephalic furrow during Drosophila gastrulation
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The cephalic furrow is an epithelial invagination that forms between the head and the trunk during Drosophila gastrulation. The cephalic furrow begins with the shortening of a single row of initiator cells. It rapidly becomes a deep fold in the lateral embryonic ectoderm. We have characterized the early steps in the formation of the cephalic furrow. The initiator cells undergo simultaneous apicobasal shortening and apical constriction at the beginning of cephalic furrow formation. Later, the initiator cell bases expand without apical constriction while apicobasal shortening continues. The anterior and posterior cells adjacent to the initiator cells also adopt a wedge-like shape when invagination is underway. Other cells that enter the fold become shorter and broader than cells remaining on the surface of the embryo. Surprisingly, we found that myosin II does not increase in the apices of initiator cells during cephalic furrow formation. Since myosin II localization is not altered, we tested whether myosin II is differentially activated in the initiator cell apices. Neither monophosphorylation nor diphosphorylation of myosin regulatory light chain increases in initiator cell apices during cephalic furrow formation. These results suggest that neither remodeling nor activation of myosin II is involved in initiator cell shape change. However, we observed that F-actin is remodeled during initiator cell shape change and cephalic furrow formation. F-actin levels increase in the initiator cell apices throughout early cephalic furrow formation and increase in other cells that enter the fold. Our results suggest that the predominant mechanism of epithelial invagination, apical constriction driven by actomyosin contraction, is not involved in cephalic furrow formation. Instead, apicobasal shortening and basal expansion of the initiator cells might be the cell shape changes that drive the earliest stages of cephalic furrow formation.

Program Abstract #138
Characterizing the downstream effectors of DMD-3 induced morphogenesis of the tail tip of C. elegans males
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The molecular mechanisms that control the changes in cell shape, migration, polarity and adhesion that drive morphogenetic events are still incompletely understood. We are studying the morphogenetic processes that direct the formation of the C. elegans male tail as a relatively simple model for morphogenesis. During the last larval stage, the pointed larval tail tip undergoes a male-specific remodeling process that generates a blunt ended adult tail that is arrayed with the sensory structures utilized during mating. Previously, we discovered that the DM-domain transcription factor DMD-3 is both necessary and sufficient to direct morphogenesis of the male tail tip. We are currently focusing on characterizing the mechanisms by which DMD-3 performs this function. Specifically, we are attempting to identify the molecular “effectors” of male tail morphogenesis and determine how DMD-3 regulates these effectors. To accomplish this, we are taking a forward genetic approach utilizing a microarray of RNA purified from laser dissected tail tips and a reverse genetic approach using a suppressor screen of a DMD-3 induced hermaphrodite tail tip retraction phenotype. Preliminary results from these experiments demonstrate that genes involved in the synthesis of chondroitin are necessary for tail tip morphogenesis and that one of these genes, sqv-4, is male-specifically expressed in the tail tip. Furthermore, preliminary experiments have demonstrated a clear role of intracellular vesicular trafficking during male tail morphogenesis. We are currently performing experiments to further characterize the function of these processes in driving tail tip morphogenesis and determining how these processes are controlled by DMD-3. Since similar morphogenetic events occur in all animals, this work has the potential to yield insights into conserved developmental mechanisms.
This work is supported by an NSF Fellowship, Award #1255877.

Program Abstract #139
Frazzled/DCC facilitates cardiac cell outgrowth and attachment during Drosophila dorsal vessel formation
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Drosophila embryonic dorsal vessel (DV) morphogenesis is a highly stereotyped process that involves the migration and morphogenesis of 52 pairs of cardioblasts (CBs) in order to form a linear tube. This process requires spatiotemporally-regulated localization of signaling and adhesive proteins in order to coordinate the formation of a central lumen while maintaining simultaneous adhesion between CBs. Previous studies have shown that the Slit/Roundabout and Netrin/Unc5 repulsive signaling pathways facilitate site-specific loss of adhesion between contralateral CBs in order to form a luminal space. However, the concomitant mechanism by which attraction initiates CB outgrowth and discrete localization of adhesive proteins remains poorly understood. Here we provide genetic evidence that Netrin signals through DCC (Deleted in Colorectal Carcinoma)/UNC-40/Frazzled (Fra) to mediate CB outgrowth and attachment and that this function occurs prior to and independently of Netrin/UNC-5 signaling. fra mRNA is expressed in the CBs prior to and during DV morphogenesis. Loss-of-fra-function results in significant defects in cell shape and alignment between contralateral CB rows. In addition, CB outgrowth and attachment is impaired in both males...
The sea urchin small micromeres arise at the vegetal pole from an unequal 5th cleavage, and their progeny are specified to become preferentially affected terminal dendrites. Among those, alleles of coordinating these different developmental processes. From a forward genetic screen we identified a number of mutant alleles that elongation and positioning, and we have used Drosophila sensory neurons as a model system to identify control points involved in coordinating these different developmental processes. From a forward genetic screen we identified a number of mutant alleles that preferentially affected terminal dendrites. Among those, alleles of raw were of particular interest because it affected multiple aspects of terminal dendrite patterning, including terminal dendrite branching, length, and orientation, suggesting that it may define a point of convergence for multiple signaling pathways that influence terminal dendrites. Indeed, we found that raw independently regulates terminal branch stabilization/orientation and elongation through distinct genetic pathways. Raw localizes to branchpoints where it promotes dendrite stabilization together with the Tricornered kinase via effects on cell adhesion. Raw independently influences terminal dendrite growth through a mechanism that involves microtubules. Using modifier screening, we have identified mutants that genetically interact with raw to promote terminal dendrite growth but not adhesion. Thus, Raw defines a point of convergence in distinct pathways shaping dendrite patterning. This work was supported by NIMH R00-MH084277, NINDS R01-NS076614, a March of Dimes Basil O’Connor Starter Scholar Award, a Klingenstein Fellowship in Neuroscience (JZP) and a Benjamin Hall Graduate Fellowship (JL and WL).

**Program Abstract #140**

**Raw is a novel membrane protein that regulates terminal dendrite adhesion and dynamics**

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Dendrite arborization pattern is a hallmark of neuronal type, but the basis for morphological stereotypy in neurons of a given type is not well understood. Dendrite morphogenesis involves the concerted action of several distinct programs, including branch initiation, elongation and positioning, and we have used Drosophila sensory neurons as a model system to identify control points involved in coordinating these different developmental processes. From a forward genetic screen we identified a number of mutant alleles that preferentially affected terminal dendrites. Among those, alleles of raw were of particular interest because it affected multiple aspects of terminal dendrite patterning, including terminal dendrite branching, length, and orientation, suggesting that it may define a point of convergence for multiple signaling pathways that influence terminal dendrites. Indeed, we found that raw independently regulates terminal branch stabilization/orientation and elongation through distinct genetic pathways. Raw localizes to branchpoints where it promotes dendrite stabilization together with the Tricornered kinase via effects on cell adhesion. Raw independently influences terminal dendrite growth through a mechanism that involves microtubules. Using modifier screening, we have identified mutants that genetically interact with raw to promote terminal dendrite growth but not adhesion. Thus, Raw defines a point of convergence in distinct pathways shaping dendrite patterning. This work was supported by NIMH R00-MH084277, NINDS R01-NS076614, a March of Dimes Basil O’Connor Starter Scholar Award, a Klingenstein Fellowship in Neuroscience (JZP) and a Benjamin Hall Graduate Fellowship (JL and WL).

**Program Abstract #141**

**Mechanisms of primordial germ cell homing in the sea urchin**

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The sea urchin small micromeres arise at the vegetal pole from an unequal 5th cleavage, and their progeny are specified to become the primordial germ cells. Throughout gastrulation, small micromeres actively extend filopodia and lamellipodia at the tip of the gut though they continue to remain part of the invaginating epithelium and express Lvg-cadherin. Once gastrulation nears completion, the tip of the gut undergoes basement membrane remodeling that facilitates the small micromeres’ epithelial-mesenchymal transition (EMT) and migration over the archenteron to the posterior halves of the forming coelomic pouches. We show that the small micromeres reach the coelomic pouches via a directed homing mechanism. Ectopically placed small micromeres are able to find their way home to the coelomic pouches from any starting position in the embryo. Spatially and temporally misplaced 16-cell stage micromeres also home upon insertion into the blastocoeel of a late gastrula host. The small micromeres home directly to the non-skeletogenic mesoderm (NSM) lineage of the forming coelomic pouch. Small micromeres are directed to the NSM via evolutionarily conserved signaling and chemoattractant mechanisms. When the NSM is not specified, the small micromeres are unable to home. Transcription factor knockdowns within the NSM lineage shed light on the regulatory underpinnings of homing mechanisms. By using the robust homing behavior of these cells, we uncovered a transcriptional circuitry responsible, in part, for directed homing mechanisms of germ cells. Current aims are to connect the underlying transcriptional regulation of the signaling with the chemoattractant mechanisms by which primordial germ cells undergo such a dramatic feat of finding their way home. Support for this project was provided by NIH R01-HD-14483 and NIH PO1-HD-037105.

**Program Abstract #142**

**Pitx2c Promotes Epithelial Polarization to Drive Left-Right Asymmetric Gut Looping**

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Intestinal malrotation is a potentially lethal disorder that occurs in 1 in 500 newborns. Normal gut looping and directional rotation is dependent, in part, on Pitx2c, a transcription factor that is expressed in several mesoderm-derived tissues on the left side of the embryo. Functional genetic studies have shown Pitx2c to influence midgut rotation by establishing left-right (LR) asymmetric cellular properties within the dorsal mesentery. However, the morphogenetic origins of other key topological features of digestive anatomy, such as the rightward rotation of the gastroduodenal (GD) loop, remain elusive. We found that, prior to overt looping, Pitx2c is expressed not only in the left gut mesoderm, but also in the left gut endoderm, and that several epithelial polarity markers (aPKC, E-cadherin, β-catenin, γ-tubulin, and α-tubulin) are apically enriched within the left, but not right, endoderm cells. These subtle cellular asymmetries suggest that Pitx2c directs GD looping by precociously inducing the apical enrichment of epithelial polarizing factors in the left gut wall, promoting epithelial maturation and tissue expansion earlier on the left side, thus driving the
formation of a curvature. In support of this model, we show that loss-of-function of Pitx2c in the left foregut endoderm results in a lack of GD looping, with a concomitant disruption of epithelial architecture and loss of apical localization of polarity markers in the left gut wall. Conversely, ectopic expression of Pitx2c on the right side also results in a lack of GD curvature, accompanied by precocious, albeit disorganized, apical enrichment of these morphogenetic factors in the right gut wall. Our results suggest that Pitx2c generates morphological asymmetry in the gut tube by promoting precocious epithelial polarization on the left side. This work provides a new inroad to understanding asymmetric organ morphogenesis and the etiology of intestinal malrotation.

Program Abstract #143

Hedgehog signaling coordinates the relative length and diameter of the developing gut tube

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The proper length and diameter of the digestive tract are critical for normal physiological function, yet the developmental mechanisms that establish the relative dimensions of the gut tube remain unknown. Early in Xenopus gut morphogenesis, concentric layers of endoderm cells within the primitive gut tube become progressively polarized and then rearrange to both open the gut lumen and form a longer, narrower tube lined by a single-layer epithelium. As Hedgehog (Hh) signaling is required for multiple aspects of radial and longitudinal gut patterning, we hypothesized that this pathway might serve to coordinate the diameter and length of the gut tube by influencing endodermal morphogenesis. Xenopus embryos were exposed to cyclopamine, a potent Hh signaling inhibitor, or purmorphamine, a compound that promotes ectopic Hh signaling, during gut morphogenesis. Cyclopamine-mediated Hh inhibition was found to interrupt endodermal rearrangement and differentiation, resulting in a short gut tube lined by an irregular epithelial monolayer, with an enlarged lumen formed by both cell rearrangement and apoptosis. In contrast, ectopic Hh signaling was found to accelerate endodermal differentiation, producing a stratified, undulating epithelium and narrow lumen; the gut tube did elongate in this context, but acquired a flattened, ribbon-like configuration and irregular surface topography. Although Hh homologues are expressed in the endoderm, components of the Hh receptor complex are confined to the outer mesoderm layer of the gut, suggesting that endoderm morphogenesis is controlled by signals from the surrounding mesoderm. Consistent with this idea, the smooth muscle layer of cyclopamine- or purmorphamine-treated guts was found to be hypo- or hyperplastic, respectively. Taken together, our results suggest that Hh signaling indirectly regulates large-scale gut topology by balancing the opposing processes of endodermal rearrangement and differentiation.

Program Abstract #144

Dynamin acts basally to promote epithelial tube expansion in the Drosophila ovary

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Epithelial tube morphogenesis is fundamental to metazoan organ and tissue development, and requires the precise choreography of tube patterning, tube formation, and tube expansion. Expansion of an epithelial tube to a terminal morphology represents the culmination of tube morphogenesis and is critical for tube function, but we still know relatively little about this process. In the Drosophila ovary, follicle cells that encase developing oocytes perform an exquisitely simple and robust tube morphogenesis program to create the eggshell’s dorsal respiratory appendages (DAs). The Tramtrack69 transcription factor promotes the expansion of the DA-tubes by positively regulating a network of genes, including the homeobox transcription factor Mirror, the focal-adhesion scaffold Paxillin, and the endocytic scissor Dynamin (Peters et al., 2013). To further explore the contribution of Dynamin, we expressed dominant-negative Dynamin only in DA-tube cells and produced a severe, penetrant DA-tube-expansion failure. Since expansion of apical (i.e., luminal) cell surfaces is an essential component of epithelial tube expansion, we predicted that Dynamin, which has characterized roles in apical membrane remodeling, was acting apically to promote DA-tube expansion. Unexpectedly, we discovered that Dynamin protein was primarily basal, not apical, in DA-tube cells, suggesting one of two potentially novel mechanisms for Dynamin-mediated promotion of DA-tube expansion: (1) turnover of specific basal targets (e.g., Integrin-based focal adhesions), or (2) general reallocation of basolateral membrane to the expanding apical membrane. To distinguish these mechanisms, we are now examining the behavior of Dynamin, known Dynamin interactors, membrane markers, and basolateral protein complexes. These studies, funded by NIH R01 GM079433, NSF GRF DGE-0718124, and UW Provost Bridge Funds, are illuminating a novel mechanism by which Dynamin can act basally to promote epithelial tube expansion.

Program Abstract #146

Integrin-mediated cell-ECM adhesion controls morphogenesis through modulation of the biomechanical properties of a tissue.

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Integrin-mediated Cell-ECM adhesion is essential for tissue morphogenesis during development. An inherent aspect of morphogenesis is that it involves massive changes in the mechanical properties of tissues. Integrins are known to integrate mechanical signals to regulate the formation of complex tissue architecture, but the mechanisms involved are poorly understood. Dorsal closure (DC), an integrin-dependent morphogenetic process that occurs during fly embryogenesis, provides a model to study integrin function during tissue morphogenesis. We have identified integrin-containing adhesive structures on the basal surface of the amnioserosa, an extra-embryonic epithelium that is essential for DC. These adhesive structures bear striking resemblance to focal
Cadherin 2 Orchestrates Tissue Self-Assembly Through Two Opposing and Spatially Non-Overlapping Mechanisms

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Patterned configurations of adherent cells and Extracellular Matrix (ECM) underlie the topological and mechanical diversity of animal tissues. We have previously shown that vertebrate trunk elongation is propelled via cell-Fibronectin mechanics within the paraxial mesoderm. This tissue self-assembles from motile mesodermal progenitors in the posterior tailbud. Here, we examine how cell-cell and cell-ECM adhesion are integrated during this tissue assembly. We specifically examined cadherin 2 (cdh2) depletion by itself and in conjunction with depletion of the primary Fibronectin receptor Integrin α5. Paradoxically, this analysis suggests that Cdh2 both stimulates and inhibits ECM fibrillogenesis. We resolve this apparent contradiction via genetic mosaics and demonstrate that Cdh2 restricts ECM formation to tissue boundaries through two distinct mechanisms: an inhibitory β-Catenin dependent

Program Abstract #147

Cell division and cell cycle arrest control the size of a basement membrane breach

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During organ remodeling and cancer cell invasion large gaps in the basement membrane are formed. How the size of these de novo gaps in the basement membrane is regulated is unknown. We have analyzed uterine-vulval attachment during larval development in 21 species of rhabditid nematodes, a morphogenetic process that requires the coordination of basement membrane breaching and gap expansion for nematodes to lay eggs. Our combined cell biological and evolutionary approach has identified that a non-dividing vulval cell always bounds the basement membrane gap created between these organs. Using cell cycle manipulation and live cell imaging in the model nematode Caenorhabditis elegans, we show that actively dividing vulval cells aid in the enlargement of the breach by promoting basement membrane movement. In contrast, cell cycle arrest halts basement membrane movement and limits gap expansion. We demonstrate that the basement membrane component laminin accumulates at the edge of the basement membrane gap and recruits integrin in non-dividing vulval cells to stabilize gap position. Together these studies reveal that regulating cell division and cell cycle arrest can function as an evolutionarily conserved mechanism to control the size of a basement membrane breach, facilitating the exchange of cells between tissues.

Program Abstract #148

A hemicentin and plectin-dependent adhesion system links tissues by connecting adjacent basement membranes

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Complex cell arrangements within a tissue can be controlled by temporally and spatially regulated cell-cell or cell-matrix adhesion systems, but a tissue-tissue adhesion system that may connect developing tissues to create functional organs is not well described. Using C. elegans uterine-vulval attachment as a model for organogenesis, we have identified an adhesion system that links the developing uterine and vulval tissues to facilitate uterine-vulval connection. Like most epithelia, the gonad and the vulva are each encased by basement membrane (BM), a sheet-like extracellular matrix. Live-cell imaging and electron microscopy reveal that the BMs surrounding the gonad and vulva are linked at the site where uterine-vulval connection will be initiated by anchor cell (AC) invasion. We find that the extracellular matrix component hemicentin (HIM-4), a secreted protein found between BMs, forms punctate accumulations under the AC and controls linkage of the gonadal and ventral BMs to promote rapid invasion. Through targeted screening, we identify an integrin-binding cytolinker protein, plectin (VAB-10A) that localizes to the AC-BM interface and tethers hemicentin to the AC. Finally we show that the integrin dimer INA-1/PAT-3 is required for the formation of BM linkages. Together, intracellular plectin, integrin and hemicentin are the founding components of a cell-directed adhesion system that connects adjacent tissues through their juxtaposed BMs. As spatially and temporally controlled tissue-tissue adhesion is essential for the development of many organs, including the blood brain barrier, lung alveoli, and the optic cup, we anticipate that this BM-BM adhesion system represents a common mechanism to align and attach tissues. This work was supported by The Pew Scholars Program in the Biomedical Sciences and NIH Grants GM079320 and GM100083 to D.R.S; and an NSF Graduate Research Fellowship to M.A.M.

Program Abstract #149

Adhesions, thus we term them Focal Adhesion-Like Structures (FALS). FALS are dynamic, motile structures; their morphology and relative stability is tied to the developmental stage of the tissue. Upon application of ectopic force, we observe striking changes in FALS morphology and dynamics. Genetic modulation of actomyosin contractility and/or FALS attachment to actin also perturbs FALS dynamics and behavior suggesting that FALS are mechano-responsive structures. Mutations in integrin and its ECM ligands disrupt FALS and lead to a failure of DC. Intriguingly, mutations that act to increase cell-ECM adhesion through FALS also result in DC defects. Using quantitative image analysis and mathematical modeling, we show that FALS regulate the mobility of amnioserosa cells during DC. Misregulation of integrin function in this context changes the biomechanical properties of the tissues involved, and in particular, alters the friction forces between tissue layers, leading to impaired cell mobility and disrupted DC. Altogether, our study illustrates how modulation of cell-ECM adhesion can alter the fundamental biomechanical properties of a tissue to regulate morphogenetic events.

*Funded by an NSERC training grant.
mechanism and a stimulatory β-Catenin independent mechanism. These data suggest Cdh2 as a key node that represses ectopic ECM among adherent cells within a tissue and promotes ECM fibrillogenesis along tissue boundaries. As Fibronectin fibrillogenesis is a mechanically driven process dependent upon actomyosin contractility, we hypothesize that Cdh2 mediates its stimulatory role by localizing the force exerted by cells on the ECM to tissue boundaries. To test this hypothesis we generate a novel mechanical force sensor that detects high magnitude tension between cells and the Fibronectin matrix. Uniquely, our probe relies upon ratiometric imaging and the mechanical stability of GFP rather than FRET. The force sensor highlights known sites of Fibronectin assembly in a manner dependent upon Integrin α5 function and Integrin binding. We are currently working to apply this sensor to measure the role of Cdh2 in organizing the mechanical environment of the developing embryo.

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Program Abstract #150
ADAMTS9-mediated extracellular versican remodeling is crucial for the morphogenesis of the mammalian feto-maternal axis
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During embryogenesis, extracellular matrix (ECM) provides a substrate for cell adhesion, migration, and constitutes the cellular microenvironment, with the potential to regulate growth and differentiation cues. Developmental ECM remodeling is therefore crucial for morphogenesis of the embryo. Here, using a gene-trap derived mouse mutant, we demonstrate that one of the most conserved matrix remodeling enzymes, ADAMTS9 (also known as Gon-1 in C. elegans, and ADAMTS-A in Drosophila) is required for the development of the feto-maternal axis. We report here that ADAMTS9 proteolysis of ECM is crucial for VSMC orientation and proliferation to ensure growth of the umbilical vasculature. We also show that ADAMTS9 is crucial for the development of the yolk sac vasculature in mice. ADAMTS9 (a disintegrin and metalloprotease with a thrombospondin type-1 motif, family member-9) is a secreted protease known to cleave versican at a specific site. ADAMTS9 proteolysis of versican is crucial for the morphogenesis of the developing yolk sac vasculature. ADAMTS9-mediated extracellular versican remodeling is crucial for the morphogenesis of the mammalian feto-maternal axis.

Developmental regulation of integrin clustering
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Integrin adhesion receptors mediate the attachment of cells to the ECM and connect to the intracellular adhesion complex through the adapter protein, talin. Talin serves as a focal point for regulating integrin activity. The N-terminal, FERM domain containing, head domain of talin is known to regulate the affinity of integrin for its ECM-ligands, a process known as inside-out signaling.

Using transgenic and classical genetic approaches we describe a novel mode of integrin regulation that is mediated by the talin head domain.
domain. Using a mutation that specifically blocks the process of talin-mediated inside-out integrin signaling, but leaves other functions of the head intact, we show that it is not essential for fly development. In comparison, we found that a mutant allele of talin that disrupts key molecular interactions within the Head domain gives rise to severe loss of function phenotype. Intriguingly, we find that this phenotype is due to defects in the reinforcement of the adhesion complex during development and for clustering the integrin receptors at sites of adhesion. Our findings thus identify a novel role for the talin head domain in controlling integrin clustering and in initiating signals that strengthen the integrin adhesion complex. We propose that this reinforcement is essential for regulating the durability of Cell-ECM adhesion during development and for the maintenance of the tissue architecture that forms during embryogenesis.

Program Abstract #153
Role of Tmem2 in organization of the extracellular matrix surrounding cardiac and skeletal muscle
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The organization of extracellular matrix (ECM) is essential to maintain the architectural complexity of both cardiac and skeletal muscle, but the pathways that manage the composition and modification of the ECM during muscle development are not fully understood. Here, we present the first evidence implicating the transmembrane protein Tmem2 in the regulation of the ECM. In zebrafish, depletion of maternal and zygotic tmem2 inhibits heart tube formation: in MZtmem2 mutants, cardiomyocytes fail to move toward the midline. In addition, MZtmem2 mutants exhibit U-shaped, rather than chevron-shaped, somites. Investigation of the somite defects in MZtmem2 mutants revealed that Tmem2 function is required for maintenance of muscle fiber attachment to the myotendinous junction. Focal adhesion proteins, which anchor muscle fibers to the basement membrane, and ECM components of the basement membrane are mislocalized in MZtmem2 mutants. Moreover, MZtmem2 mutants exhibit hypoglycosylation of alpha-dystroglycan, a major component of the dystroglycan adhesion complex. Reminiscent of the defects observed in skeletal muscle, we find irregular and disorganized deposition of laminin and fibronectin surrounding MZtmem2 mutant cardiomyocytes, which could account for their inability to migrate toward the midline. Taken together, our results implicate Tmem2 as a novel regulator of ECM modifications that are critical for both skeletal and cardiac morphogenesis. Through ongoing structure-function analysis, we will evaluate the biochemical basis for this aspect of Tmem2 function.

Program Abstract #154
Microvilli unfold to expand the cell surface during Drosophila cellularization
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Morphogenesis is driven by cell shape change, which often requires cell surface expansion. Key aspects of this remodeling are still mysterious: Where does new membrane for cell surface growth come from? How does it get to where it needs to go? According to a decades-old hypothesis, microvilli can unfold to provide membrane for cell surface expansion during embryo cleavage, compaction, and epiboly; but this was never directly demonstrated. We now find that microvilli do serve as a membrane source for cleavage furrow growth during Drosophila cellularization, the first tissue-building event in the embryo. Microvilli coat the embryo surface before cellularization but are gone after. By live cell imaging, we see that microvillar membrane is lost from the embryo surface in a slow phase followed by a fast phase, and these biphasic kinetics match the biphasic kinetics of furrow ingression. We modulate furrow kinetics and show that furrow ingression controls the rate of microvillar depletion. Finally, we directly track microvillar membrane and show that it unfolds and slides along the cell surface into furrows, independent of endocytosis. Now we are working to validate a new model whereby force generated by furrow ingression pulls on the microvillar membrane, increasing plasma membrane tension such that microvilli disassemble and their membrane slides into the furrows.
My work validates the long-standing hypothesis that microvilli unfold to expand the cell surface. Evidence from a wide array of cell shape changes and isolated cell types is consistent with an unfolding mechanism, and now we have definitively shown that unfolding can occur in the forming tissue of an intact embryo. Thus, we suggest that unfolding microvilli or other membrane projections is a fundamental mechanism of cell shape change and morphogenesis, with significant implications for development and disease.

Program Abstract #155
A maternal-zygotic module stabilizes F-actin to promote the robustness of morphogenesis
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Robustness is a property built into biological systems to ensure stereotypical outcomes despite fluctuating inputs from gene dosage, biochemical noise, and environment. During development, robustness safeguards embryos against mistakes that underlie structural and functional birth defects.
While much attention has been paid to the role of gene and signaling networks in promoting robust cell fate choices in embryos, little has been done to rigorously assay how mechanical processes like morphogenesis are designed to buffer against variable conditions. Here we show that morphogenesis is made robust by mechanisms targeting the actin cytoskeleton. We identified two novel members of the Vinculin/α-Catenin Superfamily that work together to promote robustness during Drosophila cellularization, the first tissue-building event in the embryo. We find that zygotically-expressed serendipity-α (sry-α) and maternally-loaded spitting image (spt) are paralogs that share an overlapping, actin-stabilizing activity during cellularization. spt alone is sufficient for cellularization at an optimal condition, but both spt plus sry-α are required at high temperature and when actin assembly is compromised by genetic perturbation. In addition, we find that a paralog interaction may exist, such that a reduced dose of maternal spt results in a compensatory increase in the transcriptional activity of zygotic sry-α.

Since maternal RNAs and protein are often loaded into oocytes long before they act in development, their levels may not be reliable. Expressing overlapping activities, or even the same gene, both maternally and zygotically could then ensure the robustness of early development. In addition, we show for the first time that specific cytoskeletal-based mechanisms promote robust morphogenesis. Since all morphogenesis depends on cytoskeletal remodeling, in embryos and adults, we suggest that robustness-promoting mechanisms aimed at actin could be effective at all life stages.

Program Abstract #156
Actin-Microtubule Cross-Talk at Rhabdomere Terminal Web in Drosophila Photoreceptor Cell Polarity
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Coordinated interactions between cytoskeleton and cell polarity proteins are involved in many polarized cellular processes. Cell polarity genes provide positional cues for elongating photoreceptors during photoreceptor morphogenesis. It has recently been found that stable microtubules in Drosophila photoreceptors, a non-ciliary rhabdomeric eye, were linked to photoreceptor cell polarity. Interactions between microtubules and actin are a basic phenomenon that underlies many fundamental cell biological processes in which dynamic cellular polarity need to be established and maintained. Since Spectraplakin, an actin-microtubule cross-linker, is able to bind microtubule and actin cytoskeletons, the role of Spectraplakin was analyzed in the regulations of photoreceptor cell polarity. The Spectraplakin in developing pupal photoreceptors specifically localizes at rhabdomere terminal web, a sub-rhabdomeric fibrous actin meshwork, which was recently proposed to involve in a secretory trafficking. The spectraplakin mutant photoreceptors showed dramatic mislocalizations of cell polarity markers, adherens junctions, and the stable microtubules. This role of Spectraplakin in cell polarity and adherens junction regulation was further supported by spectraplakin’s gain-of-function phenotype. Spectraplakin overexpression in photoreceptors caused a cell polarity defect including dramatic mislocalization of Crumbs, adherens junctions and the stable microtubules in the developing photoreceptors. Our data suggest that Spectraplakin, an actin-microtubule cross-linker, at rhabdomere terminal web is essential in photoreceptor cell polarity.

Program Abstract #157
Role of Rho family GTPases in morphogenetic events during sea urchin embryogenesis
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During gastrulation, when the basic body plan of the organism is established, changes in cell shape, cell adhesion and cell movements drive the formation of the primary germ layers. In the sea urchin, a complex gene regulatory network has been described that drives the specification of the mesoderm and endoderm. In contrast, far less is known about how these specification events contribute to the actual morphogenetic rearrangements during gastrulation. The Rho-family GTPases Rac, Rho and Cdc42 have been shown to be fundamental for these processes in a number of model systems, and indeed, work in other labs has implicated RhoA in the formation of the primary invagination. Given the many advantages of the sea urchin as a model organism for developmental biology, we aim to better characterize the roles of Cdc42 and Rac in morphogenetic events in the sea urchin embryo. Using a combination of live cell probes and immunofluorescence, we have identified Cdc42 as a potential regulator of Primary Mesenchymal Cell (PMC) motility and skeletonogenesis. PMCs form the larval skeleton and spicules of the larvae, and Cdc42 was enriched in PMCs following ingestion. Further, inhibition of Cdc42 using either dominant-negative Cdc42 or small molecule inhibitors had no effect on the PMC epithelial-mesenchymal transition or PMC ingestion. However, Cdc42 inhibition blocked filopodial extension, PMC alignment, fusion and spicule deposition. Additionally, expression of dominant-negative mutants of Cdc42 and Rac1 alone had no drastic effects on the early embryo, whereas co-expression of both mutants showed a dramatic effect on blastomere organization, raising the possibility that Rac and Cdc42 play partially redundant roles in regulating blastomere organization and polarity. Current efforts are focused on confirming the role of Cdc42 in regulating PMC motility as well as exploring to potential role of downstream effectors such as the formin Diaphanous-2.

Program Abstract #158
The Differential Roles of Evolutionarily Conserved DOCK Family Members in Development
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The evolutionarily conserved Dock family of proteins function as guanine nucleotide exchange factors (GEFs), which form a protein complex with the ELMO family. Upon binding, this complex is recruited to the membrane to activate the Rho GTPase Rac.
modulating actin cytoskeletal movements, including cell migration and myoblast fusion. There are 11 vertebrate Dock family members subdivided into four families, A – D, and the *Drosophila* genome encodes one ortholog per subfamily, making it an excellent model organism for this work. Subfamilies A and B have high domain homology; Myoblast city (Mbc; vertebrate Dock180) and Sponge (Spg; vertebrate Dock4). These proteins have redundant function in some processes, like border cell migration, but not in others, like somatic microtubule development. Mbc’s ability to function upstream of Rac *in vitro* and *in vivo* has been well-documented. However, the GTPase downstream of Spg remains unclear. To determine if these two Dock proteins are functioning redundantly, we explored their roles in the different tissues they are expressed. Herein we show that Spg and Mbc are not functioning redundantly in the somatic muscle, central nervous system (CNS), or the dorsal vessel (DV). Moreover, we provide evidence that Spg is functioning in concert with the Rho GTPase Rap1 to regulate aspects of adhesion. These data coincide with previously published data from our lab, which used the CNS to show the adhesion molecule, N-Cadherin, is capable of genetically interacting with Spg but not Mbc. Together these data indicate Mbc and Spg do not function redundantly and likely have differential downstream targets. Further exploration of this pathway using live-imaging, genetic analysis, and electron microscopy will reveal the specific function of these highly conserved proteins and provide insight into many aspects of development and disease. This work was supported by a pre-doctoral fellowship from the American Heart Association and the UMKC Women’s Council.

Program Abstract #159
dchs1b mediates early morphogenesis and cell fate specification in zebrafish through regulation of actin and microtubule cytoskeleton
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Dachsous (Dchs), an atypical cadherin, regulates planar cell polarity, tissue size, and cell-cell adhesion in *Drosophila* and cultured mammalian cells. Loss of Dchs1 function in mice leads to multi-organ defects and postnatal lethality. Using the zebrafish model, we characterize the embryonic phenotypes of loss-of-function *dchs* mutants to elucidate its function in early vertebrate development. In zebrafish, as in humans there are two *dchs* homologs (*dchs1* and *dchs2*), with two *dchs1* and *dchs1b* ohnologs. We generated two nonsense mutations in *dchs1b* and one nonsense mutation in the *dchs2* extracellular cadherin repeat domain that cause strong/complete loss of function. Whereas zygotic *dchs1b* and *dchs2* mutants show no obvious morphologic phenotypes, defects in cytoplasm-yolk segregation, cortical granule exocytosis, maternal mRNA translocation, and cell division are evident in maternal-zygotic (MZ) *dchs1b-/-* embryos. Epiboly as well as convergence and extension gastrulation movements are impaired in both MZ*dchs1a-/-* and MZ*dchs2-/-* mutants. In addition, Spemann-Mangold gastrula organizer and mesodermal gene expression are aberrant in MZ*dchs1b-/-* mutants. Our studies indicate that many of these pleiotropic phenotypes are caused by abnormal dynamics of the actin and microtubule cytoskeleton. Accordingly, disruption of the actin cytoskeleton in WT embryos causes minor deficiencies in expression of organizer and mesodermal genes and phenocopied the cytoplasmic segregation and cortical granule exocytosis defects of MZ*dachs1b-/-* mutants. Disruption of microtubule cytoskeleton in WT embryos alters the expression of Spemann-Mangold gastrula organizer and mesodermal genes without causing perceptible early phenotypes. Together these data establish a novel function for vertebrate Dchs in early morphogenesis and cell fate specification of one-cell zygote and early cleavage stage vertebrate embryos via regulation of both actin and microtubule cytoskeletons. 

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Program Abstract #160
The DEAD-box helicase Obelus regulates centrosome positioning and epithelial polarity
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During axis elongation in the *Drosophila* embryo, polarized cell behaviors cause the germband epithelium to double in length along the anterior–posterior axis and narrow in width along the dorsal–ventral axis, establishing the structural basis for the body plan. These cell rearrangements are dependent on the asymmetric distribution of actomyosin contractility and adherens junction proteins. The establishment of a planar polarized epithelium coincides with a reorganization of microtubules from a radial array focused at centrosomes to non-centrosomal arrays aligned with apical-basal axis. The mechanisms underlying this microtubule reorganization are not well understood. We found that prior to axis elongation, centrosomes no longer serve as microtubule organizing centers and move away from the adherens junctions to occupy a perinuclear position in the cell. We identified an essential role for Obelus, a member of the Brs/Ascc3 family of splicing factors that is conserved from yeast to humans, in regulating centrosome positioning and adherens junction organization during epithelial remodeling. In *obelus* mutants, centrosomes remain associated with adherens junctions and the core adherens junction proteins E-cadherin, ?-catenin, and Par-3 form aberrant aggregates that fail to support proper cell rearrangements during axis elongation. In addition, a subset of pericentriolar proteins are associated with mislocalized adherens junction proteins at ectopic sites on the apical cell cortex. These results demonstrate a role for Obelus in regulating the distribution of microtubule organizing centers in the cell and controlling interactions between centrosomes and adherens junctions that are important for epithelial morphogenesis. Current experiments are focused on identifying the RNA targets of Obelus required to regulate centrosome positioning and cell polarity. This work was funded by NIH R01 grant GM079340 to J.Z.
During development, the PTEN/PI3K/PDK1 pathway, independently of pAKT, is required for pseudostratification of cephalic neural plate morphogenesis of the cranial neural plate. Our results define a novel function of PTEN on cell shape changes during elongation of neuroblasts, a crucial process required for neural tube development.

Traffic and mechanics within the neuroepithelium: role and mechanism of the interkinetic nuclear migration

The neuroepithelium (NE) or ventricular zone (VZ), from which multiple types of brain cells arise, is pseudostratified. In the NE/VZ, neural progenitor cells are elongated along the apicobasal axis, and their nuclei assume different apicobasal positions. These nuclei move in a cell cycle–dependent manner, i.e., apicalward during G2 phase and basalward during G1 phase, a process called interkinetic nuclear migration (INM). Despite recent advances in understanding its underlying molecular mechanisms, the processes to which INM contributes mechanically remained unclear. We found that knockdown of the cell-surface molecule TAG-1 resulted in retraction of mouse neocortical progenitors’ basal processes. Highly shortened stem-like progenitors failed to undergo basaldward INM and became overcrowded in the subapical space. The overcrowded progenitors left the apical surface and migrated into basal neuronal territories, disrupting a normal brain structure. Similar abnormal delamination occurred when artificial overproliferation was induced by Wnt3a overexpression. These observations, together with the results of in toto imaging and physical tests, suggest that progenitors may sense and respond to excessive mechanical stress, and that INM is essential to preventing nuclear/somal overcrowding, thereby ensuring normal brain histogenesis. To further understand how the INM behaviors of individual cells are functionally assembled or coordinated to maintain a stable NE/VZ structure, we performed computer simulation of the mouse

Program Abstract #161
Modeling Redistribution Cascade of Planar Cell Polarity that Propagates without Attenuation
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Planar cell polarity (PCP) is a cell arrangement in which subcellular asymmetry is arranged in one direction among cells. PCP is a fundamental and essential phenomenon for the development and it appears in various contexts. However, this variety confuses the study of the underlying mechanism. In some cases, it seems that graded expression patterns are responsible for establishing PCP, but in other cases such gradients were not found. The boundary propagation model was proposed as alternative to the gradient model, although the studies were limited to locally induced ectopic polarity. The boundary propagation depends on a redistribution cascade and the polarity has been expected to attenuate along the cascade. In this study, we questioned whether or not the boundary propagation is really incapable of long-range patterning. We theoretically explored the required properties of adhesion components to enable PCP induction in any context, and simulated it on virtual cell arrangements. When a pair of adhesion proteins are assigned heterophilic property to the extracellular domains and homophilic/heterophobic property to the intracellular domains, long-range PCP was induced by either of graded expression patterns and boundary propagation. Surprisingly, it showed robustness against cell arrangement patterns; we could not make a cell arrangement that disturb PCP induction. We also obtained a prediction that an additional homophilic adhesion component participated in PCP induction. The moderation by the additional component differed between PCP inductions by gradients and boundary propagation. Here boundary propagation was shown to be easily achieved by a combination of seemingly simple functions. The robustness of this model should be noted because it provides a mechanism for PCP induction in tissue composed of multiple cell types. It was also suggested that the gradient model and the boundary model were not alternative but functionally distinctive.

Program Abstract #162
The PTEN/PI3K/PDK1 pathway, independently of pAKT, is required for pseudostratification of cephalic neural plate during development
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The tumor suppressor gene PTEN in known to regulate proliferation, cell size, apoptosis and cell polarity. We found that conditional deletion of PTEN in the mouse epiblast is lethal at E9.5 and mutants fail to close the cephalic neural tube. In this tissue, loss of PTEN does not affect proliferation, cell size or cell death, but instead prevents elongation of neuroblasts and prevents formation of a pseudostratified epithelium. Specific epiblast expression of an activated allele PIK3CA, a point mutation allele of PIK3 commonly found in human cancer, causes similar phenotype, demonstrating that the phenotype is caused by elevated levels of PIP3. Genetic removal of PDK1, a kinase that is activated in the presence of elevated PIP3, rescues neural plate defects of PTEN mutant embryos, demonstrating that PTEN/PI3K/PDK1 pathway is required for formation of the pseudostratified neural plate. In the standard pathway, AKT acts downstream of PDK1, but inhibition of the pathway by the pAKT inhibitor MK2206 does not rescue the PTEN phenotype, demonstrating that this role of the PTEN pathway is independent of AKT. The wild-type (WT) neural plate undergoes dramatic changes in apical ring constriction and cell packing in a short period of time, from E8.0 to E8.5, correlating with pseudostratification and elevation of the neural plate. We observed that the defects in pseudostratification and cell elongation of the PTEN mutant neural plate correlate with defects in apical constriction and fewer dynamic changes in cell packing during this stage, suggesting that PTEN/PI3K/PDK1 pathway, independent of pAKT, acts through regulation of apical shape dynamics during morphogenesis of the cranial neural plate. Our results define a novel function of PTEN on cell shape changes during elongation of neuroblasts, a crucial process required for neural tube development.

Funding: EMBO Long Term Fellowship, Beatrui de Pinós Fellowship (Generalitat de Catalunya) and MSKCC.

Program Abstract #163
Traffic and mechanics within the neuroepithelium: role and mechanism of the interkinetic nuclear migration
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The neuroepithelium (NE) or ventricular zone (VZ), from which multiple types of brain cells arise, is pseudostratified. In the NE/VZ, neural progenitor cells are elongated along the apicobasal axis, and their nuclei assume different apicobasal positions. These nuclei move in a cell cycle–dependent manner, i.e., apicalward during G2 phase and basallyward during G1 phase, a process called interkinetic nuclear migration (INM). Despite recent advances in understanding its underlying molecular mechanisms, the processes to which INM contributes mechanically remained unclear. We found that knockdown of the cell-surface molecule TAG-1 resulted in retraction of mouse neocortical progenitors’ basal processes. Highly shortened stem-like progenitors failed to undergo basaldward INM and became overcrowded in the subapical space. The overcrowded progenitors left the apical surface and migrated into basal neuronal territories, disrupting a normal brain structure. Similar abnormal delamination occurred when artificial overproliferation was induced by Wnt3a overexpression. These observations, together with the results of in toto imaging and physical tests, suggest that progenitors may sense and respond to excessive mechanical stress, and that INM is essential to preventing nuclear/somal overcrowding, thereby ensuring normal brain histogenesis. To further understand how the INM behaviors of individual cells are functionally assembled or coordinated to maintain a stable NE/VZ structure, we performed computer simulation of the mouse.
**Program Abstract #164**

**FGF, BMP, and SHH signaling pathways regulate inner ear morphogenesis**

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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. As form and function are critically interrelated, 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. We have characterized the expression patterns of SHH and BMP signaling read-out genes in the otocyst and found several useful probes, including Gli1/3, Ptc1, Id2/3, and Gata3. In addition, we are investigating the use of BMP and SHH reporters that can be bred into our signaling mutant mice. We have begun to examine the changes in these signal readouts initially in FGF3 and FGF10-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. This research was supported in part by Choose Development!, an NSF (grant IOS-1239422) sponsored program through the SDB.

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**Program Abstract #165**

**Live imaging of convergence and extension and dynamic cellular activity in the developing mammalian cochlea**

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The mammalian organ of Corti (OC) consists of a mosaic of mechanosensory hair cells (HCs), surrounded by several types of supporting cells (SCs), which extends along the length of the cochlea. The proper formation of this mosaic is critical for auditory function. In cochlear development, the domain of cells of the prospective OC is initially much shorter and broader than it is at maturity. As the cochlea grows, the OC becomes longer and narrower. This type of cellular rearrangement is often achieved via the process of convergent extension (CE). Cellular rearrangements in the cochlear duct are consistent with CE, but the movement of cells within the embryonic cochlear epithelium has not been directly observed. Using mouse cochlear explant cultures with individual fluorescently labeled cells, we visualized the movement of cells within the developing epithelium *in vitro*. Time-lapse videos generated at various times between embryonic day 14 and birth show movements of cells that are consistent with CE. Both HCs and SCs exhibit protrusive activity, suggesting an active versus passive process, but the protrusions of SCs are much more dynamic, indicating that SCs may be the driving force in the rearrangement of OC cells. Observations of cell movements over several days indicate that most convergent cell migration occurs prior to embryonic day 16, but extension continues until at least the equivalent of post-natal day 1. We observe movement of SCs and HCs whether they are explanted with the underlying mesenchyme or directly onto an artificial substrate, demonstrating that the signals and mechanisms for migration are intrinsic to the epithelium. Both the protrusive activity of OC cells and their migration are dependent on non-muscle Myosin II, as inhibiting its activity causes cells to stop moving. This study is the first to visualize the migration of living cells within the developing cochlea and indicates that active cell movements are necessary for cochlear development.

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**Program Abstract #166**

**EMT During Avian Gastrulation is Independent of Slug Function and E-cadherin Downregulation**

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Epithelial-mesenchymal transition (EMT) is an evolutionarily conserved process during which cells lose epithelial characteristics and gain a migratory phenotype. Although downregulation of the E-cadherin (E-cad) through transcriptional repression by Snail and other repressors has been considered a prerequisite for EMT, recent studies have challenged this view. Here we investigate the relationship between E-cad expression, Snail function and EMT during gastrulation in chicken embryos. IF and ISH analyses showed that although mRNAs coding for E-cad and the closely related protein P-cadherin (P-cad) are downregulated as epiblast cells approach the primitive streak, E-cad and P-cad proteins are retained on the surface of cells undergoing EMT and after they emerge into the mesoderm. Surprisingly, reduction of Slug protein levels using a morpholino, or expression of a dominant negative Slug construct, failed to inhibit EMT. Cells expressing dominant negative Slug that had migrated into the mesoderm showed
increased E-cad protein levels on their cell surface. Cells electroporated with an E-cad expression construct also underwent EMT and migrated into the mesoderm. To determine whether Snail could induce EMT in epiblast cells, wild type and dominant active forms of Snail were expressed in the lateral epiblast. Snail-expressing epiblast cells were never observed to undergo EMT. In contrast, cells expressing the Rho inhibitor peptide C3 exited the epiblast without activating Snail or the mesoderm marker N-cadherin. Finally, Slug immunolocalization in late gastrula embryos (HH stages 7-8) identified some cells that were undergoing EMT without detectable levels of Slug. Together, these experiments indicate that epiblast cells undergo EMT while retaining high levels of E-cad protein on their surface. They also raise questions about the role of Slug in regulating EMT during avian gastrulation. Supported by NIH grant P41HD064559 to PBA.

Program Abstract #167

Aquaporin-3b is required for tissue boundary formation during gastrulation

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Aquaporins are 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. In gastrulating embryos, expression is restricted to the blastocoel roof and the dorsal margin, specifically, the deeper cells of the sensorial layer. Morpholino (MO)-mediated inhibition determined that *aqp3b* protein is necessary for proper blastopore closure. Confocal microscopy of phalloidin-stained gastrula embryos revealed a lack of tissue organization and abnormal cell shape, particularly at the boundary between involuted and non-involuted cells. A major constituent of the extracellular matrix (ECM) is fibronectin (FN). Fibrin FN was absent in cells injected with *aqp3b* MO in a cell-autonomous manner. Non-fibrin, cell membrane-associated FN was not affected, suggesting that the defect is specific for FN fibril assembly or maintenance. FN is required for cell movements during gastrulation, including epiboly, radial convergence and convergent extension. Regulation of FN fibril synthesis has been linked to the PCP pathway, which can enhance cadherin-based cell-cell adhesion that, in turn, initiates polymerization of fibril FN. Preliminary analysis of *aqp3b* MO treated dorsal margin explants has identified defects in convergent extension (CE), which appear to correlate with PCP pathway (Wnt/Fz/Dsh) signaling. Thus, our results suggest a role for aqps in the ability of cells to correctly interact with and assemble the extracellular matrix. Our continued studies are focused on further defining this role at the molecular and cellular level, including signaling pathway interactions, analysis of cell shape and volume and subcellular imaging during cell migration. Funded by NSF.

Program Abstract #168

The Spadetail/Tbx16 Transcription Factor Regulates Zebrafish Mesodermal Cell Migration

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During early vertebrate embryonic development tissues become organized progressively from anterior to posterior along the body axis. In zebrafish, mesodermal precursor cells reside in the posterior end of the body, the tail bud, and gradually move out and differentiate to form somites. During this process they undergo a pseudo-epithelial to mesenchymal transition (EMT) and later undergo the reverse process, a mesenchymal to epithelial transition. Many of the precise movements these cells undergo as they mature have not been described in detail. The T-box transcription factor Spadetail/Tbx16 (Spd) is required both for mesodermal cell morphogenesis and differentiation. Previous data suggest that *spt* mutant mesodermal progenitor cells can initiate but not complete EMT, leaving the pseudo-epithelium but not acquiring all of the correct migratory properties, and therefore accumulate at the posterior end of the embryo. Wild-type cells transplanted to wild-type host pre-mesodermal region contribute to trunk and tail somites, while *spt* morphant cells in wild-type hosts contribute to the most posterior tail somites and the tail fin. I have developed a tail bud explant method to monitor the migration and actin cytoskeleton of cells in the maturing mesoderm. Fixed and live imaging of tail bud explants during somitogenesis revealed that the numbers and types of actin-based protrusions are the same in wild-type, *spt* morphant, and *Spt* overexpressing cells. However, pre-mesodermal cells with altered *Spt* levels cannot orient their cell protrusions properly. Together, these data suggest that *Spt* is required in mesodermal cells for acquisition of migratory polarity during maturation, though not for protrusion formation. Examination of larger scale cell movements in wild-type, *spt* mutants and morphants, and *Spt* overexpressing animals are ongoing.

Program Abstract #169

Discovery of a novel skull defect in a ciliopathy model

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In humans, ciliary dysfunction results in a spectrum of developmental disorders termed ciliopathies. Craniosynostosis, which is typically characterized as the premature fusion of skull bones, is a common feature in ciliopathies, where synostosis of frontal and parietal bones is most common. However, little is known about the etiology of these ciliopathic skull defects. At present, craniosynostosis is thought to occur by precocious osteogenesis between skull bones, but this has not been tested directly in ciliopathic models. The ciliopathic *Fuz* mutant mouse presents with craniosynostosis, however we find that Instead of two pairs of skull bones, frontal and parietal, the *Fuz* mutant develops only one frontal bone pair. Therefore, synostosis can occur not by
premature fusion, but defective morphogenesis at early stages in skull development. We propose a novel mechanism for human ciliopathic synostosis that is driven by early morphological defects rather than precocious osteogenesis.

Funding: NIDCR/NIH (F32DE023272)

Program Abstract #170
Role of MID1/2 in regulation of cell adhesion and facial morphogenesis.

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During embryogenesis, the lip and primary palate form from the union of freely projecting facial process. Specific orofacial epithelia orchestrate the fusion of these facial processes through dynamic changes in epithelial behavior, beginning with presumptive changes in adhesives properties and polarity, followed by contact and formation of an epithelial seam, and then disintegration by a combination of coordinated radial movement, epithelial-to-mesenchymal transformation and apoptosis to enable formation of a contiguous mesenchymal bridge that defines the primary palate. Disruptions to any part of this process results in cleft lip, one of the most common birth defects. Mutations in numerous epithelial-expressed genes, including MID1, PVRL1, and IRF6, have been found in syndromic forms of cleft lip. We are interested in understanding the contribution of the microtubule associated MID1 protein and its paralog and binding partner, MID2, in regulating oral epithelial behavior. Studies in our lab have already demonstrated that in epithelial cells, a dominant negative MID1 mutation, which disrupts both endogenous MID1 and MID2, resulted in defects in cell adhesion and collected cell migration. In this study, we use micropost technology to better understand the role that the MID proteins play in the regulation of epithelial adhesion. This technology enables accurate measurement of cell-cell and cell-matrix adhesive forces. Additionally, we have identified two novel MID2 binding partners, PLEKHA7 – a known regulator of E-cadherin endocytosis and adherens junction strength – and PLEKHA5. Our results are providing novel insight into how the MID complex may control the behavior of orofacial epithelia during formation of the primary palate. Funded by the NIDCR F30DE023697 and the Laurel Foundation Endowment for Craniofacial Research.

Program Abstract #171
Hypomorphic mutations in the novel GTPase rsg1 cause defects consistent with ciliopathies in zebrafish.

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A wide range of human diseases, termed the ciliopathies, are now known to result from genetic lesions that affect cilia structure and function. Ciliopathies have a wide range of phenotypic outcomes, and notably, many human disease alleles are thought to be hypomorphic mutations, underscoring the importance of mutational analysis for understanding these diseases.

Previously our lab has shown that knockdown of the novel GTPase rsg1 has profound effects on ciliogenesis in Xenopus embryos. When we examined a zebrafish double-point mutant in rsg1 isolated in an ENU mutagenesis screen, we found that mutants display many of the developmental and functional phenotypes associated with ciliopathies, including craniofacial abnormalities, limb defects, and loss of kidney function. We also observed cardiac abnormalities, hypopigmentation, and a high rate of retinal coloboma. Surprisingly, we found that cilia were largely normal in rsg1 embryos, suggesting that this mutation does not cause gross defects in cilia structure, but rather may impact cilia function. The rsg1 mutant fish harbor two point mutations in poorly conserved regions of the rsg1 protein, suggesting that this allele may be a hypomorph. Together, these data provide the first genetic evidence for a role for Rsg1 in cilia structure or function.

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Program Abstract #172
A new mutant mouse lines provides support for the vascular hypothesis underlying Oculo-Auriculo-Vertebral Spectrum.

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Oculo-Auriculo-Vertebral Spectrum (OAVS) is a complex condition that affects ~1:5,600 live births. OAVS is noted for its wide phenotypic variability and often asymmetric presentation. Classic features include hypoplasia of the external ear (i.e. microtia) and mandible, particularly around the temporomandibular region. Other frequently reported anomalies include maxillary hypoplasia that is often proportional to that of the mandible, pre-auricular or lateral facial tags, ear canal atresia with associated hearing deficits, and cervical vertebral anomalies. A number of hypotheses have been put forth to explain the marked facial asymmetry, including that of a vascular disruption event during early facial development (Poswillo, 1975). Despite this, there remains little evidence and few genes have been definitively implicated. Here we report a new mutant mouse line that replicates almost all of the classic OAVS phenotype. Using low-pass whole genome sequencing we successfully identified a small rearrangement within the mapping interval that disrupted only a single gene. Significantly, the candidate gene encodes a zinc finger transcription factor that is expressed in appropriate embryonic tissues including the branchial arches that give rise to many of the features seen in patients with OAVS.

Among the known downstream targets of this factor are two genes with prominent roles in vascular development and integrity, potentially providing the first genetic mechanism in support of Poswillo’s original hypothesis. This mutant mouse line provides a rare opportunity to identify the critical genetic pathways involved in this unique phenotypic presentation and thus an array of candidates for causing OAVS in patients.

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Analysis of clefts missing, a novel mouse mutation with pharyngeal arch, cardiovascular and neural tube defects.

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Craniofacial deformities are due to defects in the development of the pharyngeal apparatus, a series of transient embryonic structures that later form the bones, muscle and internal cavities of the face and neck. In both humans and mouse models, craniofacial malformations are often associated with heart and/or neural tube defects. However, a clear understanding of the developmental and genetic basis for the co-existence of developmental defects in multiple organs is not yet clear. Here, we present our results on the analysis of clefts missing (clem), a recessive lethal mouse mutation recently identified in the course of a forward mutagenesis screen in our laboratory. clem mutants were selected due to their severe pharyngeal arch phenotype at E10.5, but a detailed analysis revealed that clem embryos also have cardiac malformations, as well as exencephaly.

The phenotypic characterization of clem mutants at E10.5 indicated that the pharyngeal arches have an unsegmented appearance, a phenotype that is likely due to the absence of ectoderm clefts in clem mutants. At the gross morphological level, this pharyngeal phenotype is similar to that found in hypomorphic Fgf8 mutants or in embryos that have been exposed to high doses of retinoic acid. However, linkage analysis mapped the clem mutation to a 3cM interval on chromosome 12, and our evaluation of candidate genes in this region indicates that the clem mutation does not disrupt any gene known to affect either FGF8 or retinoic acid signaling. Consequently, our data supports that the study of clem mutants will lead to the identification of a novel factor involved in craniofacial development. Additionally, our analysis of cardiovascular and neural tube defects in these mutants is giving us clues about the developmental mechanisms responsible for co-existing developmental defects in clem embryos.

Regulation of zebrafish pharyngeal arch morphogenesis by miR-27

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Cranial neural crest (CNC) cells derived from the dorsal neural tube give rise to most of the facial skeletal elements including the pharyngeal cartilage. The role of microRNAs in the development of pharyngeal cartilage has been poorly investigated. In this study, we show that the miR-27 family is required for zebrafish pharyngeal arch morphogenesis. miR-27 is highly enriched in the pharyngeal arches and miR-27 knockdown leads to loss of all pharyngeal cartilage, as well as an abridged palate in the upper jaw. CNC cell migration is not affected in miR-27 morphants. However, pharyngeal mesenchymal condensation and further chondrogenic differentiation is severely impaired, as revealed by the severe reduction on sox9a and runx2b expression levels as well as loss of major secreted extracellular matrix components. We identify ptk2.2, a focal adhesion kinase, as a potential endogenous target of miR-27. Further studies will reveal how focal adhesion kinase signaling regulates early chondrogenesis in zebrafish.

Eye and tooth development are linked in a teleost model via Pitx2

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The vertebrate eye is a complex structure and its development is one of the earliest events in head morphogenesis. Abnormalities in eye development are associated with human brain and craniofacial abnormalities. A novel model organism in evolutionary biology, the Mexican tetra (Astyanax mexicanus) is a single species that exists as a sighted morph (surface fish) and a blind morph (cavefish). Both morphs display normal development of the eye primordia up until 24 hpf, after which point the eyes of the cavefish undergo degeneration due to lens apoptosis. Eye degeneration results in non functional eyes with altered craniofacial morphology, increased sensory organs and increased number of oral teeth. Here we manually removed the lens from the surface form, at 1-2 dpf and determined the effect of the removal on craniofacial development. There is a dramatic effect on the surrounding craniofacial skeleton such as enlarged orbital bones of the affected side and decreased number of teeth in the pre maxilla and mandible compared to surface control fish. There is an altered expression of key signalling molecules after the surgical lens removal in the mandibular arch of surgery side but not on the control side. Significant changes in Pitx2 expression could be detected in the forebrain and mandibular arch following lens removal. A similar expression pattern of Pitx2 is observed in cavefish following lens apoptosis. Lens degeneration has a negative effect on the level of Pitx2 expression in the forebrain and a positive effect in mandibular arch mesenchyme. This study uncovers previously unknown effects of lens-induced Pitx2 expression in craniofacial development in teleost fish. Additionally, we show that the Mexican tetra may be a valuable model organism in studies related to tooth and eye diseases, such as Reiger syndrome, a rare disorder that is characterized by defects in the anterior eye segment and dental anomalies, both as a result of a Pitx2 mutation.

Jaw-wide dental patterning in the leopard gecko and its modulation by canonical Wnt signaling

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Developmental patterning in the dental arch sets up tooth number, size, and spacing within the jaw. These phenotypes are
hypothesized to be controlled by a reaction-diffusion mechanism acting in the embryo. While the molecular basis for individual tooth patterning has been investigated in cichlid fish and in mice, reptiles have numerous teeth and precisely arrayed, diagonal tooth rows (termed Zahnreihen). The members of an individual Zahnreihe come from adjacent tooth families suggesting the participation of diffusible activators and inhibitors. We use the leopard gecko, *Eublepharis macularius*, to trace the ontogeny of the Zahnreihen. We also compare the prehatching patterns of the dentition to the adult condition in which the dentition is fully functional but new replacement teeth continue to develop. To perturb development, we injected gecko eggs 2–3 weeks prior to hatching with small molecules and assessed the dentition with microCT scans, histology and proliferation analysis. We modulated Wnt activity, a pathway implicated in reaction-diffusion mechanisms for feather and tooth patterning. *In ovo* injection of the Wnt antagonist XAV939 increased the total tooth number present in developing jaws compared to age-matched controls. Cell proliferation was however, decreased during the initiation phase of teeth. In contrast, treatment with Wnt agonist BIO did not affect tooth number but increased cell proliferation in the pre-initiation dental lamina. We propose that lowering Wnt signaling with XAV may derepress another signaling pathway that promotes tooth formation. Alternatively, the XAV may promote precocious differentiation of dentin and enamel that is more readily detected in CT images. Further analysis of molecular pathways and 3D morphometric analysis of the Zahnreihen in treated leopard geckos will clarify the role of Wnt signaling in putative reaction-diffusion mechanisms for tooth patterning. This work is supported by an NSERC Discovery grant to JMR.

**Program Abstract #177**

**A family of FOX genes determines precise spatial patterns of growth and differentiation within facial bone and cartilage precursors**

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FOX genes encode a large family of winged helix/forkhead transcription factors that have been shown to play multiple roles during development. While mutations in a number of FOX genes are known to cause craniofacial defects, how members of this large family coordinate development of the craniofacial skeleton remains unclear. In situ analyses in mice have led to a proposal that FOX genes form a complex expression code, much like the Dlx or Hox genes, that pattern the craniofacial primordia, yet this model remains to be tested at the functional level. In this study, we find that the homologous FOX genes of zebrafish (*foxc1a, foxc1b, foxd1, foxd2, foxf1* and *foxl1*) are also expressed in distinct patterns within the neural-crest-derived pharyngeal arches that are the precursors to the facial skeleton. By manipulating major signaling pathways, we show that these distinct expression patterns result from differential sensitivity of FOX enhancers to Hh, Fgf, Notch, Bmp and Edn signaling. This suggests that FOX genes act as integrators of multiple signaling cascades in the cranial preskeletal mesenchyme. Next, we use morpholino knock-down, TALEN mutants and conditional transgenic misexpression approaches to show that FOX genes have very specific requirements in controlling bone differentiation and cartilage growth in distinct regions of the developing face. In particular, we find that FOX genes act in region-specific manners to prevent the differentiation of dermal bone and promote the development of cartilage precursors. In summary, our evidence in zebrafish supports the model in which FOX genes act specifically on small populations of the neural crest and shape the future facial skeleton by precisely regulating the balance between the self-renewal and differentiation of skeletal progenitors. This work has been supported with funding from the California Institute for Regenerative Medicine and the National Institutes of Health.

**Program Abstract #178**

**Differential effects of Wnt proteins on initiation and maintenance of facial cartilage**

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Precise control of skeletal patterning is necessary to create the unique architecture of the craniofacial skeleton. Previous work in our lab has shown that several WNT genes are expressed in stage- and tissue-specific patterns in the developing avian facial prominences, including many of the so-called non-canonical WNTs. Mutations in one of these genes, Wnt5a, cause craniofacial defects in mice. It is not known whether other WNTs also play a role in facial morphogenesis or which signaling pathways are involved. In the present study, we used conditioned media (CM) or cell pellets expressing putative non-canonical WNT ligands to treat mandibular mesenchyme in micromass culture. Surprisingly, there was variation between responses to different ligands. Wnt4-CM and Wnt11-CM caused a substantial increase (between 2- and 4-fold) in the total proportion of Alcian-blue stainable cartilage (*n* = 16/16 and 24/24 respectively), although total cell numbers were not affected. Local effects were also observed around Wnt11-expressing cell pellets. The number of cartilage nodules near the pellet increased (*n* = 8/8). In contrast, cultures treated with Wnt6-CM or Wnt7b-CM appeared normal for the first few days of culture, but underwent degradation of cartilage after 4–5 days (*n* = 12/12). This was similar to effects previously reported by our group for Wnt5a. Treatments with Wnt5b or the WNT antagonist Sfrp2 had no appreciable effect on cartilage differentiation. Our data suggest that there are two different classes of non-canonical WNTs acting in facial morphogenesis; those that affect the initiation of chondrogenesis such as Wnt11 and Wnt4 and those that affect the long-term stability of cartilage such as Wnt6, Wnt7b and Wnt5a. Future studies will identify specific signaling pathways that mediate these effects. This project was funded by the Canadian Institutes of Health Research (CIHR).

**Program Abstract #179**

**Mutant forms of human WNT5A expressed in chicken cause craniofacial defects**

57
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Missense mutations in WNT5A (C83S or C182R) cause the dominant form of Robinow Syndrome. The main phenotypes include overall short stature, short limbs and facial abnormalities including wide nasal bridge and micrognathia. The Wnt5a null mice recapitulate many of the features of Robinow syndrome cases suggesting that the human WNT5A point mutations decrease function. Furthermore, the severity of Robinow syndrome suggests that mutant forms of the protein may act in a dominant-negative manner on wtWNT5A. Our approach was to clone the wt and mutant forms of WNT5A into avian retroviruses and misexpress them in the lower beak. The main phenotype was shortening and deviation of the lower beak towards the injected side. The most severe and consistent phenotypes were observed with the C83S mutation (7/7). The basis for the deviations was the decrease in proliferation observed 72h post injection. All forms of the virus decreased proliferation to a similar extent. Further analysis may reveal selective effects on the cartilage that differs between the types of virus. Using 3D imaging techniques, we calculated the volumetric changes at 72h on the injected versus non-injected sides of the mandible. There was a 10% decrease (p = 0.001) in the RCAS::WNT5A C83S injected halves of mandibles compared to control RCAS::GFP. To sum up, our results showed that hWNT5A mutations that cause Robinow syndrome in human, lead to shortening of mandible in chicken due to decreased cell proliferation, and probably shortening of Meckel’s cartilage. The possibility that WNT5A mutant proteins change cell elongation or migration will also be examined. Our results can contribute to a better understanding of the cellular and molecular basis for the syndrome and of WNT5A function.

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Program Abstract #180
Age-related changes of acrodont dentition
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Reptilian dentition exhibits large variability in tooth shape, size, number of replacement generations and morphology of tooth attachment. In chameleon, only one generation is initiated and teeth attach firmly to the underlying bone by mineralized attachment tissue, which is called acrodont dentition. As ankylosis is pathological condition in human leading to tooth root as well as surrounding bone resorption, we aim to analyze age-related changes of acrodont dentition in chameleon where ankylosis represents a physiological condition. First, we evaluated morphological changes in the area of surrounding the tooth-bone attachment by micro-computed tomography. We found that the density of all observed tissues was similar at post-hatching chameleon stages. Even the computer-generated 3D images could not depict the exact border between the tooth and bone mass. Border between both tissues could be predicted only based on the presence of osteocytes in lacunae. Next, we analyzed the distribution of calcium, phosphorus and magnesium in the teeth, bone and tooth attachment tissues at three different post-hatching stages. Laser Induced Breakdown Spectroscopy was carried out to map the intensity of individual elements in the transverse sections of the lower jaw. Intensity of analyzed elements exhibited significant differences not only between regions but also depending on the age of animals. Results were confirmed and expanded using microprobe, which allows the determination of element percentage in individual selected areas. In conclusion, we showed age-related changes in morphology and elements distribution, which contributed to very firm attachment of tooth to bone in physiological ankylosis in chameleons. This study was supported by the Grant Agency of the Czech Republic (14-37368G) and Grant Agency of the Univ. of Veterinary and Pharmaceutical Sciences Brno (96/2014/FVL).

Program Abstract #181
Activin-A and Bmp4 act synergistically to regulate the bud-to-cap transition during tooth organogenesis
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Tooth organogenesis depends on genetically programmed sequential and reciprocal inductive interactions between the oral epithelium and neural crest-derived oral mesenchyme. Previous studies indicate that both Activin-A and Bmp4 signaling pathways play critical roles in tooth development. Interestingly, mice lacking Activin-A and mice with neural crest-specific inactivation of Bmp4 both exhibited bud-stage developmental arrest of the lower molar tooth germs while their upper molar tooth germs continued nearly normal morphogenesis. In this study, we show that mice lacking both Activin-A and neural-crest derived Bmp4 expression exhibit early developmental arrest of all tooth germs. To test the hypothesis that Activin-A and Bmp4 act partly redundantly during tooth development, we applied recombinant Bmp4 protein to tooth germ explant cultures and found that addition of exogenous Bmp4 protein rescued morphogenesis of the Activin-A-deficient mouse mandibular molar tooth germ. Furthermore, we found that expression of several secreted Wnt antagonists was upregulated in the tooth mesenchyme lacking Bmp4, Msx1, or Activin-A. Pharmacological activation of canonical Wnt signaling via intraperitoneal lithium chloride injection into pregnant mothers partially rescued mandibular molar tooth morphogenesis in Activin-A-deficient as well as Bmp4-deficient mutant mice. Together, these data indicate that Activin-A and Bmp4 signaling pathways act synergistically to regulate Wnt signaling to drive tooth organogenesis through the bud-to-cap transition. This work was supported by NIH/NIDCR grant R01DE018401 to RJ.
Program Abstract #182
Cell adhesion through α-E-catenin regulates tooth formation by restricting YAP/TAZ activity
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The development of an organ is a highly orchestrated process that transforms an initially simple structure into a complicated form. Such morphogenesis is often driven by cell-cell and tissue interactions, accompanied by finely regulated cell proliferation and differentiation. Although there is a wealth of knowledge regarding the roles of various signaling pathways during morphogenesis, how physical interaction between cells can drive proper tissue formation and downstream signaling remains relatively unknown. The formation of the vertebrate tooth captures several key developmental processes and its relatively simple design makes it an excellent model system to address this question. We genetically perturbed cell adhesion by ablating α-E-catenin in murine dental epithelium. This results in the loss of cell polarity and oriented cell division. Dental epithelium ceases to invaginate and the enamel knot, a key signaling center, does not form and fails to induce the condensation of the surrounding mesenchyme. Yes-associated protein (YAP), a transcription co-factor in the Hippo pathway, was previously found to interact with α-E-catenin and we observed increased nuclear YAP in the enamel knot region, correlating with the downregulation of p21, a cell cycle inhibitor. Deletion of YAP and TAZ (a YAP homolog) in the α-E-catenin mutants rescued the formation of enamel knot, which subsequently promotes further epithelial invagination. These findings show that cell adhesion through α-E-catenin regulates proper YAP/TAZ localization to control proper signaling center formation and thus tissue morphogenesis. Additional experiments are currently underway to assess the relationship between Hippo signaling and cell polarity, as well as its role in the reciprocal interaction between epithelium and mesenchyme.

Program Abstract #183
Sall4, the Duane-radial ray syndrome gene, regulates autopod development through defining localized Shh expression in the mouse hindlimb
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Sall4 encodes a multi-zinc finger domain protein. Human SALL4 gene mutations cause Duane-radial ray syndrome (also known as Okihiro syndrome), in which patients exhibit forelimb skeletal defects. Previous studies in mice using a Sall4 gene trap line implied that Sall4 negatively regulates anterior digit development in the forelimb. However, the Sall4 gene trap line would generate truncated Sall4 that could function as a dominant negative form to inhibit all Sall proteins. Therefore, the role of Sall4 in limb development remains unknown. In order to identify the role of Sall4 in limb development using mouse model, we have generated conditional Sall4 mutants. Limb bud mesenchyme-specific inactivation of Sall4 resulted in no obvious defects, which contrasts to human SALL4 mutation symptoms and Sall4 gene trap line phenotypes. However, inactivation of Sall4 in the lateral plate mesoderm prior to limb development caused hindlimb-specific skeletal defects, such as the loss of anterior digits. Surprisingly, Sall4 mutants did not exhibit forelimb defects. We found that inactivation of Sall4 in the lateral plate mesoderm caused downregulation of Gli3 and upregulation of Hand2 in the nascent hindlimb bud. This alteration of gene expression pattern is correlated with an anterior shift of Shh expressing domain. Moreover, we found that Hox genes and its upstream regulator Ptzf exhibited altered expression pattern in the developing hindlimb buds of Sall4 mutants. Our results demonstrate that Sall4 specifically regulates hindlimb development, but is dispensable for forelimb development. Our data suggest that Sall4 regulates Gli3-Hand2 balance to set up proper Shh expression domain, and that Sall4 is essential for correct expression pattern of the Ptzf-Hox system.

Program Abstract #184
Peptidase Inhibitor 15 protein induces polydactyly in avian embryos via a SHH-independent mechanism
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Previous work in our lab discovered that the secreted protein, Peptidase Inhibitor 15 (PI15), is a novel downstream target and mediator of the Retinoic Acid (RA) pathway. PI15 is expressed in the chicken limb and therefore could regulate outgrowth, skeletal differentiation or patterning. To investigate function we used an embryo manipulation approach as well as proteomics to identify the types of proteins that interact with PI15. Quail cell lines (QT6) were stably transformed to express PI15-flag-IRES-GFP or GFP. Cells were pelleted and implanted into anterior or posterior regions of the hindlimb mesenchyme. Further work will now determine if PI15 effects are mediated by inhibition of HTRA1 and MMP2 or regulation of the ANZ during digit development. This work was supported by a CIHR operating grant to Dr. J.M. Richman
Program Abstract #185
Leptin promotes limb development in *Xenopus Laevis*
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In recent studies, it has been shown that leptin is an important modulator of a variety of physiological processes in vertebrates, such as reproduction, bone remodeling, angiogenesis, and immune function. Leptin treatment has also been shown to accelerate limb development in spadefoot toad tadpoles (*Spea hammondi*) which suggests that leptin plays a role in modulating the timing of early developmental processes and may have a unique role in limb patterning and differentiation. Our current research investigates leptin as a growth factor during early development. Here we show that injection of recombinant *Xenopus* leptin in the periphery (intraperitoneal, IP) (Nieuwkoop-Faber, NF, stage 52-54) caused acceleration in limb development relative to the saline-injected controls. This suggests a direct role of leptin in early limb development. By staining with antibody for phosphohistone H3, we showed that the acceleration in development was associated with increased mitotic cells. Whole-mount *in situ* hybridization showed that leptin receptor expression is distributed throughout the limb bud. To investigate whether this was a direct effect of leptin on the developing limb, we looked at leptin receptor activation through the JAK/STAT pathway. Activation of JAK/STAT pathway has been shown to be involved in physiological processes such as angiogenesis, cell proliferation and immune function. IP injection of leptin increased expression of phosphorylated STAT-3 throughout the developing limb. The expression pattern of the leptin receptor in the limb bud suggests that leptin signaling is a direct pathway involved in the promotion of limb development. These are the first described effects of leptin on morphogenesis in vertebrates, and given that leptin expression is positively correlated with nutritional state at this stage of tadpole development, it may serve as a link between nutrition and early developmental processes. (supported by NIH grant 1 R15 HD057604-01 to EJC).

Program Abstract #186
Homocysteine affects cell-cycle proteins and Pax9 and Sox9 gene products during limb development
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Hyperhomocysteinemia are related to an increased risk of the occurrence of limb defects. The aim of this study was to investigate if Hcy can affect the expression of cell-cycle proteins and gene products of Pax1/9 and Sox9 during limb development. Embryos of Gallus domesticus were treated with 20µmol D-L Hcy/50µL saline at E2 and analyzed at E6. Untreated embryos received only 50µL saline solution. No significant differences in cell proliferation were observed between Hcy-treated embryos and controls. We examined the p53 and p21 proteins, important in the progression of cell-cycle. The number of cells labeled with anti-p53 was significantly higher in limbs of Hcy-treated embryos (97.6 ±1.37) than controls (77.6 ±1.87, p≤0.0001). On the other hand, we observed a lower number of cells labeled with anti-p21 in Hcy-treated embryos (327.6 ±43.73), which differed significantly from controls (707.0 ±96.50, p≤0.05). The expression of PCNA, a protein involved in cell-cycle progression and DNA repair, is fewer in Hcy-treated embryos (59.6 ±2.73) and differed significantly from controls (144.0 ±11.60, p≤0.0001). The products of Pax1/9 genes are essential to early differentiation of mesenchymal cells in chondrocytes. The number of cells labeled with anti-Pax1 in Hcy-treated embryos did not differ from controls. In contrast, the number of cells labeled with anti-Pax9 increased in embryos treated with Hcy (69.3 ±2.14), and differed significantly from controls (51.3 ±0.96, p≤0.0001). Finally, we investigated the expression of Sox9 gene products that are involved in both proliferation and condensation of mesenchymal cells. Embryos treated with Hcy showed a significantly larger number of cells labeled with anti-Sox9 (8.6 ±0.46) compared to controls (4.0 ±0.08, p≤0.0001). Our results indicate that the Hcy-treatment changes the mesenchymal cell dynamics during limb development and provide information to better understand the cellular basis of the effects of Hcy during limb development.

Program Abstract #187
Sox11 is required to maintain proper levels of Hedgehog signaling during vertebrate ocular development
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PURPOSE: The SRY-Box transcription factor Sox11 functions as a regulator of cell fate, survival, and differentiation in the embryonic and adult nervous system. Previously, we have shown that reduced levels of sox11 in zebrafish result in in microphthalmia, coloboma, and specific reduction of rod photoreceptor cells. The goal of this study is to determine the mechanism of action of Sox11 during ocular development in zebrafish and characterize the functional activity of human SOX11 sequence variants.

METHODS: Translation blocking morpholinos were injected into 1-cell stage zebrafish embryos. Embryos were collected at different time points and processed for whole-mount *in situ* hybridization and immunohistochemical examination. Embryos were treated with 2 µM cyclopamine and 75 µM purmorphamine during critical stages of eye development. DNA samples from probands with MAC (microphthalmia, anophthalmia, and/or colobomata) were screened by sequencing the SOX11 coding.
Sonic Hedgehog controlled mechanisms of cochlear outgrowth

Program Abstract #188
Sox4 regulates ocular development upstream of Hedgehog signaling in zebrafish

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Sox4 is a member of the group C SRY-box containing transcription factors, which is required for the establishment of neural properties and neuron survival. Although it has been reported to regulate retinal neurogenesis in mice and Xenopus, its function in ocular development has not been elucidated. Zebrafish have two co-orthologs of the mammalian sox4 gene: sox4a and sox4b. The purpose of this project was to study the role of sox4 during oculogenesis using zebrafish as a model.

We found that sox4a/b was expressed in the zebrafish retina and the onset of expression coincided with the onset of retinal neurogenesis. Morpholino-mediated sox4 knockdown caused ectopic cell proliferation in the ganglion cell layer and a reduction in rod photoreceptors. The rod lineage transcription factor Nr2e3 showed prolonged expression in sox4 morphants, suggesting that rod photoreceptors were specified but did not terminally differentiate. The chordoid fissure also failed to close in sox4 morphants, causing ocular coloboma. Overexpression of sox4 mRNA in wild type (WT) embryos caused cyclopia. Coloboma and cyclopia are known ocular defects related to increased or reduced Hh signaling, respectively. Using quantitative real-time PCR (qPCR) and fluorescent in situ hybridization (FISH), we found that expression of the Hedgehog (Hh) signaling ligand ihhb was strongly upregulated in sox4 morphants. Consistently, the expression of pax2a in the optic stalk, which is induced by midline Hh signaling, was expanded in sox4 morphants. Treatment of sox4 morphants with the Hh inhibitor cyclopamine or co-injection of an ihhb morpholino rescued coloboma.

Based on the fact that sox4 knockdown causes coloboma with elevated ihhb expression, sox4 overexpression causes cyclopia, and the coloboma phenotype can be rescued by cyclopamine or ihhb co-knockdown, we conclude that sox4 controls ocular development by negatively regulating Hh signaling.

Program Abstract #189
Sonic Hedgehog controlled mechanisms of cochlear outgrowth

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The mouse cochlea derives from a ventral extension of the otic vesicle and, over the course of embryonic development, undergoes a complex sequence of morphogenetic changes resulting in its lengthening, coiling and differential patterning into sensory and nonsensory cell types that are essential for hearing. Mouse mutants lacking the secreted morphogen, Sonic Hedgehog (Shh), or the ability to respond to Shh within the inner ear (Smoecko) display a complete absence of the cochlear duct. Currently, the mechanisms by which Shh directs the initial stages of cochlear development remain unclear. To identify candidate regulators of cochlear outgrowth, we compared the expression profile of control and Smoecko inner ears at E11.5 by microarray and identified 154 genes that were significantly reduced in the absence of Shh signaling. Thus far, the expression of 23 downregulated genes was evaluated by in situ hybridization and categorized by their localized patterns in the cochlea: (i) ventral unrestricted (7); (ii) ventromedial wall (7); (iii) ventrolateral wall (6); and (iv) ventral tip (3). These genes were also evaluated for their responsiveness to loss (Smoecko) and gain (ShhP1) in Shh signaling activity within the inner ear. Gas2 stood out as a promising candidate gene for further study based on its pattern of expression along the medial wall of the cochlear duct and corresponding loss and gain in expression in response to respective modulations in Shh signaling. Gas2 encodes a protein with actin and microtubule binding domains with similarity to spectraklins, known cytoskeletal regulators of tissue morphogenesis. At E11.0, Gas2 is primarily localized to the plasma membrane of cells undergoing shape changes that may be a driving force of cochlear outgrowth. Mouse embryos lacking Gas2 function are currently being generated and will be evaluated for their role in morphogenesis of the cochlear duct.

This work is supported by R01 DC006254 to DJE and T32 GM008216-27 to AMR.

Program Abstract #190
Thyroid hormone regulates differentiation and morphogenesis of adult pigment cells

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Thyroid hormone (TH) has been recognized for more than a century as the trigger that stimulates amphibian metamorphosis. Nonetheless, the mechanisms by which this endocrine factor effects local, cell-type specific developmental outcomes remain largely unclear, particularly in vertebrates that do not undergo a discrete metamorphosis. We investigated the roles of TH in the
morphogenesis of the adult pigment pattern of zebrafish. This distinctive pattern is composed of dark stripes of black melanophores alternating with light “interstripes” of yellow xanthophores; both cell types differentiate during post-embryonic developmental stages. Using transgenesis, mutational analyses and time-lapse imaging, we demonstrate that hypothyroid or hyperthyroid states result in severe disruptions to the adult pigment pattern: TH inhibits the development of melanophores while simultaneously promoting the development of xanthophores. We further identify a role for TH in maintaining the pigment pattern during later adult life. Finally, we demonstrate that TH promotes and coordinates the development of other traits including the swim bladder, craniofacial skeleton, barbels and scales. Our studies identify TH as a critical regulator of post-embryonic zebrafish development, and provide valuable new opportunities for dissecting the mechanisms underlying cell-type specific responses to this global endocrine factor.

Program Abstract #191
Modulation of cell death by the inhibitor of apoptosis proteins is required for appropriate urogenital system morphogenesis
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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. Previously, we have shown that loss of LAR-family tyrosine phosphatases results in deficient ureter maturation due to reduced apoptosis. Using mouse embryonic fibroblasts derived from LAR-family triple knockout animals we have confirmed this defect in response to intrinsic and extrinsic apoptotic stimuli, as they are able to activate caspases 8 and 9 while failing to activate caspases 3/7. This is associated with dysregulated activity of the cellular inhibitor of apoptosis protein (cIAP) family, as apoptosis is rescued by co-administration of the cIAP1/2 antagonist BV6. Importantly, the regulation of the IAP pathway is crucial during ureter development as both treatment of E11.5 urogenital systems with BV6 in culture, and genetic inactivation of cIAP1 in vivo, results in an acceleration of apoptotic CND elimination associated with elevated levels of caspases 3/7. Developmentally this is predicted to result in the retrograde flow of urine from the bladder to the kidney, known as vesicoureteral reflux. Accordingly, ink injection assays on postnatal cIAP1/-/- pups revealed VUR in knockout animals, highlighting the importance of regulated IAP activity during ureter maturation.

Program Abstract #192
Ultraviolet-B radiation induces DNA damage and affects the expression of cell-cycle proteins during morphogenesis of freshwater prawn
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The increase of UVB radiation that reaches the Earth’s surface is associated with the reduction of the ozone layer. UVB radiation can penetrate transparent waters and therefore compromise aquatic organisms. *Macrobrachium olfersi* is a prawn that lives in clear shallow water, and their yolky eggs are exposed to environmental radiation during development. The aim of this study was to investigate whether UVB radiation induces DNA damage and interferes on cell-cycle proteins in embryos of *M. olfersi*. Ovigerous females with embryos in early post-nauplius stage (E5) were irradiated using a UVB lamp 6W (310 mW/cm²) for 30 min. Then, females with embryos in early post-nauplius stage (E5) were irradiated using a UVB lamp 6W (310 mW/cm²) for 30 min. Then, irradiation, formation of CPD was observed in embryos kept in natural light (4.73% ± 1.12) and darkness (4.83% ± 0.21). However, UVB-irradiated embryos kept 48 h in light:dark had a significant decrease of CPD (1.62% ± 0.33), in opposite to embryos kept in darkness (4.13% ± 0.68). This reduction of CPD under natural light suggests that embryos of *M. olfersi* have mechanisms to repair DNA lesions, mediated by light. Moreover, there was a decrease in the embryonic cell proliferation, mainly after 48 h of irradiation. Additionally, we observed after 1 h of UVB exposure, a upregulation of PCNA. When kept in darkness, for 48 h after UVB exposure, embryos showed an upregulation of p53. No significant changes were observed in expression of p21. Our results showed that UVB radiation induces DNA damage in embryos of *M. olfersi* and that under natural light these embryos showed a successful CPD repair.

Program Abstract #193
Trim33, a novel chromatin reader is required for the lineage potential of myocardial progenitors in the developing heart.
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In mice, homozygous loss of function of Tgf-β signaling components in early embryonic stages results in congenital heart disease (CHD) while in humans, phenotypic abnormalities associated with hemizygous mutations in Tgf-β signaling components are dose sensitive and subject to environmental modifiers. So far, the complexity of molecular mechanisms underlying CHD is poorly understood and poses challenges to identifying reliable therapeutic targets and regenerative strategies. Recently, tripartite motif (TRIM) 33, a Smad binding protein was shown to regulate Tgf-β signaling. Trim33 interacts competitively with Smad2/3 affecting transcription of Tgf-β-dependent genes via epigenetic modifications. Trim33 global knockout (KO) mice die early in embryogenesis. However, epiblast-specific Sox2-Cre/Trim33 mutants persist until after midgestation and show Ventricular Septal Defects (VSD). Mesodermal induction itself is intact in KO and Sox2-Cre/Trim33 mutants as shown by lack of expression
differences in \( T \), a T-box transcription factor required for mesoderm specification. However, progressive differentiation of a mesodermal subset is affected as demonstrated by in vivo phenotype in myocardial tissue. Cardiac-specific \( Nkx2.5 \) \( Cre/Trim33 \) mutants do not show obvious cardiac phenotypes. We have established a \( Trim33\)-expressed embryonic stem cell line which will enable us to elucidate cell autonomous functions of Trim33 and its regulatory role in Tgf-\beta signaling, using knock out culture systems for cardiomyocyte differentiation. Taken together, we hypothesize that Trim33 plays a non-redundant role in programming terminal differentiation potential of cardiac progenitors via its epigenetic role in Tgf-\beta regulation between time points \( E6.5 \) to \( E7.5 \) in mouse, when precursor cells have relative plasticity. We propose that loss of Trim33 in the epiblast results in a latent effects in morphogenesis manifesting at later time points. Funding Sources: NIH R01DE013085, NIH R01HL074862 to VK

**Program Abstract #194**

**Ectoderm-Mediated Nitric Oxide Signal Formation Regulates Epaxial Myotome Formation in Chick Embryos**

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Chicken embryo myogenesis called myotome formation occurs first in the dorsal somite dermomyotome, which contains myotome precursor cells for epaxial myotome. Preliminary work shows that ectoderm generates large nitric oxide (NO) and paracrine signals to dermomyotome layer, although the role of NO signaling there is unknown. Nitric oxide synthases (NOS) produce NO, a short-lived messenger molecule that regulates gene expression and many physiological activities primarily through NO/cGMP signaling pathway. We hypothesize that ectoderm NO is a necessary signal regulating early myogenesis. To investigate a brief 6-hours NOS inhibitor treatment was used to reduce ectoderm NO signal, given at early embryo Hamilton Hamburger stages (HH 8-19) when myotome formation was first expressed. The NO donor compound was also added to ectoderm to produce earlier NO signaling. The ectoderm NO changes on myotome formation were shown by titin immunofluorescence and confocal microscopy and assessed statistically between control and experimental specimens. The NOS inhibitor result showed a highly significant delay in start of myotome formation by 2 somites from the control, but did not slow the rate of 4 new somites expected in 6 hours incubation. In control, embryos with 1-11 somites expressed no myotome and in NOS treated absence of myotome extended to 13 somites. In both cases, embryos with a higher number of somites showed a progressive biphasic rate of myotome formation. Furthermore, in NOS treated, start of myotome formation did not deviate from the normal right triangle growth pattern. NO donor treatment resulted in strong titin expression in cells located medio-ventral in somites and atypical from the normal dorsomedial myotome formation site. The strong early presence of titin labeled cells indicates NO regulation of initial myotome formation in embryo myogenesis. We conclude that ectoderm NO is a signaling regulator over early epaxial myotome formation. NIH-RIMI (P20MD000544)

**Program Abstract #195**

**Identification of Developmental Checkpoints in Caenorhabditis elegans Regulated by Insulin/IGF and Steroid Hormone Signaling Pathways**

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Organisms in the wild develop with varying food availability. When faced with nutrient scarcity, organisms may arrest development until conditions improve. Such developmental arrest requires a means of sensing the nutritional environment and coordinately stopping diverse morphogenetic processes. The mechanisms by which this systemic arrest occurs are not well understood. The nematode *Caenorhabditis elegans* is an excellent model for studying the connection between nutritional conditions and tissue development due to its short life cycle and invariant cell lineage. We found that when starved, *C. elegans* arrests development at precise checkpoints that occur once in each larval stage. At these checkpoints, nutritional conditions dictate a systemic, all-or-none decision to either remain arrested or to continue tissue development through the larval stage to the next checkpoint. This decision is regulated by insulin and steroid hormone signaling pathways, which are conserved regulators of development and reproduction in metazoans. DAF-2, the insulin-like receptor, regulates the response to food and passage through the checkpoints. DAF-16, a FOXO transcription factor that is negatively regulated by insulin signaling, is required for timely arrest in response to starvation. DAF-16 in turn limits continued development during starvation by inhibiting the activity of DAF-9, a cytochrome P450 required for the production of steroid hormones. Our results identify a novel mode of metazoan growth in which development proceeds from checkpoint to checkpoint. These checkpoints serve the dual purpose of maintaining tissue coordination during developmental arrest, and of ensuring that morphogenesis only progresses when growth conditions are favorable.

**Program Abstract #196**

**Identification of a transporter required to support extreme growth in neurons**

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A central question in growth control of multicellular organisms is how growing organisms maintain proportionality. For example, as animals grow, dendrite arbors of many neurons must expand proportionally to sustain proper connectivity and maintain coverage of their receptive field. However, different types of neurons have different growth requirements, depending on the size and complexity of their dendritic arbors. We hypothesize that the transporter is required to support extreme growth in neurons.
of their dendrite (and axon) arbors. We have been working to identify the cellular machinery that supports neuron growth with a focus on understanding whether neurons with large arbors have specialized mechanisms to support their extreme growth requirements. From a genetic screen, we identified a series of mutants that define a pathway required to support “extreme” growth in neurons: these mutants selectively affect growth of neurons with large dendrite arbors. Remarkably, neurons bearing these mutations grow to a fixed length before growth deficits become apparent, suggesting that these gene products are specifically required to support “extreme” growth. Consistent with this notion, one of these mutants affects a putative transporter that is broadly expressed in neurons and non-neuronal cells but only required to support growth in neurons with large arbors. Using microarray-based expression profiling we found that mutation of this transporter impinges on translational output in neurons with large arbors, but not in other cells. Although the basis for this functional specificity are currently unknown, our identification and characterization of these mutants provide a framework for understanding the specialized mechanisms that support extreme growth in neurons. Funding for this research was provided by an NIMH-R00MH084277, an NINDS-R01NS076614, a Basil O’Connor Starter Scholar Award, a Klingenstein Fellowship in Neuroscience (IZP), and a Benjamin and Margaret Hall International Fellowship at the Univ. of Washington.

Program Abstract #197
Unraveling the control and regulation of vertebrate muscle cell fusion using the zebrafish model system
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Molecular pathways controlling vertebrate muscle development are largely conserved, and we are using the genetic and embryological advantages of the zebrafish model to dissect muscle development and function. Specifically, I am studying the mechanism and regulation of vertebrate muscle cell fusion. As a renewable organ, muscle tissue utilizes fusion to maintain homeostasis and continuously renew and repair damaged tissue, making fusion a vital process for muscle function. Previous work from our lab has shown that slow muscle cells activate differentiation of neighboring fast muscle cells as they migrate laterally within the developing myotome, and I hypothesize that slow muscle also triggers fast muscle cell fusion. I am characterizing fusion-promoting genes activated by smoothened (smo), a transmembrane protein crucial for transmission of Hh signaling that is necessary for zebrafish slow muscle development. Additionally, I am also identifying novel factors functioning in vertebrate muscle fusion. I have isolated zebrafish homologs of the mouse brag2 and tmem8e genes and shown that they are expressed in spatial and temporal patterns expected for muscle fusion-regulating genes. To explore their functional relevance, I am creating null zebrafish mutant alleles of both genes. I am also working to understand the regulation of jamb and jamic, two vertebrate-specific genes that have been previously shown to regulate zebrafish muscle fusion. Further insight and dissection of the zebrafish muscle fusion pathway will increase our knowledge of vertebrate muscle development and lend insight to current and future therapies and regenerative treatments for muscle disease, such as muscular dystrophy and cachexia. Supported by the OSU/NCH Center for Muscle Health and Neuromuscular Disorders Predoctoral Fellowship 2014.

Program Abstract #198
Visualizing lymphatic network morphogenesis in embryonic mouse skin
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National Institutes of Health, U.S.A.
The lymphatic system is a complex endothelial cell network responsible for maintaining tissue fluid homeostasis, immune cell trafficking, and lipid absorption in the gut. Defects in the lymphatic system can result in edema or chronic inflammation while pathological lymphangiogenesis plays a major role in metastasis of certain tumors. Although this intricate system of lymphatic vessels develops throughout the body, little is known about the molecular and cellular mechanisms regulating lymphatic network morphogenesis. To address this issue, we developed an in vivo, whole-mount, tissue imaging system to visualize lymphatic network development in mouse skin. Using this technique, in coordination with mouse genetic approaches, we can now analyze complex morphogenetic processes required for lymphatic development such as initial emergence of LECs from lymph sacs, LEC migration, sprouting lymphangiogenesis, and lymphatic vessel remodeling. The current model of lymphatic development suggests that lymphatic endothelial cells (LECs) differentiate within specified regions of the cardinal and intersomitic veins. LECs then emerge from the blood vasculature, forming a network that invades the skin and other organ systems during embryogenesis. Using our in vivo imaging system in combination with genetic labeling of LECs, we found that a subset of LECs differentiate within the local blood vessels of the skin and then incorporate into the developing lymphatic network. The concept that multiple blood-vascular beds can give rise to LECs during embryogenesis is novel, and may lend insight into mechanisms regulating pathological neo-lymphangiogenesis and regeneration of lymphatic vessels during wound healing and tissue repair.

Program Abstract #199
A novel mechanism of spontaneous fractured bone regeneration
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Medical treatment of fractured bones involves correction of displacement or angulation known as reduction. Although it is considered necessary for bone union and regeneration, in infants severely angulated long bone fractures unite and proper
morphology is often regained without orthopedic reduction. To date, there is no mechanistic explanation for spontaneous bone realignment.

To study spontaneous regeneration of fractured bones, humeral fractures were induced in newborn mice and limbs were left unstabilized. Micro-CT scans showed rapid realignment of initially angulated bones. Surprisingly, reduction of the angulation was not mediated by bone modeling; instead, it involved substantial movement of the two fragments prior to callus ossification. To uncover the mechanism that generates the force required for fragment movement we examined fracture callus, which exhibited asymmetric cartilage formation. Analysis of gene expression profiles, cell proliferation and bone growth revealed the establishment of a functional, bidirectional growth plate at the concave side of the fracture site. This growth plate acts in opposite directions and thereby generates force that drives the two fragments toward straightening, similarly to a mechanical jack. Finally, we demonstrate the regulatory role of muscle force in this process, as blockage of muscle contraction led to disruption of growth plate formation and premature callus ossification, resulting in failed reduction.

This study offers a new paradigm for bone regeneration by describing for the first time the process of natural reduction and the mechanism that mediates and regulates it. Our findings reveal a previously unknown survival mechanism for vertebrates following long bone fracture. Moreover, this novel regeneration program offers a new perspective on treatment of bone fractures.

Program Abstract #200
Frizzled10 mediates Wnt1/3a signaling in the dorsal spinal cord of the developing chick embryo
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In the chick, a dorsal-to-ventral gradient of WNT1/3A activity controls proliferation and the specification of dorsal interneurons via a β-catenin-dependent pathway. However, the identity of the receptors mediating downstream functions remains poorly understood.

In this poster, we show that the spatiotemporal expression patterns of FZD10 and WNT1/WNT3A are highly correlated and demonstrate that FZD10 expression is dependent on Wnt/β-catenin dependent signaling. We then use an in situ proximity ligation assay to reveal an interaction between FZD10 and WNT1/WNT3A. In the presence of LRP6, FZD10 promotes WNT1 and WNT3A signaling using an 8xSuperTopFlash reporter assay in HEK293T cells. Consistent with a functional role for FZD10, we demonstrate that FZD10 is required for proliferation in the spinal cord. Together, our results identify FZD10 as a receptor for WNT1 and WNT3A in the developing chick spinal cord. Funded by NSF-RIU MCB-1244602, NSF-RIU IOS-0950892, NIH-AREA 1R15HD070206-01A1, and NIH-AREA 2 SO6 GM52588 grants to LWB, NIH R01DC009236 grant to SCC, and NIH-RIMI P20MD000262 grant to SFSU.

Program Abstract #201
Functional role of Annexin A6 in trigeminal ganglia assembly
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Cell-cell interactions are required to properly pattern multi-cellular organisms, creating tissues and organs important for organism function. We are using the trigeminal ganglia of the developing chick embryo as mode to understand how two migratory cell types, neural crest cells and placode cells, intermingle and coalesce to form new structures. Because neural crest and placode cells also contribute to other aspects of the vertebrate body plan, defects in neural crest and/or placode cell formation can have severe developmental consequences, warranting further investigation of molecules controlling their formation.

Our prior work uncovered a role for Annexin A6 in mediating cranial neural crest cell epithelial-to-mesenchymal transitions through cadherin modulation, but a potential role for Annexin A6 in forming later neural crest derivatives has not been explored. To this end, we defined a detailed spatio-temporal expression profile for Annexin A6 in the chick head. Our data reveal that Annexin A6 is present in premigratory and early migratory neural crest cells, but its expression declines and is absent in later migratory neural crest cells contributing to the trigeminal ganglia. Intriguingly, Annexin A6 is instead observed in placode cell precursors in the ectoderm and in placode cells interacting with neural crest cells during trigeminal ganglia assembly. Furthermore, Annexin A6 perturbation in either premigratory neural crest cells or placode cell precursors alters trigeminal ganglion assembly. Future experiments will be performed to elucidate the sub-cellular localization of Annexin A6 and to determine how Annexin A6 regulates trigeminal ganglia assembly. Collectively, our results will provide insight into the role of Annexin A6 during the migration and coalescence of these migratory cell types during gangliogenesis. These findings will be directly translatable to the formation of other organs and tissues derived from multiple cell types.

Program Abstract #202
Contact-mediated cell-to-cell signaling during adult pigment pattern formation in zebrafish
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Cell-to-cell signaling is critical to ensure proper pattern formation during development and so the mechanisms controlling the temporal and spatial transport of signaling molecules are of critical importance. Short-range signaling is often mediated by direct receptor–ligand interactions, or pore complexes such as gap junctions. By contrast, long-range signaling is often considered to rely on the establishment of diffusible gradients of morphogens or other factors. In addition to this traditional mechanism, recent studies
have suggested that long-range signaling also can be achieved by direct contact-mediated delivery of signaling molecules via specialized filopodia. Here, we show that xanthophores, which form the yellow interstripes of adult zebrafish, extend very thin processes across multiple cell-diameters. Such processes extend most frequently during the peak stages of adult pigment pattern formation, and interact with the melanophore that will form the black stripes of the adult. Interestingly, processes extended by xanthophores stabilize after contacting melanophores, but not other cell types. Additionally, we find that vesicle-like puncta localize at the tips of these processes and can be left behind if processes retract. Consistent with previously suggested roles for Notch–Delta signaling in pattern formation, we show that a DeltaC fusion protein localizes to filopodia and is transported with vesicle-like puncta as processes extend. Moreover, inhibition of process extension by xanthophores disrupts pigment pattern formation, and mimics the effects of inhibiting Delta–Notch signaling in melanophores. These observations are consistent with the hypothesis that long-range cellular processes extended by xanthophores transmit a Delta–Notch signal to melanophores, and thereby play important roles in pigment pattern formation in zebrafish. [Supported by NIH R01 GM096096 to DMP.]

**Program Abstract #203**

**Neural crest cells act as endocytic sink to regulate FGF signaling for normal cardiovascular development**

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A subpopulation of the neural crest cells, the cardiac neural crest cells (CNCCs), are required for remodeling of the pharyngeal arch arteries (PAAs) into the great arteries of the heart, correct alignment of the outflow tract with the ventricles, and formation of the septum dividing the aorta and pulmonary outlets. CNCC-ablated embryos show an excess of FGF8 signaling in the pharynx, and outflow alignment can be rescued by blocking FGF signaling. This suggests that in the pharynx the CNCCs modulate levels of FGF8 ligand. It has been shown that levels of extracellular FGF8 are regulated during early zebrafish development via endocytosis. Here we show that endocytosis by the CNCCs regulates FGF signaling and is required for proper cardiovascular development. CNCCs endocytosed labeled FGF8 and shuttled the ligand to lysosomes for degradation. Chick embryos treated with a clathrin-dependent endocytosis inhibitor, Pitstop2, displayed alignment defects and abnormal persistence of PAAs. Surprisingly, PAA defects occurred most frequently in embryos treated prior to CNCC migration, long before remodeling occurs. Premigratory CNCCs were targeted by electroporation with dominant negative Dynamin (dnDyn) to block endocytosis. Similar to Pitstop2-treated embryos, experimental embryos showed alignment and PAA remodeling defects. Vascular smooth muscle differentiation was abnormal; adjacent to PAAs we observed ectopic smooth muscle. RNA-Seq, qPCR, and in situ hybridization show that following dnDyn electroporation, FGF8 target genes and negative regulators are upregulated, indicating an excess of FGF signaling. Our model provides a framework for investigating the mechanism by which the CNCCs remodel the paired PAAs into the asymmetric great arteries. The cardiovascular defects observed are consistent with dysregulated FGF8 signaling, and FGF signaling in the pharynx is abnormal. Regulation of signaling through endocytosis is a newly described function of neural crest cells.

**Program Abstract #204**

**R-spondin 3 Regulates Zebrafish Dorsoventral and Anteroposterior Patterning by Inhibiting Zygotic Wnt/β-catenin Signaling**

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The Wnt/β-catenin or canonical Wnt signaling pathway plays fundamental roles in early development and in maintaining adult tissue homeostasis. R-spondin 3 (Rspo3) is a secreted protein that has been implicated in activating the Wnt/β-catenin signaling in amphibians and mammals. Here we report that zebrafish Rspo3 has a unique domain structure and plays a negative role in regulating Wnt/β-catenin signaling. While mammalian and amphibian Rspo3 genes contain 2 furin-like (FU) domains, zebrafish Rspo3 contains 3 FU domain. This additional FU3 is also present in four other ray-finned fish Rspo3 studied but absent in the elephant shark Rspo3. In zebrafish, rspo3 mRNA is maternally deposited and has a ubiquitous expression in early embryonic stages. After 12 hpf, its expression becomes tissue-specific and dynamic. Forced expression of rspo3 promotes dorsoanterior patterning and increases the expression of dorsal and anterior marker genes. Knockdown of rspo3 increases ventral-posterior development and stimulates ventral and posterior marker gene expression. Forced expression of rspo3 abolishes exogenous Wnt3a action and reduces the endogenous Wnt signaling activity. Knockdown of rspo3 results in increased Wnt/β-catenin signaling activity. Further analyses indicate that Rspo3 does not promote maternal Wnt signaling. Human RSPO3 has similar action when tested in zebrafish embryos. These results suggest that Rspo3 regulates dorsoventral and anteroposterior patterning by negatively regulating the zygotic Wnt/β-catenin signaling in zebrafish embryos.

**Program Abstract #205**

**Wnt5a and Wnt11 inhibit the canonical Wnt pathway and promote cardiac progenitor development via the Caspase-dependent degradation of AKT.**

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Wnt proteins, ligands that regulate cell behavior via a canonical signaling pathway that induces β-catenin dependent transcription, promote the expansion of the second heart field (SHF) progenitors that give-rise to the majority of cardiomyocytes. However, activating β-catenin also causes loss of the SHF, highlighting the need to precisely control β-catenin. We recently reported that two
Fibroblast growth factors (Fgf8s) are critical in many aspects of embryonic development and other cellular functions including apoptosis, cell adhesion, and proliferation. We identified mRNA expression of Fgf8a in the retinal ganglion cells (RGCs) and its receptor FGFR1b in surrounding retinal vasculature of 2 day-old zebrafish. Antisense morpholino knockdown of Fgf8a resulted in a significant reduction in the number of RGCs and also a reduction in the corresponding tectal innervation. In addition, Fgf8a morphant embryos have mispatterned retinal vasculature, suggesting a role in neurovascular signaling. It has previously been reported that zebrafish survive and develop normally for 7 days without blood flow as it receives nutrients by simple diffusion. To rule out hypoxia as a cause for the observed Fgf8a morphant phenotype, we utilized the silent heart mutant fish line, which lacks cardiac troponin t resulting in embryos without blood flow, as heart contractility does not initiate. Cell counts from these fish have however, shown a decreased eye diameter and a loss in RGC numbers. Therefore, using immunohistochemistry, we looked to see if loss of RGCs was due to lack of proliferating cells using pHH3 or increased cell death using anti-active caspase 3 in both silent heart and Fgf8a mutant fish. We hypothesize that Fgf8a, from the RGCs, signals through FGFR1b on the retinal vasculature to promote β-catenin degradation. Consistent with these data, treating differentiating ES cells with AKT inhibitor increases the levels of SHF markers to levels similar to those seen after Wnt5a/Wnt11 treatment. Together, these suggest that Wnt5a/Wnt11 inhibit the canonical Wnt pathway and promote SHF development via the Caspase-dependent degradation of AKT.

Program Abstract #206
Heparan sulfate proteoglycans regulate FGF signaling in the zebrafish 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. A heparan sulfate 3-O-sulfotransferase (HS3ST) known to be involved in HSPG synthesis was previously shown to be specifically expressed within the PLLp. We 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.

Program Abstract #207
A role for Fgf8a in neurovasculature signaling in the developing zebrafish retina
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Fibroblast growth factors (Fgf8s) are critical in many aspects of embryonic development and other cellular functions including apoptosis, cell adhesion, and proliferation. We identified mRNA expression of Fgf8a in the retinal ganglion cells (RGCs) and its receptor FGFR1b in surrounding retinal vasculature of 2 day-old zebrafish. Antisense morpholino knockdown of Fgf8a resulted in a significant reduction in the number of RGCs and also a reduction in the corresponding tectal innervation. In addition, Fgf8a morphant embryos have mispatterned retinal vasculature, suggesting a role in neurovascular signaling. It has previously been reported that zebrafish survive and develop normally for 7 days without blood flow as it receives nutrients by simple diffusion. To rule out hypoxia as a cause for the observed Fgf8a morphant phenotype, we utilized the silent heart mutant fish line, which lacks cardiac troponin t resulting in embryos without blood flow, as heart contractility does not initiate. Cell counts from these fish have however, shown a decreased eye diameter and a loss in RGC numbers. Therefore, using immunohistochemistry, we looked to see if loss of RGCs was due to lack of proliferating cells using pHH3 or increased cell death using anti-active caspase 3 in both silent heart and Fgf8a mutant fish. We hypothesize that Fgf8a, from the RGCs, signals through FGFR1b on the retinal vasculature to promote growth its growth and development. Subsequently, we suggest that the retinal vasculature responds by secreting an unknown factor that make a major contribution to the elongating body axis. Retinoic acid (RA) regulation of caudal Fgf8 is thought to be essential for normal body axis extension, but how RA might influence the axial stem cell pool is undetermined. Axial stem cell fate is

Program Abstract #208
RA repression of Fgf8 governs cell fate choices of axial stem cells and their progeny
Thomas J. Cunningham1, Sandeep Kumar1, Thomas Brade1, Lisa L. Sandell2, Mark Lewandoski3, Paul A. Trainor2, Gregg Duester1
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Bipotential axial stem cells residing in the late gastrulation caudal epiblast give rise to both neural and paraxial mesodermal progeny that make a major contribution to the elongating body axis. Retinoic acid (RA) regulation of caudal Fgf8 is thought to be essential for normal body axis extension, but how RA might influence the axial stem cell pool is undetermined. Axial stem cell fate is
dependent on a balance between Sox2 that promotes generation of neural progeny in the ectodermal layer, and Tbx6 that represses Sox2 and promotes mesodermal progeny. Here, we find that Raldh2−/− embryos, deficient in RA synthesis, exhibit decreased Sox2 in the neural plate and an expanded mesodermal Tbx6 expression domain, suggesting an imbalance in the stem cell pool and providing a mechanism for ultimate failure of axis elongation in the absence of RA. We demonstrate in vivo and in vitro that normal body axis extension and repression of Fgf8 requires posterior neuroectodermal RA activity, but not mesodermal RA activity. We place the critical site of RA-Fgf8 antagonism to the neural plate (adjacent to the caudal epiblast), which enables low FGF8 signaling in the anterior primitive streak necessary for (1) normal somite formation, and (2) restriction of mesodermal progeny from the axial stem cell niche in order to maintain a sufficient level of neural differentiation and a stable stem cell pool. In conclusion, we reveal a progeny-derived feedback mechanism whereby signals (FGF8) generated from the stem cell niche are negatively regulated by signals (RA) generated from their progeny to regulate stem cell fate choices and differentiation. Supported by NIH grant GM062848.

Program Abstract #209
RAD6 promotes G1-S transition and cell proliferation through upregulation of CCND1 expression
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Protein ubiquitination plays crucial roles in protein degradation and protein functional regulation. RAD6 is an important E2 ubiquitin-conjugating enzyme, which has been substantially studied in its biochemical functions, while the biological or physical functions of RAD6 remain unclear. In this study, we reported that RAD6 promotes G1-S transition and cell proliferation by regulating the expression of cyclin D1 (CCND1), which is a critical factor in G1-S transition and cell proliferation. Meanwhile, we further demonstrated that RAD6 regulates the transcription of CCND1 probably through affecting H2B monoubiquitination and H3K4me3 levels at CCND1 promoter region. Moreover, Kaplan-Meier plotter survival rate assay and expression level assay suggested that RAD6 and CCND1 are positively correlated with each other in clinical samples of breast cancer patients. Taken together, this is the first evidence of linking RAD6 protein to cell cycle progression and cell proliferation in human cells, which would also shed light on breast cancer molecular diagnosis and prognosis.

Program Abstract #210
Differentiation of F9 Cells into Extraembryonic Endoderm is Accompanied by Metabolic Changes, Increased Levels of Reactive Oxygen Species and Canonical Wnt Signaling.
Gregory Kelly, Gurjoth Deol, Leanne Sandieson, Eugene Klimov, Benjamin Dickson, Jason Hwang
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In mouse, the differentiation of Primitive Endoderm (PrE) from ICM cells is part of an epithelial-to-mesenchymal transition. These events are mimicked in vitro when F9 cells are exposed to Retinoic Acid (RA). An increase in Gata6 and Wnt6 mRNA levels, and an increase in the levels of TRoMA-1 accompany PrE differentiation. Canonical β-catenin signaling is also activated, as evident by increased Tcf/Lef reporter activity and the inhibition of Wnt signaling with XAV-939 blocks differentiation. The level of Reactive Oxygen Species (ROS) also increases, and treating cells with hydrogen peroxide or with RA and antioxidants, which induces or inhibits differentiation, respectively, indicates that ROS are sufficient and necessary for PrE to form. Inhibiting flavoenzymes with DPI suggests that NADPH oxidases are responsible for the increase in ROS, and the specific inhibitors AEBSF and Fulvene-5 are being used to determine if Nox-1 and/or 4 are the prime candidates involved. Studies to identify the source of ROS have revealed high levels of LDHA, PDK1 and phospho-PDH in undifferentiated F9 cells, suggesting that they use aerobic glycolysis instead of oxidative phosphorylation to generate ATP. During RA-induced differentiation these levels drop, while treating cells with dichloroacetate to increase mitochondrial respiration induces differentiation without the need for RA. Together we propose that during differentiation, cellular metabolism switches from aerobic glycolysis to oxidative phosphorylation and the subsequent increase in ROS impacts on the Wnt signaling pathway required for PrE formation. GMK acknowledges research support from NSERC Canada.

Program Abstract #211
Molecular interplay between BMP4 and Fibrillin in embryonic development and Marfan syndrome
Jan Christian, Autumn McKnite, Hyung-seok Kim, Judith Neugebauer
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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 that forms during cleavage plays an essential role in stabilization of the mature ligand, as shown by the fact that mice carrying a knock-in point mutation (Bmp4S2K) that bypasses complex formation die early in embryogenesis due to reduced levels of mature BMP4. The BMP4 prodomain binds to the extracellular matrix protein Fibrillin1 (FBN1), which assembles into microfibrils that provide structural support to the aorta and other elastic tissues. We hypothesized that interactions between FBN1 and the transient BMP4 prodomain/mature ligand complex are required to deposit mature BMP4 into the extracellular matrix to promote stability. Consistent with this model, reduction of Fbn1 gene dosage in mice heterozygous for a null allele, but not the S2K allele of Bmp4, leads to early perinatal lethality and phenotypic defects not observed in either single mutant. Compound mutants exhibit developmental defects associated with reduced BMP4 activity but also show expanded airspaces in the
lungs, breakdown of the aortic elastic matrix and ruptured aortas. These defects are stereotypical manifestations of the human developmental disorder, Marfan syndrome, which is caused by mutations in \textit{Fbn1}. Collectively, our data suggest that \textit{Fbn1} is required to generate a stable, matrix anchored BMP4 ligand, and that BMP4 signaling feeds back to positively regulate expression and/or assembly of \textit{Fbn1} into microfibrils. These findings have significant implications for human health since initial breakdown of the microfibril network in Marfan syndrome would lead to destabilization of BMP4, which would exacerbate matrix disruption and disease progression.

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Program Abstract #212
Expression of A-type Eph receptors during midfacial development
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Eph receptors, the largest known family of receptor tyrosine kinases, interact with their membrane-bound ephrin ligands to direct a number of developmental processes through cell migration, repulsion and attraction. Two subfamilies of Eph receptors exist, the A-type Ephs and the B-type Ephs. While the B-type family has been characterized to be important for craniofacial development, specifically palatogenesis, the A-type family has been implicated to play a role in craniogenesis, but has not been widely studied in secondary palate development, and expression patterns of these receptors in the developing midface are not readily available. Further, recent human genetic data indicate potential importance for the \textit{EPHA3} gene in human non-syndromic cleft lip and palate (CLP). Experiments done in the lab showed distinct, but overlapping, expression of \textit{EphA3} and \textit{EphA4} in the developing palate. The possible functional roles of \textit{EphA3} and \textit{EphA4} were examined through \textit{EphA3}\textsuperscript{-/-} and \textit{EphA3}\textsuperscript{-/-}; \textit{EphA4}\textsuperscript{-/-} mutant mice. However, no overt cleft lip or secondary palate phenotype was observed in these mice. To further examine the possible role of the \textit{EphA} genes in midfacial development, the expression patterns of all nine EphA receptors was investigated across six stages of mouse development through in-situ hybridization. \textit{EphA3}, \textit{EphA4}, and \textit{EphA7} display highly specific overlapping expression patterns at E12.5, E13.5, and E14.5 in the developing tongue, palatal shelf mesenchyme, and nasal septum. \textit{EphA1} and \textit{EphA2} are expressed in the palatal epithelium, and \textit{EphA2} is highly restricted to the developing salivary gland, at all three stages. These expression patterns, together with human genetic data, strongly support \textit{EPHA} receptor involvement in the development of the midface, and overlapping expression patterns suggest that functional redundancy is likely to be at play.

Program Abstract #213
NPY/NPYR signaling is essential for lacrimal gland development
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The lacrimal gland (LG) is a branched ocular organ essential for LG tear production. Similar to the salivary glands, the LG is highly innervated by parasympathetic nerves, which are necessary for both LG function and tissue homeostasis: without innervation secretion is abrogated and the tissue atrophies. We have found that the LG is also highly innervated by these nerves during development, however the role of innervation in LG morphogenesis is not known. Here we show that nerves and neurotransmitter neuropeptide Y (NPY)/NPY receptor signaling are essential for LG epithelial development. Using gene and protein expression analysis, we found NPY and NPY receptors, NPY1R and NPY2R, to be highly expressed by developing nerves and epithelial/mesenchymal cells, respectively. Strikingly, ablation of NPY receptors in a compound mutant mouse resulted in aberrant LG morphogenesis, as shown by reduced epithelial branching, innervation, and epithelial Krt5\textsuperscript{+} progenitors, disorganized secretory acinar cells and impaired myoepithelial cell differentiation. In contrast, treatment of LG with NPY peptide in \textit{ex vivo} cultures promoted branching morphogenesis through increased epithelial progenitor cell proliferation. Together these results suggest that parasympathetic innervation along with NPY signaling are required for LG development, possibly through the regulation of epithelial progenitor cell proliferation and differentiation. Determining NPY’s role in development is crucial to further understanding of the signaling pathways that regulate glandular development.

Program Abstract #214
Deletion of the engrailed genes in rhombic lip-derived cerebellar progenitors reduces growth and alters sonic hedgehog expression
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Sonic hedgehog (SHH) signaling and the engrailed homeobox transcription factors (EN1 and EN2, referred as EN1/2) have important roles in development of the cerebellum. SHH signaling is required for cerebellar growth and foliation by inducing proliferation of granule cell precursors (gpcps), whereas the \textit{En1/2} genes regulate the timing and patterning of foliation as well as growth. A genetic interaction exists between \textit{En} gene function and SHH signaling during development of the Drosophila wing imaginal disk. Using mice in which \textit{En1} and \textit{En2} were conditionally knocked-out in rhombic lip-derived cells that give rise to gpcs and the projection neurons of the cerebellar nuclei (\textit{Rld-En1/2 CKOs}), we tested whether \textit{En1/2} interact with SHH signaling in cerebellar development. First, we made mutants carrying the \textit{Rld-En1/2 CKO} and a gain-of-function Shh transgene (\textit{P\textsubscript{1}-Shh}) that
over-expresses SHH in Purkinje cells (PCs) and results in a larger cerebellum. Surprisingly, we found the double mutants have a smaller cerebellum than Rld-En1/2 CKOs. Second, we determined the expression pattern of components of the SHH pathway using RNA in situ analysis of cerebellar sections during development. We found a decrease in mRNA levels of Shh in PCs, and the SHH target gene Gli1, a readout for SHH pathway activation, in the central zone of Rld-En1/2 CKO cerebella. We also observed defects in the PCs in the same region of mutant cerebella. We conclude that the SHH pathway interacts with En1/2 function with En1/2 being dominant over SHH, and that the PC defects in Rld-En1/2 CKOs are caused by disruption of paracrine signaling between either the cerebellar nuclei and their pre-synaptic partners (PCs) or the GCPs and their synaptic targets the PCs. Future work deleting En1/2 in each cell type will clarify which cellular interaction disrupts PC development and cerebellar growth.

We would like to thank the MSKCC SURP program for supporting this project, and NIMH grant (R37MH085726).

Program Abstract #215
Changes of TRPC6 Channels and Calcium Signaling during Differentiation of Conditionally Immortalized Mouse Podocytes
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The transient receptor potential canonical (TRPC)6 channel is an important ion channel located in podocytes, which plays an essential role in regulating calcium homeostasis of the cell signaling. Podocytes are specialized, terminally differentiated cells surrounding glomerular capillaries, and are the subject of keen interest because of their key roles in kidney development and disease. Here we wonder whether TRPC6 channels undergo developmental changes during the podocyte differentiation, and whether they contribute to the maturation of podocytes. Using morphological, immunofluorescence techniques and electrophysiological methods, we investigated the developmental changes of distribution and expression of TRPC6 and calcium signaling in conditionally immortalized mouse podocyte cell line. Our results showed that the distribution of TRPC6 channels changed with the maturity of podocyte differentiation. The fluorescent intensity of TRPC6 on cell surface increased, which was accompanied by a corresponding increase in the density of current flowing through the channels. The development changes of TRPC6 may contribute to the podocyte maturation and the physiological function. In addition, intracellular calcium fluorescence intensity increased with the maturity of podocyte differentiation. These results have implications for the physiology and development of kidney and will serve as a baseline for future studies designed to investigate developmental changes of ion channel expression in podocytes.

Program Abstract #216
BMP7-TAK1-JNK-JUN signaling pathway governs the proliferation of nephron progenitor cells
Sree Deepthi Muthukrishnan1,2
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Balancing self-renewal and differentiation of nephron progenitor cells is essential for formation of a full complement of nephrons during kidney development. The cellular fate of the nephron progenitors is controlled by multiple signaling cascades and complex molecular interactions with the ureteric bud as well as other progenitor cell types in the niche. We have previously shown that BMP7 promotes proliferation of the nephron progenitor cells through the MAPK pathway and differentiation of the progenitors via the SMAD transcription factors. However, the cellular and molecular mechanisms for these distinct effects of BMP7 on progenitor cell fate are yet to be elucidated. In this study, we show that BMP7 activates the TAK1-JNK-JUN signaling cascade in the highest order (CITED1+) nephron progenitor cells in vitro. We also demonstrate that in vivo, Bmp7 and Tak1 genetically interact and function in the same pathway to govern the self-renewal of the nephron progenitor cells. We found that conditional deletion of Tak1 in the nephron progenitor population results in strikingly smaller but morphologically normal kidneys as a result of coordinated reduction in proliferation in the nephron progenitors and the collecting duct. More importantly, we show that Tak1 is required for renewal of the CITED1+ nephron progenitor cells throughout nephrogenesis. Further, we identified Jun and Myc (cell cycle regulatory genes) as key targets downstream of TAK1- JNK signaling that may potentially be involved in regulation of progenitor proliferation. In vivo and in vitro experiments are underway to determine if Jun is required for self-renewal of nephron progenitors. Taken together, our findings suggest that BMP7-TAK1-JNK-JUN signaling pathway regulates the proliferation of highest order nephron progenitor cells during kidney development.

Program Abstract #217
Regulation of gap junctions during functional differentiation of the mammary gland
Rachael Norris, Paul Lampe
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The mammary gland is fully functional during lactation, when junctions between the epithelial cells of the mammary gland help coordinate the secretion of milk by inner luminal cells and the ejection of milk by outer myoepithelial cells. The gap junction protein, Connexin43 (Cx43) plays a crucial role in lactation, based on evidence that different connexin isoforms cannot replace its function. The C-terminal tail of Cx43 is likely important for this process because it contains several regulatory phosphorylation sites that affect gap junction assembly, turnover, and pore permeability. In support of this idea, the phosphorylation pattern of Cx43 in mammary tissue changes within hours after parturition as seen by Western blotting; however, it is unknown which phosphorylation sites are modulated and how their regulation would affect mammary gland function.

To gain a better understanding of the role of Cx43 during lactation, we used phosho-specific antibodies to investigate which regulatory sites are changed. We found that phosphorylation increases on casein kinase 1 regulated sites S325/328/330, which are
involved in gap junction assembly. Additionally, while Cx43 is reported to be expressed primarily in myoepithelial cells, our results suggest that Cx43 connects myoepithelial and luminal cells. In fact, Cx43 gap junctions are adjacent to, but separate from Cx26 junctions, which are reported to be expressed only in luminal cells. Furthermore, a subset of Cx43 in luminal alveolar cells is phosphorylated on S365 and co-localizes with the scaffolding protein ZO-1. Based on these findings, we have developed a model to account for the expression of Cx43 in both mammary epithelial cell types and for changes in phosphorylation to explain its role in mammary gland differentiation. This work was supported by NIH grants GM55632 and CA149554 to P.D. Lampe and by the UW/FHCRC NIH Interdisciplinary Training Grant T32CA80416 and a fellowship from the Fund for Science to R. Norris.

Program Abstract #218
Withaferin A (WFA)-caused the production of reactive oxygen species (intracellular ROS) modulates apoptosis via PI3K/Akt and JNKinase in rabbit articular chondrocytes
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Kongju National Univ., Republic of Korea

Withaferin A (WFA) is known as a constituent of Ayurvedic medicinal plant, Withania somnifera, and has been used for thousands of years. Although WFA has been used for the treatment of osteoarthritis (OA) and has a wide range of biochemical and pharmacologic activities, there are no findings suggesting its properties on chondrocytes or cartilage. The aim of the present study is to investigate the effects of WFA on apoptosis with focus on generation of intracellular reactive oxygen species (ROS). Here we showed that WFA significantly increased the generation of intracellular ROS as well as inhibited proliferation in a dose-dependent manner. We also suggested that WFA markedly leads to apoptosis as evidenced by accumulation of p53 via Western blot analysis. N-Acetyl-L-Cystein (NAC), an antioxidant, prevented WFA-caused expression of p53 and rescued apoptosis of chondrocytes. We also found that WFA causes the activation of PI3K/Akt and JNKinase. Inhibition of PI3K/Akt and JNKinase with LY294002 (LY)/triciribine (TB) or SP600125 (SP) in WFA-treated cells reduced accumulation of p53 and rescued fragmented DNA. Our findings suggested that apoptosis caused by WFA-induced intracellular ROS generation is regulated through PI3K/Akt and JNKinase in rabbit articular chondrocytes.

Program Abstract #219
The relationship of proteoglycans and cadherins to FGF signaling in Drosophila during gastrulation
Nathanie Trisnadi
California Institute of Technology, USA

Gastrulation of an early embryo involves coordinate cell movements supported by multiple signaling pathways, adhesion molecules, and extracellular matrix components. Fibroblast growth factors (FGFs) have a major role in Drosophila mesoderm migration, however other inputs and/or co-factors to FGF acting in this process are unclear. We performed an ectopic expression screen for genes that impact mesoderm cell migration and identified 11 genes. Here we focus on two genes: E-cadherin (Ecad) and the heparan sulfate proteoglycan (HSPG) Terribly reduced optic lobes (Trol). At gastrulation, upon epithelial-to-mesenchymal transition (EMT) of the invaginated mesoderm, Ecad is downregulated followed by N-cadherin upregulation. We found that changes in levels and distribution of these cadherins are affected by FGF. In addition, we analyzed the role of Trol, ortholog of mammalian Perlecan. Trol is the only Drosophila HSPG secreted from cells, whereas all others such as Syndecan (Sdc) are membrane-associated. trol mutant embryos exhibit defects of early mesoderm migration, where presumably long-range FGF signaling is required for collapse of the invaginated tube. In contrast, Sdc plays a role at the end of the mesoderm spreading process, when FGF signaling occurs between adjacent cells to support cell intercalation required to form a monolayer. Trol, but not Sdc, is important for yet another migratory group, the caudal visceral mesoderm (CVM); but, conversely, Sdc ectopic expression has stronger effects than Trol on pericardial cell differentiation, another FGF-dependent process. Collectively, our data support the view that HSPGs modulate FGF ligand range of action: secreted Trol is able to function at a distance to reinforce long-range ligand-receptor interactions such as those controlling large cell movements; whereas transmembrane Sdc is required in cases where short-range signaling is acting such as in defined domains of cell differentiation or to support cell intercalations.

Program Abstract #220
Mechanisms of Notch-Src synergy in Drosophila
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Notch signaling controls diverse cellular events including proliferation, differentiation, and apoptosis during development and disease. Synergistic interactions between Notch and other genes have been associated with hyperproliferative and invasive phenotypes that can lead to developmental defects and cancer. We performed a genetic screen for modifiers of Notch in Drosophila and identified 359 genes that synergize with activated Notch to cause hyperplasia and invasiveness. Among these genes are the two Drosophila Src genes, Src42A and Src64B. Co-overexpression of activated Notch and Src in the developing wing results in massively overgrown and disorganized wing discs. N/Src synergy causes deregulation of the cell cycle and induces proliferation in regions where the cell cycle is normally arrested in G1, such as the D-V boundary of the wing disc. In addition, N/Src cells display invasive behavior and changes in protein expression patterns characteristic of metastatic cells. Analysis of downstream signaling pathways reveals that both JNK and JAK/STAT are strongly activated; blocking either of these pathways rescues much of the synergistic phenotype. Finally, we performed RNA-sequencing analysis to identify transcriptional targets of N/Src synergy. In
addition to identifying a number of *de novo* target genes, this analysis also revealed that N/Src synergy results in the differential regulation of several known Notch target genes. Taken together, these results indicate that the complex synergy between Notch and Src not only leads to the *de novo* activation of signaling pathways but also affects the regulation of normal Notch-dependent processes.

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**Program Abstract #221**

**Self-association of the APC tumor suppressor is required for the assembly, stability and activity of the Wnt signaling destruction complex**

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¹Carnegie Mellon Univ., USA; ²Franklin and Marshall College, USA

The tumor suppressor Adenomatous polyposis coli (APC) is an essential negative regulator of Wnt signaling through its activity in the destruction complex with Axin, GSK3β and CK1 that targets β-catenin/Armadillo (β-cat/Arm) for proteosomal degradation. The destruction complex forms macromolecular particles we termed the destructosome. While APC functions in the complex through its ability to bind both β-catenin and Axin, we hypothesize that APC proteins play an additional role in destructosome assembly through self-association. Here we show that a novel N-terminal coil, the APC Self-Association Domain (ASAD), found in vertebrate and invertebrate APCs, directly mediates self-association of Drosophila APC2 and plays an essential role in the assembly and stability of the destructosome that affects β-catenin degradation in Drosophila and human cells. Consistent with this, removal of the ASAD from the Drosophila embryo results in β-cat/Arm accumulation and aberrant Wnt pathway activation. These results suggest that APC proteins are required not only for the activity of the destructosome, but also for the assembly and stability of this macromolecular machine. Surprisingly, we have identified a novel *in vivo* isoform of APC2 lacking the ASAD, suggesting that monomeric APC2 may also have a role in Wnt signaling or in APC’s cytoskeletal functions.

**Program Abstract #222**

**Mad linker phosphorylation controls BMP pathway activation and termination**

Abigail Aleman, Marlyn Rios, Daniel Lee, Matthew Juarez, Edward Eivers

California State Univ. Los Angeles, USA

Bone morphogenetic proteins (BMPs) are growth factors that are known to regulate a diverse range of cellular events such as stem cell maintenance, cell differentiation and organogenesis, while dysfunctional signaling of this pathway can result in severe developmental abnormalities. Understanding how rapidly dividing embryonic cells integrate multiple signals at the level of the BMP transduction factor Mad, has been an area of intense focus for many years. Here we describe how phosphorylation of serine 212 in the linker domain of Mad is required for BMP pathway activation and how subsequent phosphorylations at serines 208 and 204 are involved in terminating the BMP signal. We demonstrate these findings using cultured cells and *Drosophila* wing imaginal discs and have identified the phosphorylating kinases. In conclusion, our results describe how new players modulate BMP signals to determine cell fate in the developing *Drosophila* wing and we propose that Mad linker hyper-phosphorylation is a cellular mechanism of fine tuning the BMP-activated Mad (pMad) gradient across its signaling range.

**Program Abstract #223**

**Regulation of metabolism by DBL-1/BMP signaling in *C. elegans***

Cathy Savage-Dunn¹,², James Clark¹,², Vanessa Almonte¹

¹Queens College, CUNY, USA; ²Graduate Center, CUNY, USA

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**Funding:** American Cancer Society New England Division- Virginia Cochary Award for Excellence in Breast Cancer Research Postdoctoral Fellowship (DMH); NIH RO1CA98402 (SAT)
Program Abstract #224
The WAVE/SCAR complex interacts with the two C. elegans junctional complexes and regulates the levels and localization of alpha-catenin/HMP-1
Maria Agapito, Sailaya Mandalapu, Maryam Honarbakhsh, Falshruti Patel, Yelena Bernadskaya, Martha Soto
Rutgers Univ., USA
During C. elegans embryogenesis movements of cells and tissues depend on dynamic reorganization of the actin cytoskeleton. Genetic screens in our lab have identified a branched actin regulation pathway that includes Rac1/CED-10, the WAVE/SCAR/GEX complex and Arp2/3 as essential regulators of epithelial morphogenesis. The actin cytoskeleton is an integral component of the apical junction of epithelial cells. However, the nature of branched actin contributions to apical junction formation and maintenance is still not clear. Previous results by our lab showed that WAVE/SCAR and Arp2/3 are needed for the membrane enrichment of proteins that regulate apical junctions in developing epithelial cells of C. elegans. We will present recent results addressing how Arp2/3 and its WAVE/SCAR nucleation-promoting factor help build and maintain apical junctions. Particularly exciting is the finding that WAVE/SCAR components associate with junctional complex components. In addition, loss of WAVE/SCAR components strongly reduces the apical enrichment of alpha-catenin/HMP-1, the protein that connects the cadherin junction to F-actin. These results provide in vivo organismal evidence for the role of branched actin regulators in assembling and maintaining the apical junction during embryonic development.

Program Abstract #225
The role of ndst3 in zebrafish development
Rebecca Anderson1,2, Jacek Topczewski1,2
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Heparan sulfate proteoglycans (HSP) play many important roles in development. Modifications of HSP are key in determining with what target molecule(s) HSP will interact. The ndst genes encode the bifunctional enzyme N-deacetylation/N-sulfotransferase that modifies HS by catalyzing both the N-deacetylation and the N-sulfation of N-acetylgalcosamine residues. In mammals there are four Ndst genes; expression of Ndst1 and Ndst2 has been detected in most tissue types while expression of Ndst3 and Ndst4 appears more restricted and developmentally regulated. Little is known about the expression or function of the ndst genes in zebrafish. In zebrafish the orthologs of ndst1 and ndst2 have been duplicated. There is only one ortholog of ndst3 and no ndst4 gene. The region of the zebrafish genome containing ndst3 shares genomic synteny with the mammalian region containing Ndst3 and Ndst4, suggesting local duplication of Ndst3 gave rise to Ndst4. This unique situation allows us to investigate the role of the Ndst3/4 ortholog in vertebrate development. We found that the expression of ndst3 is dynamic and first detectable at early somitogenesis. The strongest expression domains are seen in the both the central and peripheral nervous systems, with additional areas of expression found in the pronephric duct, liver and muscle. Due to the fact that HSP have a critical role in Wnt signaling, we tested if ndst3 is regulated by Wnt signaling. Brief LiCl treatment of embryos results in expansion of ndst3 expression domains, suggesting ndst3 may be a target of Wnt. Misexpression of low levels of ndst3 during early development induces midline defects, such as cyclopia and a reduced or missing notochord, suggesting ndst3 may positively regulate Wnt signaling. To investigate the role of ndst3 in post-embryonic development, we used CRISPR technology to create an ndst3 mutant in zebrafish. We are currently characterizing ndst3 gene function in both early and post-embryonic development.

Program Abstract #226
Exploring the Possible Function of Receptor-like Kinases in Plant Epidermal Development
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Stomata are pore-like structures on plant epidermis responsible for regulating gas exchange and respiration processes. The development of stomata is tightly controlled through various signaling pathways. Among the structures involved are receptor-like kinases (RLKs), proteins characterized by an extracellular domain, transmembrane region, and a cytoplasmic kinase domain. In Arabidopsis, receptor-like kinase genes account for nearly 2.5% of protein coding genes and are known to have broad roles in signaling and cell differentiation. However, few studies have identified specific biological functions of these many RLKs and their mechanisms of action. Previously, our group took advantage of genetic resources that specifically enrich stomatal precursor cell state and performed transcriptomic analysis (Pillitteri et al. 2011 Plant Cell). Based on the transcriptome data, we selected three RLKs, tentatively named MV1, MV2, and MV3, that are highly expressed in stomatal precursors. In order to explore their function we have generated transgenic plants expressing MV1, MV2 and MV3 under the control of their respective promoters carrying c-terminal yellow fluorescent protein (YFP) tags. Preliminary screening of the first-generation YFP transgenic lines has shown that all genes are expressed in the epidermis of young developing tissue, confirming their annotated expression patterns. Furthermore, MV1 is enriched in undifferentiated stem cell-like epidermal precursors whereas MV3 appears enriched in discrete locations in the membrane of stomatal precursor cells. In order to explore the function of these RLKs we are generating lines expressing kinase domain ATP-binding site mutants. We expect the mutations to interfere with signaling networks in which the kinases are involved and to incur a dominant negative mutation within each respective RLK, thereby providing a quantifiable phenotype for future measure. This work was sponsored by the Howard Hughes Medical Institute.
Program Abstract #227
Gene duplication and neo-functionalization in the APETALA3 lineage of floral organ identity genes in a non-core eudicot
Kelsey Galimba, Jesus Martinez-Gomez, Veronica Di Stilio
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The duplication of floral MADS-box transcription factors is considered a key contributor to the rapid diversification of flowering plants. These genes underlie the identity and development of the distinct floral organs and, when duplicated, may cause novel morphologies to arise. The B-class genes APETALA3 (AP3) and PISTILLATA (PI) are necessary for the development of stamens and petals in Arabidopsis and other model plants. Loss of function of either one of these genes causes homeotic conversions of petals to sepals and stamens to carpels, leading to a phenotype consisting of two whorls of sepals surrounding two whorls of carpels. The non-core eudicot T. thalictroides (Ranunculaceae) has three orthologs of AP3: ThtAP3-1, ThtAP3-2a and ThtAP3-2b. This species is apetalous, but interestingly, exhibits expression of all three B-class genes in both stamens and petaloid sepals. We ask whether any of the ThtAP3 genes has neo-functionalized to promote ectopic petaloidy of sepals in this species. Using Viral Induced Gene Silencing (VIGS) in T. thalictroides, we down-regulate each gene individually and in combination in order to dissect the role of each paralog.

Program Abstract #228
Gene duplication in the B-class gene affecting petal and stamen development
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In the core eudicot Arabidopsis thaliana, the B-class genes, APETALA3 (AP3) and PISTILLATA (PI), of the ABC model of flower development are responsible for the patterning of petals and stamens along the flower axis. The floral quartet model proposes that the protein products of these genes form heterodimers with each other and tetramers with the E-class protein SEPALLATA3 (SEP3). Functional tests of this model are scarce outside of the traditional model plant systems in the core Eudicots. In order to gain insight into the ancestral condition for protein-protein interactions of these floral patterning factors, we tested the floral quartet model in Thalictrum thalictroides, a perennial woodland herb representative of the sister lineage to all other eudicots. T. thalictroides has four B-class genes, one belonging to the PISTILLATA lineage (ThtPI) and three belonging to the APETALA3 lineage (ThtAP3s). Interestingly, all four B-class genes ThtAP3s and ThtPI are expressed in sepals, a deviation from the classical ABC model. This divergent expression lead us to hypothesize that one or more of these genes may have acquired a role related to the ectopic petaloidy of the sepals. We used a Yeast-Two Hybrid assay to characterize the ability of these B-class proteins to form homo- and hetero-dimers with themselves and each other as well as with ThtSEP3. The investigation of B-class protein interactions in this non-core eudicot provides insight into the role of gene duplication in increasing the network of interacting proteins that in turn affect diverse floral phenotypes. This research was supported in part by Choose Development!, an NSF (grant IOS-1239422) sponsored program through the SDB.

Program Abstract #229
Notch signaling is necessary for cell fate conversion in sponge development
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In animals, transformation of a cell from one differentiated state to another is a well-established phenomenon in regeneration and oncogenesis, but the evolutionary origin of the transformation mechanisms remains unclear. Notch signaling has been implicated in cell fate conversion of epithelial tumor cells during hypoxia-induced metastasis in humans, of cardiomyocytes during heart regeneration in in zebrafish, and of circumoral ectodermal epithelial cells during metamorphosis in a sea anemone. This raises the possibility that Notch signaling was involved in cell fate conversion in the last common ancestor of bilaterians and cnidarians (Eumetazoa). To test whether Notch signaling directs cell fate conversion in animals that diverged prior to the origins of eumetazoan lineages, we examined the subcellular localization, function and the mode of activation of the Notch receptor during development of the sponge Amphimedon queenslandica (Porifera, Demospongiae). Here we show that the A. queenslandica Notch intracellular domain (NICD) localizes to the nuclei of larval sensory epithelial cells and juvenile choanocytes (feeding cells) during transformation into pluripotent stem cells, the archeocytes, and that nuclear translocation of NICD is required for cell fate conversion in these cells. In addition, we reveal that nuclear translocation of NICD in the larval sensory cells requires external environmental stimuli, and subsequent actin-driven cell shape changes mediated by calcium signaling. Our data support an ancient evolutionary origin of Notch signaling-dependent cell fate conversion in animals, and suggest that environmentally regulated cell fate conversion may have been integral to the development of the early animal ancestors.

Program Abstract #230
Evo-devo on coral reefs: evolutionary changes in bone remodeling during late development have been critical to the adaptive radiation of the damselfishes
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Understanding the developmental changes that underlie adaptive radiations is a major goal of evolutionary-developmental biology. Comparative developmental studies of multiple, closely related species are needed if we are to determine how divergence in
morphogenesis has produced adaptive radiations in form, function and ecology. The ideal study group for such an endeavor would meet the following criteria: 1) their phylogeny is well-resolved; 2) there are thorough descriptions of the lineage’s anatomical diversity; 3) there are thorough descriptions of the lineage’s ecological diversity; 4) the relationship between morphology and ecology is known; and 5) multiple, diverse species from the same lineage are amenable to developmental study. The marine damselfishes (Pomacentridae) meet all of these criteria. This highly successful group of near-shore reef fishes is one of the dominant fish fauna on all coral reefs and it contains such well-known species as the anemonefishes (“clownfishes”). Although the functional morphology of damselfish feeding (i.e., skull morphology) has evolved rapidly, this has not been the result of changes in cranial patterning during early development. It is rather post-metamorphic shifts in cranial remodeling during late development that appear to hold the key to their adaptive divergence. This is likely due to the fact that, like most marine fishes, all damselfish larvae are zooplanktivorous regardless of their adult feeding ecology. We discuss ongoing work aimed at determining the evolutionary changes in bone remodeling that have produced the existing functional diversity seen among damselfish skulls. This research has important implications for understanding the adaptive divergence of thousands of marine fish species.

Program Abstract #231
Determining the Presence of Mesoderm in the Ctenophore P. bachei through Gene Expression Analysis
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There has been a long debate in the scientific community over the oldest surviving metazoan lineage. Traditionally it has been taught that Porifera, the sponges, occupy that niche possessing a body plan without true tissues; however recent phylogenetic analysis suggests that Ctenophora, free floating planktonic predators with a possible mesoderm layer and complex organs, may be the oldest extant metazoan lineage. In this study we searched for the presence of mesoderm in Ctenophora by examining the genome of Pleurobrachia bachei for the muscle and mesoderm specific tropomyosin, calponin, and β-catenin. Expression of all genes were found in the muscular contractile regions of P. bachei, however there was weak expression in the ectoderm as well. When phylogenetic analysis was performed, homologues of all genes were found in choanoflagellates (single celled ancestors of Metazoa) as well, indicating that these proteins could have served an unknown function in the common ancestor of Metazoa and were co-opted for muscle either in the common ancestor or independently in the different lineages. Although this evidence points to a common origin for all muscle in Metazoa (which would have to come from a mesoderm), it is not definitive proof against independent evolution. New analyses will be performed using genes specific to the mesoderm in bilaterians that have been identified in the P. bachei genome (such as WNTs, brachury, and MIER) and determining their spatio-temporal expression to resolve if they are used to produce mesoderm in Ctenophora, and to extrapolate the body plan of the common ancestor of Metazoa.

Program Abstract #232
Iontropic Glutamate Receptors In The Developing Nervous System of Invertebrates
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Ctenophores are predatory gelatinous zooplankton that swim using synchronized beating of eight rows composed of fused cilia. To manage this behavior the animal uses a sole neurotransmitter, glutamate. Ctenophores exhibit an expansion of ionotropic glutamate receptors (iGluRs) when compared to other metazoans. These receptors are known to be involved in synapse development in other organisms, but neuronal development is poorly understood in ctenophores. We have shown that iGluRs are differentially expressed in early embryos. Not only is iGluR expression evident in the embryos of ctenophores but also in embryos of other invertebrates such as ascidians. It is not known if these iGluRs are functional in developing invertebrates. I am taking a comparative genomics approach to ask if the expression of iGluRs is related to the development of the nervous system and the ctenophore apical organ. By using antagonistic and inhibitory studies I plan on investigating whether glutamate is modulating the development of the nervous system in invertebrate embryos.
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Program Abstract #233
Whole body regeneration in the sea-anemone Nematostella vectensis: A transcriptional blueprint
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Regeneration ability is essential for the homeostasis of all animals as it allows the renewal and repair of tissues and body parts upon normal turnover or injury. This ability varies greatly in different animals - while mammals have a limited capability for regeneration which is restricted to tissues, basal animals such as cnidarians can undergo whole body regeneration upon dissection into smaller parts. In this study we have used the sea anemone Nematostella vectensis, a cnidarian model animal, wherein adult polyps were dissected into two parts and the transcriptional response was studied over the course of regeneration by analyzing the regenerating regions at certain time points after dissection. The regeneration transcriptional profiles of responsive genes were studied using RNA-seq performed on the Illumina platform so as to define different clusters of genes in terms of their time course, such as genes whose
expression increases or decreases along the process and genes with early or late response patterns. The different groups of genes thus defined were characterized according to their GO (gene ontology) assignations and according to their affiliation to the major developmental signaling pathways. While some of the genes thus identified are known to be active in several regeneration models such as planaria, we have revealed novel and sometimes surprising candidates that may participate in this interesting developmental program. The evolutionary comparison between regeneration in the different model systems may reveal the basic mechanisms playing a role in this fascinating process and can potentially contribute to enhance injury treatments in man.

Program Abstract #234
Understanding animal polarity: Two phases of partitioning during early embryogenesis of the sea anemone Nematostella vectensis
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How animal-vegetal (A-V) polarity is specified during early development of non-bilaterian animals is unclear. In some bilaterian animals, Lethal Giant Larvae (LGL) and PARtitioning-defective proteins (Par) are conserved components of cellular polarization during early embryogenesis. Par proteins and their role in establishing embryonic asymmetry have been widely studied in bilaterian development but not in more basally branching animals; begging the question of whether the same molecular mechanisms are conserved for specifying A-V polarity. We address this question by characterizing the localization of different components of the Par complex during early development of the basally branching cnidarian sea anemone Nematostella vectensis. Immunostaining using antibodies made against NvLGL and NvαPKC shows that these proteins distribute throughout the egg and embryo without any clear polarization. However, in later stages when the embryo is an epithelial layer, they become asymmetrically distributed. Similar to some bilaterians, NvLGL localizes in the basolateral cortex and NvαPKC at the apical zone of the cell. Confirming results were obtained when we over expressed mRNA-reporter constructs. In addition, we tested the cytoskeletal dependency of their intracellular localization by treating embryos with drugs against microtubules and actin cytoskeleton. During earlier stages the localization of both NvLGL and NvαPKC is not affected by either treatment with no clear polarization observed. Likewise, during gastrulation, the basolateral localization of NvLGL and the apical distribution of NvαPKC remain, suggesting that the localization of both proteins is associated with either stable microtubules or actin cytoskeleton. These two phases of embryonic/cell partitioning suggest that different mechanisms establish A-V polarity in N. vectensis.
Founding Source: NSF

Program Abstract #235
G protein regulation of the actin cytoskeleton in the early sea urchin embryo
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Eggs and early embryonic blastomeres are characterized by their large volumes and minimal actin cytoskeletons, with large stores of contractile proteins sequestered in the cytoplasm. The actomyosin cytoskeleton in the early embryo is primarily utilized to increase membrane surface area (through microvilli) and for cytokinesis, but with the exception of a few model organisms, little is known about how the actin cytoskeleton is regulated during the earliest stages of development. Rho GTPases (RhoA, Rac and Cdc42) are critical modulators of actin, and while the role of RhoA in cytokinesis is well established, much less is known about how Rac and Cdc42 contribute to cytoskeletal organization in a spherical, nonpolarized cell. To better understand the regulation of actin in the early embryo, we performed live cell imaging of actin dynamics during the first cell division of the sea urchin embryo. Studies to date revealed that in addition to microvilli and the contractile ring, there are other distinct populations of actin filaments that underwent dramatic changes during the first mitotic division. A thin layer of cortical actin could be detected that underwent a transient thinning upon anaphase onset, only to recover shortly before cytokinesis. Additionally, there was an explosive elaboration of cytoplasmic actin occurring just prior to the metaphase-anaphase transition, which initiated at the cell surface, and extended inward towards the cell center. Interestingly, RhoA activity was required for all actin structures in the egg, whereas neither Rac nor Cdc42 were required. However, expression of activated Rac mutants resulted in cytokinesis failure through a mechanism distinct from models proposed in adherent cultured cells. Current efforts are focused on identifying the downstream effectors by which Rho modulates actin in sea urchin eggs, as well as the mechanism by which Rac antagonizes cytokinesis. This work was funded by NSF MCB0818729.

Program Abstract #236
The permeability barrier in Pristionchus pacificus nematode embryos is disrupted by its host’s sex pheromone
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Understanding the necromenic interaction between the nematode Pristionchus pacificus and its beetle host will offer insight into nematode-invertebrate relationships that can be applied to developmental processes on a broader scale. P. pacificus is attracted to (Z)-7-tetradecen-2-one (ZTDO), an oriental beetle pheromone. Wildtype embryos become arrested in the presence of ZTDO, though the mechanism behind ZTDO’s effects is unclear. We hypothesize that the molecule either clogs pores and prevents gas exchange or enters the embryos and disrupts cellular function. Using the lipophilic dye FM4-64 that is known to stain membrane layers of pre-anaphase II oocytes in C. elegans, we stained ZTDO-exposed embryos and found that the dye passes the permeability barrier and
stains the embryos’ plasma membranes, suggesting that ZTDO is disturbing the permeability barrier of the eggshell, rather than simply preventing gas exchange. In ZTDO unexposed embryos, only the trilaminar eggshell is stained by FM4-64: the dye does not pass the permeability barrier. It is also possible that ZTDO is preventing embryogenesis by degrading the eggshell, so ZTDO-exposed eggs would easily have their plasma membranes stained by FM4-64. The gene that mediates the nematode’s interaction with ZTDO has been identified, obi-1. The permeability of obi-1 deficient ZTDO-exposed embryos will be determined, as will the effect of ZTDO on different cell stages of the embryos and their degrees of permeability. This information can provide insight into the developmental stages of the embryo that are susceptible to prevention techniques of parasitic nematodes.

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Program Abstract #237
It's all in the timing: pre-hatching juvenile development in the necromenic nematode Pristionchus pacificus
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Molting, or the periodic shedding of the larval cuticle, appears to be a highly constrained system in nematodes. Although even single gene mutations in heterochronic genes in Caenorhabditis elegans can cause changes to the molting cycle, such as supernumerary molts in lin-4 mutants, all nematodes appear to have a conserved series of 4 juvenile molts. Pristionchus pacificus is a beetle-associated nematode in the family Diplogastridae whose first molt, unlike most other nematodes, occurs prior to hatching. Previous studies have shown this pre-hatching molt to occur in multiple genera within the Diplogastridae, but a clear timeline of these events in an individual species has yet to be shown. To precisely characterize the timing of pre-hatching development in P. pacificus, we tracked individual worms from the middle J1 pre-hatching stage through the J1/J2 transition and hatching. We found that active movement ended 21 hours after eggs were laid, followed by lethargus, a restful period required for proper molting, and hatching immediately afterwards. We inferred that edysis, or exiting of the old J1 cuticle, occurred between the termination of lethargus and the onset of hatching. We further corroborated the pre-hatching molt with the isolation of two genetic mutants that exhibited molting deficiencies at every juvenile stage, including a protracted and often-aborted pre-hatching molt. Finally, we examined the expression profile of an ultraspiracle homolog, Ppa-pnhr-1, as a developmental marker for P. pacificus molting cycles. Our study indicates the presence of a pre-hatching molt in P. pacificus and supports the hypothesis that this developmental novelty is due to a shift in the relationship between developmental timing and hatching. Funding provided by the Peter Bellinger Student Research Award (VML) and the CSUN Probationary Faculty Support Program (RHL).

Program Abstract #238
Identification of new genes in the nematode Caenorhabditis briggsae that limit the response to EGF signaling during vulval development
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Tight regulation of cell proliferation is critical for proper development. Loss of control in this process can result in cancerous growth. The reproductive organ of the nematode Caenorhabditis briggsae (C. elegans), the vulva, is an established paradigm to study cell proliferation during organogenesis. Studies in C. elegans have shown that Multivulva (Muv) mutations cause uncontrollable and excessive cell division. This phenotype is caused by mutations in genes that regulate the division and proliferation of vulval cells. Molecular genetic studies have shown that some of these genes belong to conserved signal pathways mediated by EGF-Ras, Notch, and Wnt.

In our laboratory Caenorhabditis briggsae (C. briggsae), a close relative of C. elegans is used to understand evolutionary changes in vulval development. To this end, the roles of Muv genes and their genetic networks are being investigated. So far 14 new mutations have been identified in 7 C. briggsae genes that give rise to a Muv phenotype. To localize these genes to small genetic intervals on chromosomes and facilitate functional studies, a new cost-effective mapping method has been developed. Using this method, the identities of 3 novel Muv genes that encode orthologs of C. elegans lin-1 (ETS), lin-31 (Winged-Helix) of the EGF-Ras pathway, and pry-1 (Axin), a component of the Wnt pathway have been determined. Mutations in the remaining 4 genes map to regions that lack orthologs of known C. elegans Muv genes and they also function in an EGF signaling-dependent manner. Overall, the findings demonstrate that the genetic program underlying the vulval developmental process in C. briggsae include both conserved and divergent components. In the future, the mutant strains will allow further exploration of genetic differences between C. elegans and C. briggsae. The results will lead to a better understanding of the mechanisms of cell proliferation during normal development as well as during disease.

Funding: NSERC

Program Abstract #239
Arthropod segmentation: when does it end?
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Variation in the number of posterior segments is a major source of arthropod morphological diversity. Posterior segments are defined in arthropods as the hexapod abdomen, crustacean and myriapod trunk, and chelicerate opisthosoma, and while segment
number varies widely, there appears to be a general phylogenetic trend toward reduction in posterior segments. While the general features of segmentation are understood (e.g., long vs. short germ band growth), the developmental genetics that govern segment number are not as well understood. In several species, loss, or partial loss of function, of the genes caudal, wnt8, or even-skipped results in a loss of posterior segments, suggesting a conserved role for these genes in segment generation. However, the mechanisms that restrict arthropod segment number are unknown. The spatial and temporal collinearity of vertebrate Hox gene expression are believed to provide both temporal and spatial control over axial elongation (i.e., counting the number of segments). Functional genetic studies in mice have shown that termination of somitogenesis, which is at least superficially similar to arthropod segmentation, is regulated by the time of expression of the vertebrate homolog of the Hox gene Abdominal-B (AbdB). Interestingly, we observe an unexpected addition of terminal segments in the adult fly Photuris subsequent to experimental depletion of AbdB expression during metamorphosis. Here we present our work toward understanding the relationship between the most posterior acting Hox gene and the mechanism that controls arthropod segment number in the red-flour beetle Tribolium castaneum and branchiopod crustacean Thamnocephalus platyurus.

Program Abstract #240
Detection of 18-Wheeler in developing Drosophila melanogaster embryos
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3% percent of children born in the U.S. have a birth defect, most commonly affecting the heart, lip and palate, or spinal cord. Development of these organs begins during the first month of development and require extensive cell migration. To understand the genetic and molecular mechanisms controlling organ formation, we study salivary gland formation in the Drosophila melanogaster embryo. Work by others has identified genes required for positional specification of the glands. However, the link between gland specification and invagination remains unclear. Previous observations by Kolesnikov and Beckendorf (Dev. Biol. 307: 53-61 (2007)) showed that embryos lacking the Toll-like receptor, 18-Wheeler (18W), have defects in gland invagination. We hypothesize that 18W acts as a cell surface receptor and signals to the cytoskeleton to drive the changes in cell morphology affecting invagination. To test this hypothesis we are generating a polyclonal antibody against the 18W protein to detect its location in early embryos. We used PCR to amplify a fragment of C-terminal region of the 18W protein. The PCR product was digested and ligated into the bacterial expression vector, pTYB21 (New England Biolabs), and transformed into E. coli strain DH5a. pTYB21 + 18W plasmids were transformed into E. coli strain ER2566 to induce protein expression. The fusion protein will be purified and by chitin column affinity chromatography and intein autocatalysis. We report the successful cloning, expression, and purification of the C-terminal domain of the 18W protein. Determining when and where the 18W protein is expressed will allow us to make testable predictions about its function and possible interaction partners. This will contribute to understanding how developmental signals lead to coordinated changes in cell behavior that are required for organ development.

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Program Abstract #241
Tribolium segmentation: what's going on in the growth zone?
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Segmented animals have been very successful evolutionarily and are found in major clades as phylogenetically distant as vertebrates and arthropods. Developing body segments during embryogenesis has typically been viewed as a regular process, relying on a segmentation clock to pattern budding segments and high levels of mitosis in the posterior to drive axial elongation. Our analysis of the flour beetle, Tribolium, throughout the entire period of segment addition, demonstrates that segmentation is an irregular process. Segments are added at varying rates. Furthermore, much of the rapid segment addition is driven by unexpectedly high rates of cell rearrangement, demonstrated by the differential behavior of marked anterior and posterior blastoderm cells. Although our computer model of the posterior growth zone without any cell division successfully mimics the pulse of rapid segmentation late in germband elongation, pharmacological experiments with inhibitors of cell division suggest that cell division is nonetheless required for both segmentation and elongation. This work was funded by NSF.

Program Abstract #242
Timing and concentration dependent actions of Ultrabithorax during wing development in the red flour beetle, Tribolium castaneum
Ferran Borras-Castells, Yoshinori Tomoyasu
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The two pairs of wings that are characteristic of ancestral pterygotes (winged insects) have often undergone evolutionary modification. In the fruit fly, Drosophila melanogaster, differences between the membranous forewings and the modified hindwings (halteres) depend on the Hox protein Ultrabithorax (Ubx). The Drosophila forewings develop without Hox input, while Ubx represses genes that are important for wing development, promoting haltere identity. It has been shown that, instead of acting as a master regulator residing on the top of the wing gene network, Ubx acts as a micromanager directly regulating a variety of genes at several different hierarchies of the wing gene network in Drosophila.
In beetles, such as Tribolium castaneum, it is the forewings that are modified (to form elytra), while the hindwings retain relatively ancestral flight wing morphology. We have previously shown that elytra result from a Hox-free state, despite their diverged morphology, and Ubx promotes wing development by canceling the modification. This counteracting effect of Ubx in beetle hindwings presents a previously uncharacterized mode of Ubx action during insect wing diversification. To gain further insights into the mode of Ubx action in beetles, we have analyzed the timing and concentration dependency of Ubx in the Tribolium wing development. Ubx RNAi with a variety of dsRNA concentrations and injection timings revealed that different characteristics of the hindwing require different concentrations of Ubx at different developmental timings. These results support the idea that Ubx also acts as a micromanager in the Tribolium wing development despite the varying role of Ubx between flies and beetles.

Program Abstract #243
Lobes or Gills: Insights into Insect Wing Origin Provided by Functional Analysis of vestigial in Tribolium
Courtney Clark-Hachtel, David Linz, Yoshinori Tomoyasu
Miami Univ., USA
Despite accumulating efforts to unveil the origin of insect wings, it remains one of the principal mysteries in evolution. Currently, there are two prominent models regarding insect wing origin: one connecting the origin to the paranotal lobe and the other to the proximodorsal leg branch (exite). However, neither hypothesis has been able to surpass the other. To approach this conundrum, we focused our analysis on vestigial (vg), a critical wing gene initially identified in Drosophila. Our investigation in Tribolium (Coleoptera) has revealed that, despite the well-accepted view of vg as an essential wing gene, there are two groups of vg-dependent tissues in the “wingless” first thoracic segment (T1). We show that one of these tissues, the carinated margin, also depends on other factors essential for wing development (such as Wingless signal and apterous), and has nubbin enhancer activity. In addition, our homeotic mutant analysis shows that wing transformation in T1 originates from both the carinated margin and the other vg-dependent tissue, the pleural structures (trochantin and epimeron). Intriguingly, these two tissues may actually be homologous to the two proposed wing origins (paranotal lobes and exite bearing proximal leg segments). Therefore, our findings suggest that the vg-dependent tissues in T1 could be wing serial homologs present in a more ancestral state, thus providing compelling functional evidence for the dual origin of insect wings. We are currently testing our model by (i) analyzing the nature of the Tribolium T1 wing serial homologs via RNAseq, and (ii) evaluating the presence of the T1 wing homologs in several additional insect species. This work is supported by the National Science Foundation (IOS 0950964 to Y.T., and GRF to C.C-H.)

Program Abstract #244
Exploring the molecular basis of insect wing evolution: a transcriptomic approach
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We are studying the gene regulatory network of wing development in Tribolium (the red flour beetle) and comparing it to that of the fruit fly, Drosophila, to understand the molecular basis of morphological evolution. The wings of these two insects have become vastly different over evolutionary time. The fly has typical flight wings on the second thoracic segment (T2), but has intensively modified wings (halteres) on T3. In contrast, the beetle has a pair of hardened protective structures (elytra) on T2, and uses the T3 hindwings for flight. We have been analyzing the function of potential “wing genes” (selected from previous Drosophila studies) in Tribolium wing development: i.e a candidate gene approach. However, as these studies have progressed, the choices of candidate genes have become increasingly limited and also created a fly-biased view of insect wing evolution. To gain a less biased view of insect wing evolution, we have started exploring genes that could be uniquely important for the beetle wing development, and thus not present in Drosophila wing development. We first examined a class of developmental genes (toolkit genes) that are known to be important for embryonic segmentation in Drosophila. These toolkit genes tend to show a high degree of pleiotropy, therefore increasing the likelihood of finding novel wing genes in the beetle. Despite this, we found no definitive examples of beetle wing genes whose orthologs in fly are not important for wing development. To obtain further insight into the molecular basis of insect wing evolution, we adopted a bona fide non-candidate gene approach, RNA sequencing. We are currently analyzing the Tribolium wing transcriptome (both elytron and hindwing), and comparing them to those of other insects, which will allow us to identify both conserved wing genes as well as wing genes uniquely co-opted in the beetle lineage. This work is funded by the National Science Foundation (IOS 0950964 to YT).

Program Abstract #245
Allometry and patterning in the polyphenic wings of a true bug
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Polyphenic traits develop different final states due to environmental influences on the developmental process. However, it is unclear how developmental mechanisms are altered to achieve distinct morphs, particularly in instances where two alternative morphologies occur, rather than simply the absence of structures in one morph. The red-shouldered soapberry bug Jadera haematoloma exhibits polyphenic wing morphs. Both sexes may develop as adults with complete wings and functional flight muscles or as adults with shorten wings and vestigial or atrophied flight muscles. In this species, short wings result from a combination of alternative patterning and growth specifically within the distal membrane region of the wing. Wing development is negatively correlated with
gonad development, producing a classic trade-off for individuals in fecundity versus dispersal ability. While wings exhibit this polyphenism, other appendages are more canalized in their size, and a study of ontogenetic allometry finds a steep positive scaling coefficient for the wing of both morphs, modest positive scaling for legs and antennae, and isometric growth for the beak (labium). Juvenile-stage RNA inference was used to knock down several genes with roles in wing patterning and organ size regulation in model insect species. Distal-less is required for growth and patterning within the membrane region of the J. haematoloma wing, while other genes show conserved wing patterning functions. FoxO RNAi disrupts the development of body and organ sizes and increases oogenesis. Surprisingly, while wings and beaks have very different scaling coefficients during growth, both appendages increase in relative size after FoxO RNAi, while legs and antennae remain proportional. These findings suggest that insulin signaling may regulate patterning and growth in this species to direct polyphenic developmental outcomes. This work is supported in part by Colby College and NSF (IOS-1350207).

Program Abstract #246
Transcriptome-wide analysis of the effects of temperature on global gene expression and temperature-dependent sex determination in the red-eared slider turtle Trachemys scripta elegans embryo.

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The study of temperature dependent sex determination (TSD) has long suffered from the difficulties inherent to restriction to non-model organisms. Many recent advances in the field of genetic sex determination have been made through system level approaches, made possible by large ‘omic data sets that can be collected and analyzed with relative ease in these systems. With the recent rise in efficiency and accuracy of de novo transcript assembly and decline in the cost of next-gen sequencing, it is now possible to start applying these same system wide analyses to the study of TSD, even in non-model organisms. Here we present a transcriptome wide analysis of TSD in the red-eared slider turtle Trachemys scripta elegans. Through de novo transcript assembly and homolog based annotation from gonad and non-gonadal tissue samples at the male and female producing temperatures we generated a transcript database for the assessment of gene and transcript level differential expression. We identify a comprehensive list of differentially expressed genes, then using a multiple sample statistical model for identifying evenness and tissue specificity we were able to identify genes enriched during testis or ovary development and distinguish them from those involved in global temperature response.

Program Abstract #247
Regulation of neural crest cell emigration in turtle embryos

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Turtle plastron bones develop by intramembranous ossification, suggesting that they are derived, like facial bones, from neural crest cells. Using cell-labeling and neural tube explant cultures, we have shown that cells expressing neural crest markers emerge from the trunk neural tube in the turtle Trachemys scripta for a greatly extended period compared other model amniotes. The neural crest cells that emerge late, well beyond the stage of neural crest emigration in chick or mouse embryos, appear to migrate ventrally to form an ectomesenchymal dermis that gives rise to the bones of the plastron. The specification of premigratory neural crest cells and the epithelial-mesenchymal transition that produces migratory neural crest cell is controlled by a gene regulatory network including the transcription factors Snail2, FoxD3, Sox9, and Sox10. We are currently examining the expression of markers of premigratory and early migratory neural crest cells to examine whether the premigratory domain persists during the period in between the early and late migratory phases. If the expression of these markers persist throughout this period, it will suggest that the premigratory region is maintained, and that the lack of neural crest cell migration may be due to the lack of a supportive environment. In contrast, if these genes are only expressed during the periods of active neural crest cell emigration, then the second wave of neural crest cell migration would require a second inductive signal not found in chick embryos.

Funding: Millersville Univ. Faculty Research Grant, Neimeyer-Hodgson Grant, Student Research Grant, Biology Student Investigator Award.

Program Abstract #248
Evolution of post-embryonic neural crest lineage contribution to adult pigment pattern in Danio fishes

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The diversity of vertebrate form is in large part attributable to changes in the patterning of neural crest cells and their derivatives. Pigment patterns of fishes in the genus Danio are a tractable system in which to uncover how cellular and molecular changes in neural crest lineages lead to phenotypic diversity among closely related species. Adult zebrafish, D. rerio, have horizontal stripes of black melanophores derived mainly from post-embryonic, neural-crest derived latent precursors. However, the closely related species D. nigrofasciatus has an evolutionary reduction in latent-precursor derived melanophores, and its pigment pattern is composed largely of persisting embryonic melanophores. Here, we identify evolutionary changes in endothelin signaling as a candidate mechanism for altering the relative contributions of embryonic and post-embryonic neural crest lineages. A previous study indicated that endothelin receptor b1 (ednrb1) mutant zebrafish have a pigment pattern similar to D. nigrofasciatus, whereas cell transplants showed the differences between species to be non-autonomous to melanophore lineages. We therefore assessed
expression of genes encoding Ednr1 ligands and found much lower abundance of endothelin-3b (edn3b) transcript in *D. nigrofasciatus* than in *D. rerio*. By analyzing gene expression in *D. rerio* x *D. nigrofasciatus* hybrids, we further showed that evolutionary changes in *edn3b* expression have resulted from cis-regulatory evolution at this locus. We are now testing whether *D. rerio* edn3 can restore latent-precursor derived melanophores to *D. nigrofasciatus*, as well as the roles of endothelin signaling—and its abrogation—in the development of melanophores and other lineages in these species.

Research supported by: NIH R01 GM062182 (DMP) and NIH Predoctoral Training Grant 2T32GM007270-39 (JES)

Program Abstract #249

**Comparative analysis of rudimentary colons in *P. marinus, M. glutinosa* and *Polyodon.***

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The development of a colon was vital for vertebrates to transition from an aquatic to terrestrial environment 380 million years ago. The colon permitted animals to absorb water and salt, thus maintaining constant body fluid volume and preventing desiccation on land. As a result, colon formation was thought to evolve after the transition of animals to land. Histologic and physiologic evidence suggests that an ancient colon existed in the cartilaginous fish *Leucoraja erinacea*. In addition, the colon genetic markers Hoxa13 and Hoxd13 are conserved in the skate. To determine if cartilaginous fish were pre-adapted to conserving water prior to the tetrapod transition, we examined species from gnathostomes (*Petromyzon marinus* and *Myxine glutinosa*) and ray-finned fish (*Polyodon*) for colon developmental markers and histology. Acid-mucin producing cells indicative of a colon are expressed in the distal gut epithelium of the lamprey (19%) and paddlefish (16%), at similar levels to the skate (19%). Further, an one-way ANOVA analysis indicates a significant difference in acid mucin distribution between proximal and distal regions of the gut in both fish. In contrast, acid mucins are expressed throughout the length of the hagfish intestine. Together, these results suggest a rudimentary colon in the lamprey and paddlefish, indicating that an ancient colon existed prior to and following the existence of cartilaginous fish. Current research is focusing on the conserved roles for Hox genes in patterning the early colon.

Program Abstract #250

**Developmental constraints on endoderm morphogenesis underlie the evolution of gut length***

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There is tremendous variation in the length of the digestive tract to accommodate diverse diets. To discern the developmental mechanisms that generate such variation during evolution, we assessed gut morphogenesis in an array of non-model frog species with distinct larval gut morphologies and feeding ecologies. Herbivorous tadpoles, the ancestral state, are typically derived from small eggs and have rapidly elongating guts. As their yolk reserves are quickly depleted, all the yolky endoderm cells that initially fill the gut tube become polarized and rearrange to lengthen the gut and form the epithelial lining. In contrast, tadpoles derived from slightly larger eggs exhibit delayed or decreased gut lengthening. In some species, this delay is a consequence of a larger endoderm mass taking longer to rearrange and complete epithelial morphogenesis, thus extending dependence on yolk reserves. However, in rapidly-developing cannibalistic species, the core of the endoderm never integrates into the gut epithelium; instead, many yolky endoderm cells are discarded by apoptosis, resulting in a shorter intestine with a larger lumen, a morphology adaptive for a carnivorous diet. Finally, the largest eggs harbor the greatest yolk reserves and often belong to direct-developing species that form a short adult-like digestive tract. In this context, cell polarization and organization occurs slowly within the bulky mass of yolky endoderm cells. As in herbivores, the endoderm becomes polarized; however, cell rearrangement contributes to increased epithelial surface area but does not drive tube lengthening. In general, tadpoles derived from larger eggs tend to have delayed gut lengthening or form shorter guts. Taken together, our data suggests that variation in gut morphology may have arisen due to developmental constraints on endoderm cell polarization and rearrangement and that changes in scale during tubulogenesis may be an unexpected source of novel form and function during evolution.

Program Abstract #251

**Betta splendens as a model organism for studying the evolution and development of pigmentation***

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Perhaps one of the most striking features of animal morphology is the presence of diverse colors. Among vertebrates, teleost fish exhibit some of the most varied pigmentation. The genetics of pigmentation via melanocytes has been well studied in zebrafish and shows many similarities to that in mammals, but much less is known about the genetics of the other pigmentation cell types. In an effort to learn more about the evolution of the molecular and cellular basis of coloration we have begun a research program that employs Betta splendens as a model system. Despite numerous studies regarding *B. splendens* behavior, very little has been published about breeding, development, and genetics. However, the brilliant colors selected by breeders for aquaria use provide ideal phenotypes for pigmentation studies. After trying numerous methods we have identified a number of parameters to increase the likelihood of successful breeding. Our methods allowed us access to isolate and fix embryos and fry at variable time points. Specimens were viewed and photographed under a light microscope. We compared offspring from differently colored parents (mainly blue and red) for variations in early pigment patterns. We have determined that pairs of *B. splendens* can be reliably bred to produce adequate numbers of large, transparent embryos and fry for pigmentation analysis. Conveniently, developmental stages
appear to be similar to the well-studied zebrafish, which allows for easier morphological characterization. When present, melanocytes are present by two days post fertilization and are easily visualized during early development. Our results demonstrate that early pigmentation patterns are dependent on the coloration of the parents. We conclude that B. splendens has excellent potential as a model organism for studying the development and evolution of diverse pigmentation.

Program Abstract #252
Cellular mechanisms of adult pigment pattern evolution in Danio fishes
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Understanding how evolutionary changes in development lead to the diversity of adult form requires knowing how differences at the molecular level lead to variation in differentiation and morphogenesis. Pigment patterns among Danio fishes offer an interesting system in which to study post-embryonic development and evolution as externally visible patterns are critical for teleost behavior and have played important roles in speciation. The adult pigment pattern of zebrafish, Danio rerio, consists of dark stripes of black melanophores separated by light interstripes of yellow xanthophores and iridescent iridophores. In the closely related species, D. albolineatus, the same pigment cell types are intermingled leading to a more uniform pattern. Previous studies of D. rerio suggest a conceptual model for pattern formation that includes the timing and differentiation of pigment cells, interactions between pigment cells, and environmental influences. We are using this model to test the hypothesis that evolutionary changes in one or more of these interactions are responsible for the very different pattern of D. albolineatus. Interspecific cell transplants using both single pigment cell types and combinations of pigment cells between species will elucidate if evolutionary differences are autonomous or non-autonomous to pigment cell lineages. Additionally, we are using time-lapse imaging to test how potential changes to these lineages manifest in pigment cell behavior in D. albolineatus as compared to D. rerio. These experiments will identify the bases for species differences and will allow more efficient targeting of gene discovery efforts and rescue experiments to identify the mechanistic bases for stripe loss in D. albolineatus.

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Program Abstract #253
Pigment cell heterochronies underlying pattern evolution in Danio species
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Teleost fishes have diverse and striking pigment patterns, yet we know little about how evolutionary changes in pigment cell development generate pattern variation. As an adult the zebrafish, Danio rerio, has a pattern of dark horizontal stripes of melanophores alternating with light interstripes of yellow xanthophores and iridescent iridophores. In contrast, D. albolineatus has an evolutionarily derived, uniform pattern in which melanophores, xanthophores and iridophores are intermingled. Here we show that iridophores not only initiate and orient stripes in D. rerio, but also form boundaries that terminate developing stripes. In D. albolineatus, however, very few iridophores develop whereas xanthophores develop precociously and over a much wider area than in D. rerio. This difference in xanthophore development is associated with similarly early and widespread expression of the xanthogenic factor Colony stimulating factor-1 (Csfl), owing to cis-regulatory evolution at the Csfla locus. Finally, we show that expressing Csfl similarly to D. albolineatus in D. rerio results in melanophore and xanthophore intermingling, loss of interstripe iridophores and a pattern resembling that of D. albolineatus. Together our analyses indicate that evolutionary changes in Csfl expression and concomitant alterations in the development of all three pigment cell classes have likely contributed to the loss of stripes in D. albolineatus. These results further suggest that changes in the timing of pigment cell differentiation can have cascading effects on pattern development and may have contributed to evolutionary diversification in this group.

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Program Abstract #254
Defining the regulatory loci and their target gene interactions for a model developmental and evolutionary trait
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Morphological traits are the developmental products of networks of genes whose activities are interconnected at the level of gene regulation. These connections, or regulatory linkages, consist of transcription factors interacting with binding site sequences in target gene cis-regulatory elements within a dynamic chromatin environment. The genes and regulatory linkages structuring several gene networks, and their concomitant chromatin environments have been well studied. However, the mechanisms by which network structure and chromatin environment evolve remains poorly understood. Ideal traits to study network evolution are those for which phenotypic diversity has evolved over short evolutionary time scales. One suitable developmental and evolutionary network is that governing fruit fly abdominal pigmentation. These patterns have diversified at the intraspecific and interspecific levels, which include the derived male-specific pattern possessed by the model organism species Drosophila melanogaster. While abdominal pigmentation is known to be controlled by a few prominent transcription factors that assign spatial and sex-specific patterning information, the breadth of network transcription factors and chromatin remodeling proteins has remained poorly characterized. Thus, we have evaluated over 558 and 20 genes that respectively encode transcription factor and chromatin modifying proteins for loss-of-function effects on this dimorphic trait. From this screen, to date this screen has identified over 20 transcription factors and
Patterns in Evolution: Tracing the Genetic and Molecular Basis for Convergent Pigmentation Pattern in *Drosophila* species

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

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**Program Abstract #256**

The correlated and divergent evolutionary histories for two cis-regulatory elements controlling pigmentation enzyme expression.

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A major goal of evolutionary-developmental biology research is to make a connection between the understanding of development and how development evolves. At the heart of development are gene regulatory networks (GRNs), where each network is composed of a set of transcription factors that control the expression of target genes through physical interactions (regulatory linkages) with binding sites in cis-regulatory element sequences (CREs). While the regulatory linkages have been mapped for several GRNs, lacking is a characterization of a GRN for a rapidly evolving trait whose origin, diversification, and loss can be studied. *Drosophila melanogaster* fruit flies have a male-limited pattern of abdomen pigmentation which evolved from a monomorphic ancestor, and abdomen pigmentation has diversified and been lost between related species. Here, I studied the CREs and interacting transcription factors controlling the expression of two pigmentation enzymes with similar male-specific patterns of expression. Though these CREs direct similar patterns of expression and are controlled by some of the same transcription factors, we found that the underlying composition and organization of regulatory linkages significantly differ. We provide evidence that the repurposing of an ancestrally monomorphic pigmentation GRN to a derived dimorphic state required the de novo evolution of these two CREs. While these CREs seemed likely targets of mutations responsible for diversification and losses of pigmentation, we show that these phenotypic changes largely were driven by changes at other GRN loci. Collectively, these results show how evolution can forge similar gene expression patterns from dissimilar CRE encodings, and how a trait’s origin can require the evolution of new CREs, but that these CREs may not be the preferred GRN target for subsequent evolution.

**Program Abstract #257**

Investigating the deep ancestry of and the mechanism of co-option for a pleiotropic fruit fly cis-regulatory element

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A common theme of animal evolution is the diversification of traits by the modification of evolutionarily conserved genes. Thus, understanding how conserved genes evolve new functions while maintaining existing functions is essential to understand the genetic basis for diversity. It has been reasoned that morphological evolution favors mutations in *cis*-regulatory elements (CREs) in order to minimize negative pleiotropic effects. An expectation supported by several studies which found CRE modifications to be the path of trait evolution. However, little attention has been given to the ancestral function(s) of evolutionarily relevant CREs and whether such functions impose a constraint of pleiotropy upon their paths of functional evolution. In *Drosophila melanogaster*, the Bric-a-brac (Bab) transcription factors control the development of several sexually dimorphic traits on the posterior abdomen. Female-specific Bab expression is regulated by a CRE called the dimorphic element that possesses binding sites for body plan (ABD-B) and sex-determination (DSX) transcription factors. Interestingly, the dimorphic element’s activity in pigmented abdomen regions changed, while activity in the more posterior regions and the ABD-B and DSX binding sites remained conserved. We suspect that these binding sites are constrained by the ancestral and pleiotropic functions for this CRE. Here we present our efforts to determine
this CRE’s pleiotropic functions, depth of ancestry, and manner of co-option for use in regulating pigmentation. Collectively, this study will provide insights on how a CRE balances ancestral functions while being co-opted for a new use. Funding sources were the American Heart Association (11BGIA7280000) and National Science Foundation (IOS-1146373).

Program Abstract #258
The Target Problem in Characterizing Early Metazoan Developmental Sequences
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In 1978, Riedl described what he called “the paradox of teleological evolution.” Today the paradox remains unsolved. Evolution by natural selection is not an end-directed process, but metazoan development is. Normally-developing embryos “head towards” functional targets (e.g., differentiated cell, tissue, organ, and body plan states) lying temporally in the distance. The paradox arises when one employs an undirected process such as evolution to explain the step-by-step acquisition of developmental stages, such as initial cleavage patterns, which, while not themselves viable endpoints, are necessary to reach distant functional target states. This is the target problem: characterizing and explaining the origin of developmental stages required for organismal viability and reproductive capability, where the selectable consequence is a future (developmentally remote) state.

Program Abstract #259
Role of semaphorin-1a in the developing visual system of the disease vector mosquito Aedes aegypti
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Despite the devastating global impact of mosquito-borne illnesses on human health, very little is known about mosquito developmental biology, including development of the mosquito sensory nervous system. Here, we functionally characterize visual system development in the dengue and yellow fever vector mosquito Aedes aegypti, which we are developing as an emerging model for mosquito development. Mosquitoes, unlike Drosophila and other holometabolous insects, possess adult compound eyes as larvae, a trait that makes them an interesting model in which to study comparative arthropod eye developmental genetics. Immunohistochemical analyses revealed the progression of visual sensory neuron targeting that generates the retinotopic map in the developing A. aegypti optic lobe. Chitosan/siRNA nanoparticles were then used to target the axon guidance gene semaphorin-1a (sema1a) during larval development. Loss of sema1a function resulted in optic lobe phenotypes, including defective targeting of visual sensory and second order neurons and failed formation of the retinotopic map. These sema1a knockout phenotypes correlated with a larval photoavoidance behavioral defect. The results of this investigation indicate that Sem1a is required for A. aegypti optic lobe development and highlight the behavioral importance of a functioning visual system in pre-adult mosquitoes.

Program Abstract #260
Macf1 is required for development of the retina
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Microtubule-actin cross linking factor 1 (Macf1) is one of two vertebrate spectraplakins that associate with F-actin and microtubules, thus integrating cytoskeletal networks during intracellular trafficking and cell migration. Macf1 is hypothesized to couple the microtubule network to membrane-associated junctions, allowing for the trafficking of components from dynamic actin-rich focal adhesions during cytoskeletal remodeling required for cell migration and differentiation. Microtubule and actin interactions are required for several processes during the development and function of photoreceptors and other sensory neurons/epithelia, particularly during the generation and maintenance of adherens junctions and polarization of later born neurons. Because Macf1 is known to assist focal adhesion/adherens junction dynamics in vertebrate cells and its homolog is required during photoreceptor morphogenesis in drosophila, we propose that Macf1 is important for mammalian neural retinal development due to important functions at apical junctions, compromising visual function. In mouse, Macf1 localized to apical junctions of the developing neural retina. These structures require microtubule-actin interactions for their dynamic assembly and disassembly and are critical for the maturation of late-born neurons, including photoreceptors and bipolar cells. Conditional knockout out of Macf1 in the developing retina resulted in a severe disruption of retinal lamination that primarily affected photoreceptors and bipolar cells, which resulted in a loss of visual function. These findings suggest a crucial role for Macf1 in developing sensory neurons that are dependent upon the development and maintenance of junctional polarity. Current investigations are focused on whether Macf1 plays additional distinct roles in mature photoreceptors for the trafficking of proteins en route to outer segments via the connecting cilium.

Program Abstract #261
Pattern of developmentally regulated DNA loss in the sea lamprey, Petromyzon marinus
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Embryogenesis involves the regulation of genes and their products in strict temporal and spatial patterns. Genes must be expressed in specific areas of the embryos at specific times for embryogenesis to proceed normally and perturbation of these patterns can have deleterious effects on development and survival of the organism.

The sea lamprey, Petromyzon marinus, undergoes programmed genome rearrangement (PGR) during early embryogenesis. This process results in a deletion of 20% of its genome from the somatic cell lineages whereas the genome remains unaltered within the germline cells (which go on to form gametes). However, given genomic and transcriptomic data, DNA loss may not be simply a consequence of embryogenesis but may instead be integral to the proper development of the organism.

These germline restricted DNA elements contain coding and regulatory sequences that may be necessary for early development prior to their deletion from the somatic genome. Transcriptomic data provides evidence for expression of several genes that are presumably germline restricted. It is possible that while these germline elements are removed from all somatic cells, they may follow differential rates of deletion resulting in varying patterns of spatial and temporal loss. Identifying the pattern of DNA loss in the lamprey will provide insight to its contribution to early development and also to the understanding of how the organism is able to maintain its genomic integrity in the presence of wide scale DNA rearrangement and deletion while allowing for proper embryogenesis.

Program Abstract #262
Utilizing genome editing to explore the genetic basis of evolution in the cavefish Astyanax mexicanus
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The Mexican cavefish, Astyanax mexicanus, is an excellent system for studying the genetic basis of evolution. Astyanax mexicanus exists in two interfertile forms, a surface-dwelling form and multiple independently evolved cave-dwelling forms. Cavefish have evolved a number of morphological and behavioral traits, including loss of eyes and pigmentation, increase in number of taste buds and neuromasts, and decrease in schooling and shoaling behaviors. Multiple quantitative trait loci (QTL) analyses have been performed to identify QTL for these traits. These studies, combined with the recent sequencing of the cavefish genome, provide a unique opportunity to identify and test candidate genes for these cave-specific traits. We used transcription activator-like effector nucleases (TALENs) in surface fish and zebrafish to modify two genes that underlie QTL for pigmentation and may affect behavior. These genes, oclocutaneous albinism II (Oca2) and melanocortin 1 receptor (Mc1r), contain coding changes between cavefish and surface fish. We found that we can mutate surface fish genes using these methods. Furthermore, we can test the effect of exact cavefish genetic modifications in zebrafish using genome editing due to the high level of protein sequence conservation between Astyanax mexicanus and zebrafish. This technology will provide us with powerful tools for studying the role of candidate genes and precise genetic modifications in the evolution of cavefish morphology and behavior.

Program Abstract #263
In situ hybridization during late larval stages of zebrafish development reveal a reverse collinear hoxA expression pattern in the paired pelvic fins
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Hox genes are regulatory developmental genes that are expressed in a collinear pattern during the development of multiple embryonic axes. The posterior hoxA and hoxD genes also pattern the developing paired appendages of vertebrates. Interestingly, the hoxD genes are expressed in a reverse collinear (RC) pattern in the distal portion of the paired appendages of jawed vertebrates including cartilaginous fishes, tetrapods, and ray-finned fishes. This RC pattern has not been found in the paired appendages of the most diverse groups of vertebrates, the teleost fishes. However, developmental studies of the molecular patterns of hox expression have only investigated early stages of pectoral fin development in zebrafish and medaka. Looking beyond early stages of development, we evaluated expression patterns of the posterior hoxA and hoxD genes during the patterning of the paired pelvic fins, which encompasses stages of development that have not been previously investigated for RC expression. Using in situ hybridization, we investigated the expression patterns of the posterior hoxA and hoxD genes during later stages of larval development, specifically during the emergence of the medial and pelvic fins. Surprisingly, we found no hoxd13a expression in the developing pelvic fins of zebrafish. Instead, we observed a reverse collinear expression pattern of hoxa13a and hoxa11a. Analysis of these expression patterns in a comparative context with other vertebrates provides insight into the evolution of these structures and suggests that the regulatory mechanisms of hoxD RC expression patterning was present before the two rounds of genome duplication in the stem lineage of the jawed vertebrates.

Program Abstract #264
Deep conservation of autopod enhancers in a non-teleost bony fish
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The evolution of the autopod (wrist/ankle and digits) in the limbs of tetrapod vertebrates represents a key morphological innovation that was crucial for the colonization of land. While the fossil record suggests that the wrist and digits formed through the progressive elaboration of the distal endoskeleton of the fin, there is no obvious morphological counterpart to the autopod in living fishes. Thus, comparative molecular data may provide insight into understanding both the homology of vertebrate appendages as well as the
genetic processes behind the fin to limb transition. The molecular mechanisms underlying tetrapod limb development have been well studied in mouse, with a particular emphasis on Hox genes of the A and D clusters. In mice, an initial “early” phase of Hoxd gene expression helps pattern the arm and forearm, and is followed by a “late” phase crucial for development of the digits. Regulation of Hoxd expression in developing digits is well understood and is orchestrated by a set of enhancers located upstream of the HoxD cluster. Here, we have identified and functionally characterized HoxD enhancers in the genome of the spotted gar, Lepisosteus oculatus, an actinopterygian fish that represents a sister group to teleosts. By means of zebrafish transgenic assays, we show that gar autopod enhancers drive expression in the distal portion of developing fins, and respond to the same functional cues as their murine orthologs. Furthermore, we demonstrate through mouse transgenic assays that gar enhancers drive reporter gene expression in both the wrist and digits of mouse in a pattern indistinguishable from their murine counterparts. These data suggest that tetrapods and fish share deeply conserved Hoxd autopod enhancers, and that our understanding of these processes may have been mired by a focus on the derived genomes of teleost fish.

Program Abstract #265
Fibroblast Growth Factor Diversity in the Developing Bat Limb.
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Evolutionary variation in the regulation of developmental control genes has been proposed to underlie morphological diversity among related species. Variation in protein coding sequences involved in significant adaptations have been identified in a few cases in mammals. These are involved in vision, behavior, and immunity diversity rather than morphological diversity. We have identified examples of conservation and divergence in both regulatory and coding sequence among Fibroblast Growth Factor (Fgf) gene family members expressed in the Apical Ectodermal Ridge (AER) of developing limb buds in bat relative to mouse. Fgf genes encode a large family of secreted signaling molecules. In mouse, Fgf8 expression is induced in the AER shortly after limb buds form, and is later joined by Fgf4, 9 and 17. In the bat, we are unable to detect Fgf4 expression, but find Fgf19. The mouse ortholog of Fgf19, Fgf15 is not expressed in limb buds. Fgf8 and 9 are conserved in protein coding sequence (100% and 98.5% identical, respectively) and both are expressed in the AER. Cloning and analysis of bat Fgf17 is currently in progress. In addition to divergence in expression pattern of bat Fgf19, we have obtained evidence for adaptive changes in its coding sequence, which may be contributing to divergent morphology of bat limbs. We propose that protein coding variation and regulatory variation are both important in the evolution of morphological diversity and may be selected in the same gene at the same time.

Program Abstract #266
Interdigit BMP signaling directly regulates programmed cell death during mouse limb development
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Shaping of the embryonic limb involves many processes including growth, differentiation, and programmed cell death (PCD). Furthermore, these processes integrate complex information from multiple signaling cascades such as the BMP and FGF pathways. Our previous work showed that BMP signaling regulates interdigit (ID) PCD indirectly by modulating the secretion of apical ectodermal ridge FGFs, which act as cell survival factors to the ID mesenchyme. Nevertheless, this indirect model does not exclude a direct role for BMPs in PCD. Due to lack of genetic evidence, it is still unclear whether ID BMPs directly trigger PCD. To test whether BMPs act as direct triggers of ID PCD, we inactivated the gene encoding receptor BMPR1A within the ID tissue with Osr1-Cre. The resulting mutants show a decrease of ID PCD. To test redundancy between BMPR1A and BMPR1B in PCD we inactivated ID Bmpr1A in a Bmpr1B null background. This compound mutant has a further decrease in PCD. During this analysis we serendipitously discovered a potential role of the ID in digit formation. Bmpr1B null digits are short with abnormal development of their phalanges. The defect is completely rescued in digit one when we inactivate Bmpr1A in the ID and to a limited degree in digits two through five. Marker analysis for RNA expression of various components of the BMP signaling pathway revealed a dramatic upregulation of Growth Differentiation Factor 5 (GDF5), a potential alternate ligand, in the ID mesenchyme. Currently, GDF5 is not implicated in limb PCD. To fully understand the role of ID BMP signaling on normal limb development, we are also inactivating in the ID mesenchyme Bmp2, 4, and 7, in various combinations, with Osr1-Cre. Analysis of these mutants reveals that BMP7 is necessary for ID PCD during normal development. Furthermore, ID BMP 2 inactivation sensitizes the ID tissue to furthere BMP7 perturbation.
This work establishes that BMP signaling directly regulates ID PCD.

Program Abstract #267
A Common Developmental Origin of Limbs and External Genitalia in Amniote Evolution
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The transition of vertebrates to a terrestrial lifestyle required major adaptations in their locomotory apparatus and reproductive
organs. While the fin-to-limb transition has received considerable attention, little is known about the developmental and evolutionary origins of external genitalia in vertebrates. Similarities in patterning genes expressed during their development have been proposed as evidence for a potential evolutionary link between the two anatomical structures, yet without providing any underlying developmental mechanism. Here, we have reexamined this question using comparative micro-Computed Tomography (\textsuperscript{\textmu}CT)-data, lineage tracing in three amniote clades and RNA-Seq based molecular profiling. We show that the developmental origin of external genitalia has shifted through evolution, and in some taxa limbs and external genitalia share a common primordium. We find that in the extreme case of modern snakes, the early hindlimb bud initiates but subsequently is entirely coopted to form genitalia, whereas in mice the genital tubercle develops from the ventral and tail bud mesenchyme. This recruitment of different cell populations for genital outgrowth is associated with a change in the relative position of the cloaca, which we verify serves as the genitalia organizing center. Ectopic grafting of the cloaca demonstrates the conserved ability of different mesenchymal cells to respond to these genitalia-inducing signals. Taken together, our results support a limb-like developmental origin of external genitalia as the ancestral condition and suggest that a change in the relative position of the cloacal signaling center during evolution has led to an altered developmental route of external genitalia in mammals, while preserving some of the ancestral limb molecular circuitries due to a common evolutionary origin.

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Program Abstract #268
Comparative analysis of developmental modes of vertebrate hypaxial musculature
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The hypaxial musculature of the vertebrates includes body wall muscles (e.g., intercostal muscles), the muscles associated with paired limbs and the girdles, tongue muscles and the diaphragm. These muscles undergo two distinct developmental processes as they differentiate from the embryonic somites. One is the direct ventral extension of the somitic muscle plate, giving rise to the body wall muscles. Another is the particularly complicated developmental pathway that involves the extensive distal migration of mesenchymal myoblasts from ventral edges of the somites. In this work, we chose several key vertebrate species and compared the morphology and molecular characteristics of the hypaxial muscles. In the cyclostome lamprey, which lacks paired fins, the hypobranchial muscle emerges as a stream of myoblasts originated from the anterior somites. The hypobranchial myoblasts express Pax3/7 and \textit{Lbx} genes and migrate ventrally into the pharyngeal region. A similar behavior of myoblasts was observed in the teleosts, in which the anterior somites give rise to the pectoral fin muscles and so-called posterior hypaxial muscles. In the amphibian \textit{Xenopus} embryos, Pax3- and \textit{Lbx}-positive migratory myoblasts undergo delamination from the somites and give rise to the larval abdominal muscle that ventrally covers the yolk sac and anteriorly connects to the cervical region. This cellular behavior is distinct from that of the amniote abdominal muscles, which develop as the direct ventral extension of the non-migratory muscle plate. These insights suggest that the recruitment of the migratory mode of myogenesis in different skeletal muscles would have contributed to the complexity and diversification of vertebrate morphology.

Program Abstract #269
The muscle-specific histone methyltransferase Smyd1 is necessary for myofiber maturation
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Centronuclear myopathies (CNM), also known as myotubular myopathies, comprise a cohort of similar muscular dystrophies characterized by progressive muscle weakness with age and a high percentage of centralized nuclei in muscle fibers. To date, very few genes have been linked to CNM and the underlying genetic cause is unknown for many patients. Here we report that loss of the \textit{Smyd1} gene product in mouse skeletal muscle produces a phenotype similar to human CNM. \textit{Smyd1} is predominantly expressed in cardiac and skeletal myocytes and encodes a multifunctional transcriptional coregulator and myosin-interacting regulator of sarcomereogenesis. Because \textit{Smyd1} knockout mice die midgestation due to heart malformation, we generated a conditional knockout (CKO) allele to study the role of \textit{Smyd1} in skeletal muscle development. \textit{Smyd1} was deleted specifically in skeletal myocytes using \textit{Myf6-cre} and phenotypic analysis was done at 6 weeks of age. Compared to control littersmates, \textit{Smyd1} CKO mice were smaller, weighed less and exhibited reduced muscle mass as determined by whole body composition MRI and measuring the weight of individual muscles. \textit{Smyd1} CKO mice underperformed control littersmates in strength tests. Males appeared to be more severely affected than females. A high percentage of centralized myofiber nuclei were observed in the tibialis anterior, gastrocnemius, triceps and quadriceps. The soleus was much less affected. Alterations in expression of genes linked to CNM and contractile proteins were found in the tibialis anterior, but not the soleus, suggesting a fast-twitch fiber specific effect. Muscle repair was assayed 14 days after cardiotoxin injection to the tibialis anterior. \textit{Smyd1} muscle CKO mice suffered greater muscle damage than control littersmates. These data support an important role for \textit{Smyd1} in muscle fiber maturation and reveal a novel genetic cause of CNM.

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87
**Program Abstract #270**

**Conditional deletion of Smyd1 at the myoblast stage results in reduced muscle mass**

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Smyd1 is a bifurcated SET and MYND domain-containing protein predominantly expressed in striated muscle throughout development from the myoblast/cardiacmyocyte progenitor stage to fully differentiated cardiac/skeletal muscle. Smyd1 appears to be a dual function transcriptional corepressor. It may promote transcription through intrinsic histone H3 lysine 4 (H3K4) methyltransferase activity or repress transcription via direct protein-protein interaction of the MYND domain with corepressor complexes. In zebrafish, the Smyd1a/b genes serve a critical role in sarcomerogenesis in both skeletal and cardiac muscle; which was attributed to a structural role at the M-line. In mice, complete knockout of Smyd1 (a single gene in mice) causes severe cardiac malformation, including absence of the right ventricle, resulting in embryonic death by E10.5. To determine the role of Smyd1 in mammalian skeletal muscle development, we developed a conditional knockout (CKO) allele of Smyd1 and used Mef5-cre to specifically delete Smyd1 in skeletal myoblasts. Smyd1 CKO embryos appear normal at E10.5. By E15.5, Smyd1 CKO embryos exhibit decreased muscle mass and subdermal edema. Smyd1 CKO embryos die before birth and the cause of death remains to be determined. We complemented the animal studies using a mouse myoblast cell line - C2C12, which upon induction of differentiation form multi-nucleated myotubes. Short hairpin RNA-mediated down-regulation of Smyd1 in C2C12 cells led to the formation of poorly differentiated and significantly thinner myotubes as compared to C2C12 cells transduced with a control lentivirus. These data suggest an important role for Smyd1 in myoblast differentiation and myofiber development. This work was supported in part by grants from the American Heart Association.

**Program Abstract #271**

**Scaling Pattern to Variations in Size during Vertebrate Neural Tube Development**

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Mechanisms of morphogen mediated pattern formation are largely conserved across vertebrate species, however, little is known about how they are adapted to generate proportionate morphological structures in varying embryonic sizes. We explored this question by analyzing the dynamics of patterning in zebra finch (T. guttata), chick (G. gallus) and emu (D. novaehollandiae), three species that are drastically different in embryonic size starting at early in development. We focused on patterning of the neural tube dorso-ventral axis, where cell fate specification induced by Sonic hedgehog is one of the best-studied examples of morphogen mediated organization.

Our findings suggest that there is a difference to cellular morphogen response in the three avian species. In vivo, temporal dynamics of patterning are shifted, where the neural tube of the smaller bird, finch, is patterned fastest and the neural tube of the bigger bird, emu, is patterned slowest. In vitro, naive neural plate explant assays suggest that morphogen sensitivity decreases as embryonic size increases: Finch cells are most sensitive to a given Shh concentration or duration, while the emu cells are the least sensitive. This differential response is intrinsic, as suggested by generation of chimeric embryos. Cells from different species in neural tubes of chimeric embryos retain their potential of differential response.

We believe this difference in response is crucial to scaling pattern to size in three species and that temporal adjustment is essential for conserving spatial organization of cell types in the neural tube dorso-ventral axis. We are further exploring the mechanism of this differential response and have strong evidence that it is due to intrinsic differences in GLI activity. A mechanistic understanding of differences in morphogen mediated patterning in species of different sizes will provide important insights into how pattern is adapted to size throughout evolution.

**Program Abstract #272**

**Brain development is accompanied by alterations of neural oscillatory patterns in anaesthetized Wistar rats**

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Numerous developmental neurobiologists paid great attention to the embryonic and early postnatal periods, but little is known about altered neural oscillation coupling between two brain areas during late postnatal stage. In this study, local field potentials were recorded from both ventral hippocampal CA1 (CA1) and medial prefrontal cortex (mPFC). The patterns of neural oscillation in 1.5-month-old (Young, n=8) and 3-month-old (Adult, n=8) groups were measured. Power spectrum analysis shows that more energy is distributed within lower frequency band in Young group compared to that in Adult group in both CA1 and mPFC regions. In both θ and γ bands, there are greater coherences between CA1 and mPFC in adult rats than that in young ones (θ: 0.51±0.01 vs. 0.46±0.01, p<0.05; γ: 0.36±0.01 vs. 0.33±0.01, p<0.05). PLV measures show that the above differences could partly be attributed to the enhanced phase synchronization in Adult group (θ: 0.43±0.03 vs. 0.36±0.02, p<0.05; γ: 0.18±0.01 vs. 0.12±0.01, p<0.001). The direction of neural information flow was measured by evolution map approach (EMA) and conditional mutual information (CMI). In θ rhythm, the unidirectional coupling CA1→mPFC in A group was significantly higher than that in young animals (EMA: 0.24±0.02 vs. 0.18±0.01, p<0.05; CMI: 0.12±0.01 vs. 0.10±0.01, p<0.05). In CA1 region, phase-amplitude couplings (PAC) between θ and low γ (LG, 30~50Hz) / θ and high γ (HG, 50~90Hz) were measured by modulation index, and it shows that the PAC strength is higher in adult rats than that in young ones (LG: 3.28±0.15 vs. 2.96±0.06, p<0.05; HG: 3.20±0.18 vs. 2.78±0.06, p<0.05).
The data suggest that a stronger transmission of phase information on CA1-mPFC pathway and an enhanced strength of cross-frequency coupling could be an indicator of more effective communication and network coordination in adult rats. This work was supported by grants from the National Natural Science Foundation of China (31171053, 11232005).

Program Abstract #273
A forward genetic screen in zebrafish identifies multiple loci important for normal spine development
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Adolescent Idiopathic Scoliosis (AIS) is characterized by mild to severe spine curvature, without malformations of the vertebral units in otherwise healthy children. The prevalence of idiopathic scoliosis is estimated to affect 2-3% of the pediatric population. Surprisingly, there is a paucity of information of the genetic traits and pathophysiology underlying this disease. In part, this is due to a lack of good, genetically tractable models of AIS. Here we report our results of a forward genetic screen in zebrafish to discover genetic traits affecting embryonic and post-embryonic spine development. We have screened ~300 F3 genomes, uncovering three distinct phenotypic classes of adult spine defects including: (1) short body plans, with vertebral malformations, without scoliosis; (2) late-onset scoliosis, without vertebral malformations; and (3) severe thoracic lordosis.

We are underway with classification of these mutant alleles into complementation groups. We will utilize Illumina sequencing to map and identify causative lesions in representatives of each complementation group. As of this date, we can report the identification of a novel mutant allele of the zebrafish kinesin member 6 (kif6695) gene, acting as a critical regulator of the adult spine structure. This kif6695 missense mutation disrupts an evolutionarily conserved leucine residue in the switch II region of the Kinesin motor domain, thus providing an experimentally derived candidate gene for targeted re-sequencing in human scoliosis patient samples. Experiments are underway to define the cell autonomy and molecular function of kif6 prior to and at the onset of scoliosis. Ultimately, these mutants will provide a foundation for the elucidation of the genetic traits of normal spine development and homeostasis.

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Program Abstract #274
Molecular mechanisms underlying craniofacial variation in New World Leaf-Nosed bats
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New World Leaf-nosed bats perform ecologically important functions associated with diverse skull morphologies, which remarkably and uniquely encompass much of the diversity of eutherian mammals. From an insectivorous ancestor, they have evolved to specialize on insects, nectar, fruit, small vertebrates and even blood. We report that striking morphological differences in bat craniofacial morphology associated with various feeding strategies emerge during prenatal development. Our 3D geometric morphometric analysis reveals differences in facial length. At one extreme, fruit-eating bats have flat faces, and on the other extreme, nectar-feeding bats have highly elongated faces. Facial length in mammals is controlled by interactions between the developing cranial base and the surrounding dermal bones. The cranial base has two or three regions of localized growth known as synchondroses. The synchondroses are similar to the growth plate of limb long bones, the best-studied skeletal development process. The length of limbs is determined by a genetically controlled mechanism called endochondral ossification (E0). We hypothesize that such a growth zone exists at the anterior extension of the cartilaginous cranial base in the face of bats and EO at the growth zone determines facial length. We have cloned many of the molecules involved in the growth plate during long-bone development using cDNA from Carollia perspicillata, specifically in proliferative chondrocytes (Pthrp, Fgfr3, Bmp7, Col2a1), hypertrophic chondrocytes (Fgfr1, Col10a1), and chondrocyte differentiation (Ihh, Bmp4). Bat embryo gene expression at Carnegie stages 22, 24, and 26 will be examined by in situ hybridization between closely related species representing variation in facial length. These analyses will provide the foundation for further functional experiments to reveal how specific changes in gene expression explain particular morphological alterations and novelties during bat evolution.

Program Abstract #275
Common phenotypes in multiple species identifies an Hmx1 enhancer important for lateral facial development
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The oculoauricular syndrome (OAS) in humans, and the “dumbo” and “misplaced ears” strains in laboratory mice, are both due to loss of function coding region mutations in the homeodomain transcription factor, HMX1/Hmx1. Micro-CT imaging of Hmx1 null mice reveal additional lateral facial defects aside from the abnormal external ears, including a hypoplastic paroccipital process and ramus, and variable facial asymmetry. We recently discovered that the phenotypically similar “dumbo” strain of rats is not caused by an Hmx1 coding mutation but rather a 5.7kb deletion ~80Kb downstream of Hmx1. Strikingly, expression of Hmx1 in the dumbo rat embryos was lost in the lateral mesenchyme of branchial arches (BA) 1 and 2 while other sites of expression were unaffected.
consistent with this being a regulatory mutation. Within the deleted region, we identified an evolutionarily conserved core segment of ~550bp, suggesting it functions as an enhancer for the Hmx1 locus. To test this, we performed transient transgenesis with two constructs: one containing the whole deletion region (WDR) and the other only the evolutionary core region (ECR) inserted into the Hsp68-lacZ reporter vector. Analysis of transgenic ECR and WDR embryos at E11.5, showed all exhibited a strikingly similar staining pattern that included the posterior-lateral mesenchyme of BA1 and BA2 (regions that contribute to the tempo-mandibular region and external ear, respectively) and a band of mesenchyme between the frontonasal prominence and forebrain, consistent with IHC staining for endogenous Hmx1 protein. The transient transgenesis experiments thus confirm that the core ECR has all the regulatory elements to drive expression of Hmx1 in the lateral facial mesenchyme. The ECR offers an unprecedented opportunity with which to begin to decipher the pathways coordinating lateral facial development and contributing to the oculo-auricular-vertebral spectrum, the 3rd most common group of craniofacial disorders.

Program Abstract #276
Distinct Pattern of Primary Palate and Nasal Cavity Ontogeny in Reptile Embryos
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In amniotes, the embryonic face passes through similar morphogenetic steps to give rise to the nasal cavities and primary palate. In mammals, nasal cavities are initially separated from the oral cavity by the oronasal membrane. The membrane ruptures, forming the choana, an essential connection between the oral and nasal cavities. Failure of the choanae to open or choanal atresia can be lethal to obligate nasal breathers. The presence of an oronasal membrane in non-mammalian amniotes has not been confirmed. We used optical projection tomography to make 3D reconstructions of the nasal cavities in reptile embryos and found that birds, turtles and lizards do not form an oronasal membrane. Instead they maintain an open choanal groove around which the facial prominences fuse, similar to basal vertebrates such as amphibians and lungfish. We studied proliferation patterns during choanal groove formation in the chicken embryo. Proliferation was lower (~40%) at the base of the invaginating nasal cavity than at the tips of the facial prominences (~60%). Areas of high proliferation are correlated with strong expression of FGF8 and its target gene SPROUTY2 but neither gene is expressed at the base of the nasal pit. The proliferation patterns regulated by FGF signaling may have a role in forming the choanal groove. We therefore placed an ectopic FGF soaked bead into the mesenchyme beneath the nasal pit. Surprisingly, no increase in proliferation was observed around the bead although adjacent regions were significantly increased. The choanal groove was wider suggesting that we had altered the invagination process. In normal development the groove becomes narrower due to focused proliferation at the tips of the frontonasal mass and maxillary prominences. Mammals have likely derived the oronasal membrane in association with other derived morphological traits such as the fused secondary palate. JA is funded by an F32 fellowship. The project is supported by an NSERC grant to JMR.

Program Abstract #277
Tooth replacement patterns and the effect of LiCl in the leopard gecko (*Eublepharis macularius*)
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Many reptiles are able to replace their teeth throughout life however the molecular controls of the shedding and replacement are unknown. The replacement pattern of teeth in reptiles is thought to occur in waves from posterior-to-anterior, alternating tooth positions. Here we characterize the natural history of tooth replacement in the leopard gecko and then attempt to perturb the patterns of replacement by manipulating the Wnt signaling pathway. To collect baseline tooth replacement data in the leopard gecko, upper dental arch impressions were taken weekly on 3 leopard geckos and the present/missing tooth positions were recorded over an 8 month period. During the 8 months, the palate was injected bilaterally with 1 M LiCl or the control solution, NaCl. Animals were followed for 14 weeks after injections to determine the effect on the replacement pattern. During the 14 week control period, the average tooth is replaced every 7 weeks. There are slower cycling teeth interspersed between faster cycling teeth. A posterior-to-anterior wave of replacement emerged in some regions of the mouth but this pattern was not maintained consistently. During the 14 week post-treatment period, LiCl increased the tooth replacement frequency in one individual but was not consistent among all animals. This may be due to variation in exposure and uptake of the drug. LiCl also appears to disrupt the replacement pattern. The posterior-to-anterior waves become irregular and reverse direction in some areas of the mouth. These phenotypes could be mediated by increases in cell proliferation and by dysregulation of the developmental clock controlling the timing of tooth replacement. We are presently evaluating the effect of LiCl on proliferation and the developmental status of the teeth.

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Program Abstract #278
Evolution of flight feathers with novel shaping mechanisms for bilateral asymmetry
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 Feather evolved in non-avialan dinosaurs and birds through stepwise generation of novel functional forms: periodically branched downy feathers, vaned contour feathers, and finally bilaterally asymmetric feathers adapting to flight. The meristic barb ridge formation in downy feathers is a self-organized process emerges from activator-inhibitor interactions involving BMP/Noggin/Shh. While the anterior-posterior and lateral-medial polarity in contour feathers and flight feathers, respectively, imply presence of
boundary-organized patterning using fixed landmarks as reference points. A Wnt3a gradient was found to specify the anterior-posterior polarity in vaned feathers but the molecular mechanism underlying feather lateral-medial asymmetry has not been identified. Here we compare four different feather forms, and found two key parameters determining feather vane width and asymmetry: the position and size of barb generative zone (BGZ) and the barb-rachis insertion angles. With transcriptome profiling and candidate analysis, we identified the molecular pathways associated with the above parameters: (i) Grem1 expression pattern matches the location and size of BGZ. Mis-expression of Grem1 expanded BGZ size and induced ectopic BGZ. Both endogenous and exogenous BGZ show suppressed BMP activity and cell proliferation rate. (ii) Retinoic acid (RA) metabolizing enzyme (Cyp26b1) and associated proteins (Crabp1) are differentially expressed in vanes of different widths. Perturbing RA signaling decreased barb-rachis angle and feather vane width. Meanwhile epithelial cells took more elongated appearance. We further used mathematical modeling to propose mechanisms linking these phenotypes together and generated a continuum of asymmetry. Thus different levels of integration between the self-organized patterning and various types of boundary-organized patterning drive the striking diversification of feather forms.

Program Abstract #279
Toxicological impacts of cypermethrin on Zebrafish (Danio rerio)
Grace Okuthe
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The increased use of synthetic pyrethroids to maximise agricultural production will inevitably lead to increased levels of discharge into the environment. The fate of pesticides into the aquatic environment, their interactions with biotic and abiotic components, and their potential to cause harm are still poorly understood. Synthetic pyrethroids are some of the widely used pesticides because of their effectiveness against a wide range of insect pests. These pesticides are expected to pose a significant risk in aquatic environments and their potential harmfull effects on non–target organisms are unclear. The goal of this project was to elucidate the possible impacts of short–term developmental exposure of zebrafish embryos and larvae to cypermethrin. Fertilized embryos were exposed to 0.5 µg/l of cypermethrin for 72 hrs, while larvae were exposed to 0.031, 0.063, 0.125, 0.25 and 0.5 µg/l for 50 days. All experiments were carried out under 24 hr static renewal systems. Results indicated a mean hatching rate of 93% and 73.5% for control and cypermethrin treated embryos respectively. Overall, cypermethrin, delayed time to hatch, reduced larval growth, and also caused morphological anomalies in both embryos and larvae. These results suggest that concentrations of cypermethrin used here may cause irreversible damage during early life, that could potentially alter the course of development in fish with possible ecological consequences. Further, results highlight the need for greater understanding of the molecular mechanisms by which short–term developmental exposure of embryos to cypermethrin may cause to physiological and behavioural abnormalities later in life.

Program Abstract #280
Bisphenol A affects early embryonic development in the pond snail Helisoma trivolvis
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Pond snails, such as Helisoma trivolvis, are excellent model systems to study development and neuromotor control and also serve as indicator species for changes that occur in the environment. In this study we show that chronic low dose exposure to Bisphenol A (BPA), a xenoestrogen from plastics, affects early embryonic spinning in the developing snail through a putative catecholaminergic pathway. While BPA’s role as an endocrine disruptor has been well established in many vertebrate species, its effects in pond snails have not been studied in great detail. Aside from its estrogenic and reproductive effects, BPA has also been linked to disrupting dopaminergic locomotory systems in many different species. In pulmonate snails dopamine and other catecholamines have been shown to be important in the locomotor circuit, including ciliary cells controlling embryo rotation. Here we exposed Helisoma embryos to a chronic low dose of BPA (200mg/ml) from the point of egg laying and observed them over development through hatch using a stereo-microscope with a camera. Snail embryos spin within their eggs during development to enhance nutrient and oxygen intake and to create a strong shell. BPA treated embryos (n=10) showed significant reduction in embryonic spinning by 55.15% compared to control embryos (n=14). Further the hatch rate of BPA-treated snails was 13.4% less than controls. Given that locomo motor neurons in the snail contain catecholamines, and their presence was found in the ciliary cells controlling embryonic spinning, we measured the levels of catecholaminergic staining in the early embryo (between 2-3 dpf) using glyoxylic acid histofluorescence. BPA treated snail embryos (n=6) showed 27% decreased fluorescence compared to controls (n=8), suggesting decreased catecholaminergic signaling or slower development in these embryos. The study shows that BPA affects embryonic spinning in Helisoma, potentially through an underlying catecholaminergic pathway.

Program Abstract #281
HIF and Notch Noncanonical Pathways Affect the Emergence and Differentiation of Cardiovascular Progenitor Cells
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Adaptive responses to low Oxygen (O2) tension (hypoxia) are integral to embryogenesis, tumorigenesis, and tissue ischemia. Developing embryos begin to experience a hypoxic micro-environment promoting the development of the cardiovascular system.
Early differentiation events focus the generation of cardiovascular progenitor cells, hemangioblasts, that are Flk-1 and Brachyury double-positive, which have hematopoietic, endothelial, and cardiomyocyte potential. Our model system utilizes embryonic stem (ES) cells differentiated into embryoid bodies (EBs) mimicking early development, which can be cultured under physiologic O2 conditions. Proper signaling is required temporally for the differentiation of these cells. Hypoxia Inducible Factor (HIF) is a major transcriptional player for these events. We propose that the Notch signaling pathway also plays a significant noncanonical role in mediating the generation and differentiation of hemangioblast cells. Using chemical and genetically modified mouse embryonic stem cells, differentiation experiments are being developed to explore the connection between these two pathways. Our present data demonstrate that hypoxia influences the expression of Notch receptors (Notch-1 and -4), ligands (Delta-4) and downstream Notch targets.

Program Abstract #282
Postsynaptic Neurobeachin is required for electrical and chemical synaptogenesis
Adam Miller, List Voelker, Arish Shah, Cecilia Moens
Fred Hutchinson Cancer Research Center, USA

Neural circuit organization underlies all of behavior. In order to make circuits, neurons must direct their processes to the correct location, recognize synaptic partners, and transport proteins to sites of synaptogenesis. Synapses can be chemical, where signals are transmitted via neurotransmitter release and reception, or electrical, where signals pass directly through gap junctions. To identify genes required for synaptogenesis we performed a forward genetic screen using the zebrafish Mauthner (M) circuit. M mediates a startle response to acoustic stimuli using both electrical and chemical synapses. The screen identified a mutant, disconnect4, which causes reduced localization of electrical (Cx36, ZO-1) and inhibitory chemical (glycine receptor, gephyrin) synaptic proteins at M circuit synapses. Mutants also fail to initiate startle responses to auditory stimuli, have defects in balance, and are generally less responsive than wildtype animals. RNaseq-based mapping of mutants identified a nonsense mutation in the autism-associated gene neurobeachin (nbea) and non-complementation with TALEN induced deletions confirmed its causal role. Nbea is a highly conserved, multidomain protein expressed pan-neuronally where it localizes to tubulovesicular membranes found from the Golgi to the synapse. Mouse genetics and biochemistry suggest that it plays a role regulating cargo transport to GABAergic and Glutamatergic chemical synapses. Whether Nbea acts pre- or postsynaptically, or both, remains unknown. We analyzed chimeric animals in which the pre- or postsynaptic neuron was nbea mutant while its partner was wildtype and found that Nbea is necessary and sufficient in the postsynaptic neuron for electrical and chemical synaptogenesis. We conclude the Nbea acts postsynaptically to broadly control synapse formation and its localization to the endomembrane system suggests it regulates dendritic transport of synaptic cargo.

Supported by NINDS K99NS085035 and R21NS076950.

Program Abstract #283
The genetic basis of molecularly asymmetric electrical synapses in zebrafish
Alex Whitebirch, Adam Miller, Arish Shah, Cecilia Moens
Fred Hutchinson Cancer Research Center, USA

Electrical synapses are specialized sites of adhesion that allow neurons to communicate directly with each other through gap junction (GJ) channels. Both the pre- and postsynaptic neurons contribute hemichannels composed of hexamers of Connexin (Cx) protein to the GJs. Despite this apparent simplicity, GJs can contain multiple different Cx proteins, and the functional properties of electrical synapses are influenced by the molecular composition of their constituent channels. Homotypic channels contain identical hemichannels, while heterotypic channels pair different Cx hemichannels. Although mammals have ~20 cx genes, most neuronal GJs are thought to be homotypic channels of Cx36. We found that zebrafish have four orthologous cx36-like genes, cx34a, cx34b, cx35a, and cx35b, but their roles in synaptogenesis are unclear. We generated mutants for all four genes and found that pan-Cx36-like staining at electrical synapses in the Mauthner escape circuit is normal in cx34b or cx35b mutants but was lost in cx34a and cx35a mutants. We reasoned that Cx34a and Cx35a are both essential for GJ formation and could be interacting either within individual neurons or across the synapse as apposed hemichannels. We distinguished these possibilities by generating embryos in which individual neurons in the Mauthner circuit were mutant for either cx34a or cx35a. We found that pan-Cx36-like staining is retained at synapses formed by cx34a mutant presynaptic neurons but is lost when postsynaptic neurons are mutant for cx34a. By contrast, synapses formed by cx35a mutant presynaptic neurons show a loss of pan-Cx36-like staining, while this staining is retained at GJs formed onto a mutant postsynaptic neuron. We conclude that electrical synapses in the Mauthner circuit are primarily heterotypic GJs composed of presynaptic Cx35a and postsynaptic Cx34a, and we speculate that this molecular asymmetry confers rectification to the GJs in this neural circuit. NINDS K99NS085035, R21NS076950

Program Abstract #284
Gene Knockout with CRISPR-Cas System
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In recent decades, many techniques of genetic engineering have been developed and widely adopted to study the function of genes, such as Cre-Lox recombination system and transcription activator-like effector nucleases (TALENs). Recently, another technique,
CRISPER-Cas system has been discovered and attracted increasing interest. Clustered regularly interspaced short palindromic repeats (CRISPR) loci were discovered in 1987 in Escherichia coli. And CRISPR loci together with Cas genes compose the CRISPR-Cas system, which provides bacteria and archaea with acquired immunity against viruses and plasmids by RNA-guided silencing of the targeting nucleic acid in a sequence-specific manner. In our study, we designed plasmids, PU6::klp-12_sgRNA and Peft-3::cas9. Then we injected both plasmids into the genital gianas of Caenorhabditis elegans, so that we built CRISPR-Cas system in C.elegans, which targets klp-12. Successfully, we knocked out klp-12 in C.elegans. Furthermore, we will try to knock out specific genes in specific tissues and cells with CRISPR-Cas system in C.elegans and explore the function of genes. Our further study will try to build conditional knockout C.elegans and show that CRISPR-Cas system has the potential for boosting the development of conditional gene knockout.

Program Abstract #285
Genetic screening using CRISPR/Cas9 in zebrafish
Arish Shah, Adam Miller, Cecilia Moens
Fred Hutchinson Cancer Research Center, USA

Classical forward and reverse genetic approaches have identified key molecular pathways involved in many aspects of life. However, particularly in vertebrates, the methods suffer from the time, cost, and number of animals required. The efficiency of using engineered CRISPR (clustered regularly interspaced short palindromic repeats) to mutate specific loci presents an alternative method to screen for genes involved in a process of interest. In zebrafish, injection of Cas9 RNA and a guide RNA (gRNA) into single-cell embryos can cause biallelic mutations that phenocopy known mutant phenotypes. We reasoned that new genes could be identified in a pathway given a sufficiently robust CRISPR system. Using optimal concentrations that limited toxicity, we phenocopied known mutants across a wide range of phenotypes: those that act autonomously or with few interacting cells (synapse formation, ciliogenesis), those that require many, non-autonomously, interacting cells (neuronal migration, jaw formation), and those that require large numbers of interacting cells (mesodermal convergent extension). Multiplexing six gRNAs in a single injection generated multi-phenotype embryos. Using this method, we targeted five genes related to a known gene required for synaptogenesis, and found two new candidates; one confirmed in animals homozygous for a 5kb deletion, the other is in progress. Encouraged, we are currently screening a targeted set of genes for defects in synapse formation. Multiplexing gRNAs greatly reduces the bottleneck of manual injections and demultiplexing easily identifies candidate genes. We conclude that CRISPR can be used in a powerful genetic screening strategy providing an efficient, cost effective approach, addressing issues of gene redundancy and multigenic phenotypes while maintaining the broad application for rapid identification of new genes involved in many biological processes and should apply to many model systems.

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Program Abstract #286
The GEISHA Database: A Comprehensive Gene Expression Resource for the Chicken Embryo
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GEISHA (gallus expression in situ hybridization analysis; http://geisha.arizona.edu) is a comprehensive, searchable database containing whole mount in situ hybridization (ISH) gene expression information and associated metadata for the chicken embryo. ISH expression data is obtained from day 0.5-5 embryos through large-scale in house ISH screens, from other researchers, and curated from the published literature. The database currently houses more than 39,000 images showing expression patterns of approximately 3,000 developmentally regulated genes. The database and user interface have been reorganized to integrate enhanced genomic related information for chicken, and to incorporate expression information for other model organisms. Gene summary pages provide genomic, transcript and protein metadata, and direct links to all major gene expression databases for mouse, Xenopus, Zebrafish and Drosophila are now integrated with chicken ISH expression information. A gene family Quick Search organizes data according to transcription factor, growth factor, growth factor receptor, and microRNA gene families. A new Human Disease Resource permits searching and browsing of disease-gene associations and corresponding gene expression data. Ongoing upgrades include the addition of serial section viewing and incorporation of high throughput sequencing expression data. The GEISHA database provides a comprehensive gene expression resource for the avian and broader research communities. Supported by NIH Grant P41HD064559 to PBA.

Program Abstract #287
GXD: The Mouse Gene Expression Database for Developmental Biologists
Constance Smith, Jingxia Xu, Jacqueline Finger, Ingeborg McCright, Terry Hayamizu, Janan Eppig, James Kadin, Joel Richardson, Martin Ringwald
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By integrating large amounts of mouse developmental expression information, and by making these data readily accessible and easily searchable, the Gene Expression Database (GXD) supports investigators in their quest to understand the molecular mechanisms of development and disease. GXD contains expression information from wild-type and mutant mice. It covers all stages of development and integrates different types of expression data, including those derived from RNA in situ, immunohistochemistry and RT-PCR experiments. GXD acquires data through curation of the literature, electronic submissions and collaborations with
groups performing large-scale expression studies. Currently GXD contains nearly 1.5 million expression results for over 13,800 genes. As well, GXD has over 260,000 images of expression data, allowing users to retrieve the primary data and interpret it themselves. By being an integral part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with other genetic and disease-oriented data. Thus, GXD can provide tools that allow users to evaluate expression data in the larger context and search by a wide variety of parameters and in ways unavailable elsewhere. Recent interface enhancements include an enhanced anatomy ontology and browser tool and the addition of data filters to interactively refine search summaries. GXD and its data are available through the MGI web site at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

Program Abstract #288
Examination of the effect of a DNA repair defect on the efficiency of ENU mutagenesis
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ENU mutagenesis is a powerful method for generating novel lines of mice that are informative with respect to both fundamental biological processes and human disease. Rapid developments in genomic technology have made the task of identifying causal mutations by positional cloning remarkably efficient. One limitation of this approach remains the mutation frequency achievable using standard treatment protocols, which currently generate approximately 1 sequence change per megabase when optimized. In order to increase this efficiency, we have initiated a study in which mice carrying a mutation in the DNA repair enzyme Msh6 are treated with ENU. Of note, heterozygous mice tolerate treatment well, while homozygous mutant mice did not survive, even at lower doses. Mutation efficiency is being assessed by next-generation sequence analysis of the progeny; specifically, exome sequencing is being done to sample the mutagenized genomes with high fidelity in an unbiased and cost-effective manner. As part of the same study, and using the same method for genome sampling, we will examine whether serial treatment of progeny of mutagenized mice can yield founders carrying large numbers of heterozygous mutations. We presently have multiple independent lines, both heterozygous for Msh6 and wild-type, that are on the third round of ENU treatment. Preliminary data suggests that these approaches do increase the mutational load, which can potentially increase the productivity of ENU mutagenesis screens in the mouse.
This work was supported by NIH grant R01 HD036404.

Program Abstract #289
Whole transcriptome comparisons between stable and unstable CHO cell lines for high level of recombinant protein production
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Chinese hamster ovary (CHO) cells are the most commonly used mammalian cell system for production of recombinant biological drugs, particularly therapeutic antibodies. In order to develop CHO cell lines for large scale protein product, a key criterion is that the cloned CHO cell line must be stable during the length process of cell expansion in various scales of cell culture in bioreactors. It is common that in many clones protein expression levels are dramatically reduced or even lost before reaching a final production volume in bioreactor. In fact, CHO cell clones exhibited high level of clonal variations in terms of stability of protein expression even in the subcloned cell lines in which all clones were derived from a single parental clone. Studying genetic/functional variations in cloned CHO cell lines can help understanding the heterogeneity of CHO clones in their stability of protein production and find potential regulation mechanism associated with the stability. In this study, we performed whole transcriptome analyses on two subcloned CHO cell lines with functional heterogeneity. These two subclones were generated from a parental CHO clone that has been transfected with a plasmid encoding a recombinant Fc fusion protein followed by gene amplification by methotrexate (MTX). More than 100 genes were identified as differentially expressed between stable and unstable cell lines through RNA-seq analyses. The RNA-seq data was further verified by qPCR. Our study showed that most of these altered genes are involved in biological processes, including protein binding, catalytic activity, metal ion binding and cation binding. Taken together, our study used whole transcriptome analyses to illustrate the underlying molecular mechanism related to stability of protein production in CHO clones. This information may be used to improve the stability of CHO cell lines for large scale production of biopharmaceutical products.

Program Abstract #290
Transcriptional landscape of the prenatal human brain
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1Allen Institute for Brain Science, USA; 2Univ. of Washington, USA; 3Massachusetts General Hospital, USA; 4MIT, USA; 5Yale Univ., USA; 6UCLA, USA; 7Seattle Children’s Research Institute, USA; 8UT Southwestern Medical Center, USA; 9Univ. of Southern California, USA; 10Yale Univ. School of Medicine, USA

The anatomical and functional architecture of the human brain is largely determined by prenatal transcriptional processes, many of which differ from other animals. We present an anatomically comprehensive atlas of the mid-gestational human brain, including de
These data provide a rich resource for understanding human brain development, and are freely-accessible through the Allen Brain mouse, and that these alignments match a general model of neural development described for observable developmental events. Atlases we demonstrate that gene expression can be used to align the neurodevelopmental stages of human, rhesus monkey, and enrichment in frontal lobe. We also find that many neurodevelopmental disorder and human evolution-related genes show patterned expression, potentially underlying unique features of human cortical formation. Finally, by comparing data from several Allen Brain Atlases we demonstrate that gene expression can be used to align the neurodevelopmental stages of human, rhesus monkey, and mouse, and that these alignments match a general model of neural development described for observable developmental events. These data provide a rich resource for understanding human brain development, and are freely-accessible through the Allen Brain Atlas Data Portal (www.brain-map.org). Funding sources include NIH Award Number 5R24HD008836 and NIMH Award Number RC2MH089921.

Program Abstract #291

Cytoarchitectural differences between human and mouse have molecular correlates, including species differences in gene expression in subplate, although surprisingly we find minimal differences between the inner and outer subventricular zones even though the outer zone is expanded in humans. Both germinal and post-mitotic cortical layers exhibit fronto-temporal gradients, with particular enrichment in frontal lobe. We also find that many neurodevelopmental disorder and human evolution-related genes show patterned expression, potentially underlying unique features of human cortical formation. Finally, by comparing data from several Allen Brain Atlases we demonstrate that gene expression can be used to align the neurodevelopmental stages of human, rhesus monkey, and mouse, and that these alignments match a general model of neural development described for observable developmental events. These data provide a rich resource for understanding human brain development, and are freely-accessible through the Allen Brain Atlas Data Portal (www.brain-map.org). Funding sources include NIH Award Number 5R24HD008836 and NIMH Award Number RC2MH089921.

Program Abstract #292

Transcriptional Regulation of Gli Target Genes in the Limb Bud

Hedgehog (Hh) signaling acts through Gli transcription factors to regulate growth and patterning in the developing limb bud. Here we performed experiments to distinguish genes that transiently require Hh versus those that require sustained signaling. In parallel experiments, we identified gene expression patterns for approximately 200 putative Gli target genes. Approximately 50 of these genes have localized expression patterns within the Hh-responsive domain of the limb. These patterns cluster into three major expression groups. The different groups have distinct requirements for Hh signaling. One domain requires sustained Hh signaling over time, whereas most genes within the other domains do not. To identify transcriptional co-regulators, we looked for enriched sequences in Gli binding regions associated with these genes. The most enriched motif within all domains was one corresponding to SP transcription factors. Using several approaches, we find that SP1 is required for the activation and maintenance of Gli target genes. We speculate that SP1 acts to mediate enhancer-promoter interactions for Gli responsive genes.

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Program Abstract #293

single bar, a MEF2 target of myoblast fusion

In Drosophila, myoblast fusion is a conserved process in which founder cells (FCs) and fusion competent myoblasts (FCMs) fuse to form a functional and syncytial muscle fiber. Mutants for the myogenic regulator Myocyte enhancer factor-2 (Mef2) show a failure of myoblast fusion, indicating that MEF2 regulates the fusion process. Indeed, chromatin immunoprecipitation studies show that several genes involved in myoblast fusion are bound by MEF2 during embryogenesis. Of these, the MARVEL domain gene singles bar (sing), is down-regulated in MEF2 knockdown pupae, and has five putative MEF2 binding sites within a 9000 bp region. To determine if sing is a direct MEF2 target during muscle development, we identified a 315bp myoblast enhancer of sing. This enhancer is active during myoblast fusion, and mutation of two MEF2 sites significantly decreases enhancer activity. We show that
lack of sing expression results in adult lethality and muscle loss due to a failure of fusion. Additionally, we sought to determine if sing was required in either FCs or FCMs to support fusion. Interestingly, knockdown of sing in either population did not significantly affect fusion, however, knockdown in both FCs and FCMs resulted in muscles with significantly reduced nuclei numbers. Finally, we illustrate that MEF2 regulates sing expression at the embryonic stage through the same enhancer sequences, indicating that sing is a MEF2 target at both critical stages of myoblast fusion. Our studies define for the first time how MEF2 directly controls fusion at multiple stages of the life cycle, and begin to demonstrate that the mechanisms of fusion characterized in Drosophila embryos is also used in the formation of the more complex adult muscles.

Program Abstract #294
Nuclear Factor One proteins as potential regulators of Hedgehog target genes
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The Hedgehog (Hh) signaling pathway is essential for the growth and patterning of many tissues, and results in birth defects and cancers when damaged. The Hh pathway works by modulating the activity of Gli transcription factors to activate or repress target genes. In the developing cerebellum, Sonic Hedgehog secreted from Purkinje neurons acts on granule neuron progenitors (GNPs) to drive proliferation. Chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) in GNP's revealed Gli-bound regions are enriched for Nuclear Factor One (Nfi) consensus binding sites. Three of the four Nfi genes, NfiA, NfiB, NfiX, are highly expressed in the developing cerebellum. NfiA and NfiX mutant animals display defects in GNP proliferation and maturation. ChIP-seq using antibodies specific to each Nfi protein showed considerable overlap in Nfi binding throughout the genome. Overlapping ChIPseq results combined with co-immunoprecipitation experiments support the idea that Nfi proteins stably form heterodimers in vivo. Several observations support a role for Nfi proteins in Hh target gene regulation. Nfi binding overlapped considerably with Gli binding, and NfiA, NfiX binding to chromatin is reduced when Hh is inhibited. Nfi proteins can be co-immunoprecipitated with Gli proteins. NfiA−/− cerebellum shows similar gene expression changes to cerebellum treated with Hh antagonist. Together these findings show Nfi proteins modulate the transcription of a subset of Hh dependent genes in the cerebellum, and likely function either in complex with Gli proteins or as targets of Gli.

Program Abstract #295
Epigenetic activation of Sox2 enhancer on embryonic neural plate
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During vertebrate development, the first and definitive neural marker is the Sox2 gene. The spatiotemporal control of Sox2 expression is regulated by multiple enhancers. The N-1 enhancer initially activates Sox2 expression in response to inducing signals from Hensen’s node; then the N-2 maintains Sox2 expression in regions rostral to the node. Here, we characterize the in vivo function of the H3K9me3 histone demethylase, JmjD2A, during Sox2 activation in the early neural plate. In situ hybridization analysis reveals that JmjD2A expression precedes that of Sox2 in the neural territory. To study the effects of JmjD2A knock down, we performed gain- and loss-of-function experiments, by electroporating either the full-length enzyme or a translation-blocking hybridization

Program Abstract #296
Long-range integration of repressive and patterning inputs at the Drosophila even-skipped locus
Jemma L. Webber, Jie Zhang, Aaron Mitchell-Dick, Ilaria Rebay
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Developmental robustness, such that almost every embryo gives rise to a fully functional adult, depends on precise regulation of both the levels and spatiotemporal patterns of gene expression. To achieve this, most genes are controlled by multiple enhancers. How transcriptional inputs are integrated across multiple regulatory regions is not known. Our work centers on the Drosophila ETS transcriptional repressor Yan, which directs cell fate specification downstream of receptor tyrosine kinase signaling. Focusing on the heart identity gene even-skipped (eve), we find that in addition to binding a muscle-heart enhancer (MHE) which drives expression in cardiac precursors in the embryonic mesoderm, Yan occupies two previously uncharacterized elements that we name D1 and D2.
Hooking the D1 or D2 enhancers up to a minimal promoter and reporter revealed that unlike the MHE, they do not drive a heart-specific expression pattern. Instead, both serve as general repressive elements that recruit and maintain the high local Yan concentration required to prevent aberrant MHE-driven activation of eve. Supporting this model, targeted genomic deletions of the D1 or D2 regions compromise Yan-mediated repression, leading to increased and more variable eve expression, and ultimately impaired cardiac function. Mechanistically, deletion of any individual Yan-bound element reduces occupancy at the other two regions, while chromatin conformation capture reveals a direct contact that could facilitate long-range interactions between the D1/D2 and MHE. Together our work identifies a new class of repressive element that communicates in the 3D environment with the pattern-generating MHE to ensure precise eve expression during heart development. We suggest the strategy of integrating repressive inputs with patterning information may be widely used to fine-tune the expression of critical developmental genes. Supported by awards from the American Heart Association to Jlw and from the NIH to IR.

**Program Abstract #297**

**The control of short-stature homeobox (Shox) gene expression in developing limbs by long-range enhancers**  
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The mammalian Shox genes code for two transcription factors (SHOX and SHOX2) vital for normal limb development and chondrogenesis. Yet the proteins and genetic elements controlling their expression are poorly characterized. Studies of these genes are especially relevant to human health since deficiencies of the SHOX gene cause the limb deformities characteristic of Turner syndrome, Léri-Weill dyschondrosteosis (LWD) and Langer mesomelic dysplasia, which together affect as many as 1:1000 people. SHOX deficiencies cause shortening of the radius/ulna of the arms and the tibia/fibula of the legs. In contrast, the mouse Shox2 gene is required for the development of the humerus and femur. Importantly, deletion of a presumed enhancer far downstream of the SHOX gene causes as many as 35% of LWD cases. To date, we have used transgenic mouse experiments to narrow down the SHOX limb enhancer activity to a 3.3 kb sequence 250kb downstream of the gene. Surprisingly, the enhancer sequence we have identified does not include the highly conserved sequences that were previously assumed to contain the enhancer activity. In parallel experiments, we have sought to determine whether a similar discrete enhancer controls mouse Shox2. In contrast to human SHOX, we have found that mouse Shox2 appears to be regulated by a dispersed set of enhancers scattered over a large genomic region. We are using circular chromosome conformation capture (4C-seq) technology to map the chromosomal regions that interact with the Shox2 gene during limb development. We have found a striking correspondence between GLI3 binding regions (Vokes et al., 2008) and genomic regions that interact with Shox2. We are using transgenic and genetic experiments to test the identified regions for limb enhancer activity and regulation by GLI3. Taken together, our studies will provide an important tool for helping to decipher how regulatory inputs result in the pattern of the tetrapod limb. Funding: CIHR grant MOP-93562.

**Program Abstract #298**

**Machine-learning identifies predictive signatures of enhancer activity in individual cardiac cells**  
Julian Haimovich¹, Brian Busser¹, Di Huang², Ivan Ovcharenko², Alan Michelson¹  
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The *Drosophila* heart is composed of multiple cardiac cell subtypes, which differ from each other in morphology, function, and gene expression patterns. The activities of cell-specific gene regulatory sequences known as enhancers contribute to the fate of these cells during cardiogenesis. Previous studies from this and other laboratories have demonstrated that histone protein modification and transcription factor (TF) binding sites can be used to identify unique genetic signatures of enhancers that are functional in cardiac tissues during embryogenesis. Here, we developed and applied a machine-learning approach to identify unique signatures of enhancers active in individual cardiac cell subtypes during embryogenesis. To begin, we undertook a large-scale screen of *Drosophila* enhancers having activities in cardiac cell subtypes. These enhancers were then used as a training set for construction of a series of cell-specific support vector machine classifiers based on integrated histone protein modifications, in vivo TF binding, and TF motifs. Using these classifiers, we identified unique enhancer signatures and predicted novel pericardial-specific enhancers in the *Drosophila* genome. Furthermore, hierarchical clustering of classifier weights for TF motifs revealed differentially weighted TFs amongst the cell-specific classifiers, which we hypothesize discriminate individual heart cell fates. Taken together, our data demonstrate that cell-specific classification of transcriptional enhancers is a powerful approach for identifying both novel cardiac regulatory activity in *Drosophila* and genetic features that play a role in cell-fate specification.

**Program Abstract #299**

**Red Light, Green Light: A Novel Approach to Studying Interactions between Enhancers and Gene Promoters**  
Mary List, Eric Camino, Jordan Vellky, Thomas Williams  
Univ. of Dayton, USA

The expression of animal genes is highly regulated, and changes in gene regulation have played a prominent role in animal evolution. The mechanisms of gene regulation have been well studied, yielding a simplified model of regulation where gene sequences known as “enhancers” interact with gene “promoters” in order to switch “ON” or “OFF” gene expression in certain cell types, developmental time points, and/or environments. Moreover, changes in the sequence encodings of enhancers and promoters can result in evolved patterns of expression. While promoters are located just upstream of a gene’s transcriptional start site, enhancers reside in more diverse locations. These include introns, upstream and downstream of regulated genes, and often in closer
Sites (IRES), are positioned close to the AUG of subsets of Hox mRNA to interact with ribosomal proteins and directly recruit the ribosome. By making the first targeted deletion of an IRES element, we show that HOXA9 protein is undetectable in neural tube and confer ribosome-mediated control of gene expression. These structured RNA elements, resembling viral Internal Ribosome Entry RNA regulons in Hox 5'UTRs confer ribosome specificity to gene regulation and body plan formation.

Program Abstract #300

Aret: A novel regulator of alternative splicing in the mRNA of adult muscles in Drosophila
Sandy Duong, Anton Bryantsev, Richard M. Cripps
Univ. of New Mexico, USA

Drosophila melanogaster has been used as a model organism for understanding the complexities of many biological processes due to its short generation time and conservation of genes with vertebrates. We have used adult flies to understand the genetic mechanisms determining differentiation of somatic muscles into specific fiber types. Drosophila flight and jump muscles are distinct functionally and biochemically. This distinction is due to differentially expressed genes and differentially spliced mRNA transcripts unique to each muscle type. Using comparative dataset analysis and literature search, we found that the gene, aret, encodes for three RNA-binding domains, and is differentially expressed across different muscle types. We aimed to determine if aret is an important and novel alternative splicing regulator in the adult fly musculature. Here, we show endogenous Aret protein is localized to the nuclei of the flight muscles within the thorax. Without this regulator, the flies are incapable of flight due to ultra-structural changes within the flight muscles. Remarkably, the presence of Aret is required for flight muscle specific splicing patterns, and ectopic expression of Aret in the jump muscle and in cultured cells promotes flight muscle specific splicing. This indicates Aret works autonomously as a regulator of alternative splicing in flight muscles. We have identified novel binding sites for this protein in a model transcript of a muscle-specific gene. The mammalian ortholog of Aret, the CELF family proteins, are implicated as regulators of alternative splicing, and improper dosages of these proteins result in cardiomyopathies and muscular dystrophies. This study suggests an evolutionarily conserved pathway for controlling muscle tissue diversification.

Program Abstract #301

RNA regulons in Hox 5’UTRs confer ribosome specificity to gene regulation and body plan formation
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The ribosome is generally considered to translate mRNAs similarly across cell types and developmental stages. However, recent studies suggest that individual ribosomal proteins confer greater specificity towards the translation of select transcripts, although how this is achieved remains largely unknown. Here we uncover unique RNA regulons embedded in Homeobox (Hox) 5’UTRs that confer ribosome-mediated control of gene expression. These structured RNA elements, resembling viral Internal Ribosome Entry Sites (IRES), are positioned close to the AUG of subsets of Hox mRNA to interact with ribosomal proteins and directly recruit the ribosome. By making the first targeted deletion of an IRES element, we show that HOXA9 protein is undetectable in neural tube and somites of Hox9^{-IREN/IRES} mouse embryos, where it is normally expressed, and there is a fully penetrant homeotic transformation. Remarkably, despite numerous layers of Hox gene regulation, these cellular IRES elements still act as essential RNA “switches” in converting Hox transcripts into proteins to pattern the mammalian body plan in vivo. The unexpected requirement for Hox IRES-dependent translation is further explained by the identification of a novel Translational Inhibitory Element (TIE) within the same transcripts, which blocks cap-dependent translation, thereby enabling a unique mode of translation initiation in these key developmental regulators. Together, these studies uncover a new paradigm of translational regulation that is achieved through a combination of unique RNA regulons within the mRNA template, to promote ribosome-mediated control of gene expression and organismal development.

Program Abstract #302

Hoxa2 ultraconserved element is required for normal expression of neighboring Hox genes
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Hoxa2 is a member of Hox family of transcription factors and is required for the hindbrain patterning and the formation of the skeletal structures. While multiple cis-regulatory elements have been identified within the Hoxa2 locus, little is known if these elements can regulate expression of other Hox members. In this study we utilized Hoxa2-Cre allele where Cre insertion ablates ultraconserved element required for Hoxa2 expression in the rhombomere 4. The Hoxa2 mutants show reduced Hoxa3 and Hoxa4 expression in neural crest-derived mesenchyme and pharyngeal endoderm. The intercross between Hoxa2-Cre^{+/-} and Hoxa3^{+/-} heterozygotes revealed that the compound heterozygotes display phenotype similar to Hoxa3-deficient mutants, indicating that Hoxa2 regulates Hoxa3 expression in a cis-manner. In addition to previously reported defects, Hoxa2 mutants display hypoplastic and ectopic thymus together with altered parathyroid morphogenesis, a feature that has not been previously reported. Analysis of Foxn1, an early thymic epithelial marker, and Gcm2, a parathyroid specific marker, demonstrated delayed expression and overall
Snai1 (plag1) (pleiomorphic adenoma gene 1) is a zinc finger transcription factor and an oncogene. Similar predicted Plag1-binding transitions, cancer, and pluripotency. We discovered evolutionally conserved putative Plag1 binding sites in the promoter region of Miller Lee1, Ashley Bonneau1, Valeria Yartseva1, Carter Takacs1, Ariel Bazzini1, Florencia del Viso1, Mustafa Khokha1, Hiroyuki genome activation is thought to take place. Snai1 is a zinc finger transcription factor associated with epithelia-to-mesenchymal supported by NIH grants T32GM007499 (A.R.B), F32HD071697-02 (MTL), R01GM081602-06, R01GM103789-01, pluripotency and differentiation. Conserved gene regulation during the maternal-to-zygotic transition.

Program Abstract #303
Identifying regulatory elements in 3'-untranslated regions of mRNAs
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Regulation of gene expression in eukaryotes is a complex process required for proper functioning of all cellular process. The p38 mitogen activated protein kinase (MAPK) pathway regulates the stability of many proinflammatory cytokines, like Tumor Necrosis factor (TNF)-? . One mechanism of action of p38 MAPK is through the regulatory protein tristetraprolin (TTP). In its unphosphorylated state, TTP binds to AU-rich elements (AREs) in the 3'-untranslated regions (UTRs) of the mRNAs of some cytokines and promotes their degradation. Bioinformatics tools can predict the presence of AREs in the annotated 3' UTRs of genes, but due to the lack of a strict consensus sequence for AREs and lower quality of annotation of 3’ UTR regions as compared to the coding regions, experimental validation of the predictions is necessary. Zebrafish is an excellent model organism used commonly in developmental biology for its transparent embryos, short generation time and large clutch size. Previous work has shown that upstream TTP inhibitor MAPAPK2 is active in the yolk syncytial layer (YSL) of early zebrafish embryo, making it a convenient target to study TTP regulation. Mix-type homeobox (mxtx) 1 and 2 are homeobox containing transcription factors that have modest ARE scores but their role in zebrafish epiboly suggests that they could be potential TTP targets. Here, we show that when fluorescently tagged RNA containing only the 3'UTR sequence of mxtx1 and mxtx2 is expressed in zebrafish, it localizes primarily to the YSL as opposed to the control that is expressed uniformly in the YSL and the blastoderm. Further work involves eliminating alternate interactions of the 3'UTR with other RNA binding motifs and narrowing down the sequence to particular regulatory elements bound by TTP.

Program Abstract #304
Conserved gene regulation during the maternal-to-zygotic transition
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Embryogenesis is guided first by factors provided maternally, and subsequently by factors generated de novo in the embryo. This transfer of developmental control, called the maternal-to-zygotic transition (MZT), encompasses two major activities: (i) transcriptional activation of the zygotic genome; and (ii) regulated clearance of maternal RNAs. Although the MZT is universal among animals, the mechanisms that regulate genome activation and maternal clearance in vertebrates remain largely unknown. Here, we adopt a multi-species, high-throughput strategy to elucidate conserved modes of transcriptional and post-transcriptional regulation during the MZT. We show in zebrafish that maternally provided core transcription factors Nanog, Pou5f1 (Oct4) and SoxB1 regulate activation of a first wave of zygotic genes, which are required for gastrulation and maternal clearance. Loss of these factors resulted in developmental arrest and a failure to activate >75% of zygotic genes, including the conserved microRNA miR-430, which regulates maternal clearance. Comparison with the fish medaka (Oryzias latipes) and amphibian Xenopus tropicalis revealed conserved first-wave expression of a subset of genes, including miRNAs orthologous to zebrafish miR-430. We found that these miRNAs also regulate maternal mRNA clearance in their respective species, using miRNA knockdown. We also identified a subset of orthologous maternal RNAs that are cleared in a manner depending on zygotic transcription but not depending on miRNAs, suggesting deep conservation of the mechanisms regulating maternal clearance. Together, our results help to elucidate the conserved components of the embryonic gene regulatory network, which is key to understanding the mechanisms that drive pluripotency and differentiation.
Supported by NIH grants T32GM007499 (A.R.B), F32HD071697-02 (MTL), R01GM081602-06, R01GM103789-01, R01HD074078-02, the Yale Scholars Program and Pew Scholars Program (AJG).

Program Abstract #305
Plag1 is a maternally provided transcription factor that regulates the expression of Snai1 and Slc6a8
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Our work aims at discovering transcription factors that regulate the earliest steps of pre-implantation development in human. Our human embryo transcriptome data suggest that the expression of Snai1 (snail family zinc finger 1) increases at 4-cell stage when the genome activation is thought to take place. Snai1 is a zinc finger transcription factor associated with epithelia-to-mesenchymal transitions, cancer, and pluripotency. We discovered evolutionally conserved putative Plag1 binding sites in the promoter region of Snai1. Plag1 (pleiomorphic adenoma gene 1) is a zinc finger transcription factor and an oncogene. Similar predicted Plag1-binding
motifs were also found in the promoter of Slc6a8 [solute carrier family 6 (neurotransmitter transporter), member 8]. To test whether Plag1 regulates these genes, transient over-expression experiments were carried out. In the breast cancer cell line MCF7 and in the human embryonic stem cell line HS401 Plag1 increased Snai1 and Slc6a8 expression. Igf2, a known Plag1 target, was likewise up-regulated in these cell lines in response to Plag1. We next studied the expression of Plag1 in early development. We found that Plag1 is maternally loaded to mouse and human oocytes. Furthermore, Plag1 localizes to the pronuclei of mouse zygotes. During the 2-cell stage, when the mouse zygotic genome activation takes place, the expression of Snai1 and Slc6a8 increases. Similar expression patterns of Plag1, Snai1, and Slc6a8 can be seen in independent human pre-implantation gene expression datasets published by others and us. Collectively, our results suggest that Plag1 is a maternally provided transcription factor that may participate in the zygotic genome activation by regulating Snai1 and Slc6a8.

Program Abstract #306
Independent regulation of vertebral number and vertebral identity by microRNA-196 paralogs
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The total number of vertebrae, and the identity of each segment, are highly reproducible within a given vertebrate species. MicroRNA-196 (miR-196) has been implicated in regulating vertebral pattern, potentially acting through the regulation of Hox genes, but the specific roles miR-196 plays in establishing vertebral number and identity has not been clear. Here we show that the miR-196 family of microRNAs are essential both in defining the total vertebral number and in shaping correct regionalisation of the vertebral column in mouse. Using an extensive allelic series of mouse knockouts, we show that miR-196a2 and miR-196b act in a dose-dependent manner to control the number of rib-bearing vertebrae and positioning of the sacrum. Independent of this, the three miR-196 family members (miR-196a1, miR-196a2, and miR-196b) act redundantly to restrict axis length, in part via modulation of Wnt activity, a signal that has been implicated downstream of Hox gene activity in axis specification. Moreover, we reveal unappreciated complexity in miR-196 regulation of Hox cluster expression dynamics. Loss of miR-196 leads to a collective upregulation of numerous trunk Hox target genes with a concomitant delay in posterior Hox gene activation, genes which are proposed to signal the end of axis extension and whose delayed activation would support regionalised thoracic expansion. By feeding into, and thereby integrating, multiple genetic networks controlling vertebrae formation and patterning, miR-196 is a critical player defining morphological output.

Program Abstract #307
miR-34/449 miRNAs are required for motile ciliogenesis in vertebrate mucociliary epithelia by direct repression of cp110 and regulation of basal body function
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Cilia are tubulin-based cell protrusions, which can be non-motile and serve sensory functions in signaling, or can be motile and required for the generation of extracellular fluid flows. Multiciliated cells (MCCs) harbor hundreds of motile cilia that beat in a coordinated fashion to transport mucus in mucociliary epithelia, e.g. in the vertebrate airways. The Xenopus embryonic epidermis is also a mucociliary epithelium, and has emerged as a valuable model to study transcriptional and signaling control of MCC induction, differentiation and function. In addition to transcriptional networks consisting of factors like Notch, Multicilin and Foxj1, post-transcriptional mechanisms also control ciliogenesis in vertebrate MCCs. The miR-34/449 family consists of six homologous miRNAs located at three genomic loci. Redundancy of miR-34/449 and their dominant expression in multiciliated epithelium suggest a functional significance in ciliogenesis. Here, we report that mice deficient for all miR-34/449 exhibited postnatal mortality, infertility, and strong respiratory dysfunction caused by defective mucociliary clearance. In both mice and Xenopus, miR-34/449-deficient MCCs exhibited a significant decrease in cilia length and number, due to defects in basal body maturation and in docking to the apical membrane. miR-34/449 function in ciliogenesis, at least in part, by direct post-transcriptional repression of Cp110, a centriolar protein that suppresses cilia assembly. Knockdown of cp110 in miR-34/449-deficient MCCs restored ciliogenesis by rescuing basal body maturation, Centrin incorporation and docking to the apical membrane. Our findings provide key insights into the evolutionarily conserved functions of miR-34/449 and elucidate the mechanism through which miR-34/449 enable motile ciliogenesis.

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Program Abstract #308
The Role of Long Noncoding RNAs in Regulating Chicken Limb Patterning
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Recently, it has been observed that long noncoding RNAs (lncRNAs) influence development and differentiation by regulating gene
Program Abstract #309
JmjD2B histone demethylase regulates otic placode invagination via epigenetic control of Dlx3
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The inner ear is one of the most sophisticated sensory organs in vertebrates, relaying both acoustic and motion/balance information to the brain. The inner ear development involves an intricately regulated series of events from otic placode induction, to invagination and overt differentiation. Here, we show that the epigenetic modifier JmjD2B, a histone demethylase, plays an important role in chick inner ear development. In situ hybridization (ISH) reveals expression of JmjD2B in the otic placode during its induction and later at the rim of the invaginating otic vesicle. Consistent with this, immunostaining reveals clear variations in the spatiotemporal expression of JmjD2B substrates, the epigenetic marks H3K9me3 and H3K36me3, in ectodermal, invaginating and post-invaginating otic cells. Unilateral electroporation of JmjD2B morpholino into stage 8 chick embryos reduced the expression of several ear markers, including Pax2, Dlx3, Soho1 and Sox10 by stage 13. Moreover, JmjD2B knock-down caused a clear defect in the placode cell invagination, accompanied by loss of apicobasal polarity, disorganization and multilayering of placode cells, and misexpression of cell adhesion molecules like E-cadherin. To identify potential direct targets of JmjD2B, we performed in vivo chromatin immunoprecipitation (ChIP) on dissected otic placodes and otocysts. The results show that JmjD2B interacts with regulatory regions of the Dlx3 locus, but not with Pax2 or Soho1. Taken together, the results reveal for the first time an epigenetic modification, via histone demethylation, that influences expression of key genes implicated in inner ear invagination in vertebrate embryos.

Program Abstract #310
HDAC Inhibition Disrupts Cellular Differentiation and Patterning in the Organ of Corti
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The mammalian auditory sensory organ, known as the organ of Corti, is made up of two general cell types, hair cells and supporting cells, which are specified and differentiate during embryonic development. Hair cells and supporting cells have distinct cellular phenotypes that are important for auditory function and are patterned in a stereotyped manner along the length of the cochlea duct. Considerable work has shown that various signaling pathways converge to direct cell-specific gene expression profiles that orchestrate the differentiation and patterning of these cells within the organ of Corti. However, the mechanisms by which these cell-specific gene expression profiles are maintained are largely unknown. Post-translational modification of histones, such as acetylation, is one way that gene expression profiles can be regulated. Using an in vitro organ culture model of mouse cochlea, we applied histone deacetylase (HDAC) inhibitors to determine what role regulated acetylation dynamics play in the developing organ of Corti.

Treatment with HDAC inhibitors at early developmental stages, when many cells are still making cell fate decisions, leads to a stalling of the gradient of differentiation that normally occurs along the length of the duct. Treatment at later developmental stages, after cell fate decisions have been made and individual cells are already taking on their characteristic morphologies, results in changes in both hair cells and supporting cells. Within hair cells, characteristic mechanosensory stereociliary bundles degenerate and cell-cell contacts change, leading to overall patterning defects. Supporting cells undergo changes in expression of multiple characteristic cellular markers and in their cell shape, which results in a flattening of the organ of Corti into a monolayer. These results suggest that cell-specific gene regulation through histone acetylation is essential for the normal development and maintenance of the organ of Corti. Funded by NIH IRP.
Program Abstract #311
Epigenetic alterations by NuRD and PRC2 in the neonatal mouse cochlea
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Mammalian cochlear supporting cells remain quiescent at postnatal ages and age-dependent changes in supporting cell proliferative capacity are evident. Very little is known about epigenetic regulation in the mammalian inner ear, and almost nothing is known about the histone modifications. Our data indicate that cofactors of repressive complexes such as NuRD and PRC2 are present in the neonatal organ of Corti. These NuRD cofactors are present throughout most of the organ of Corti from E18.5 until P4. By P6, these NuRD cofactors are mostly undetectable by immunofluorescence and completely lost by P7, but are detectable again at P8 and continue to be present through P21. The PRC2 enzymatic subunit, EZH2 is also highly present from E18.5 to P0 in the organ of Corti, but lost between P2 and P4. However, EZH2 staining is evident again throughout the organ of Corti by P6 and persists through P21. Our data provide evidence that HDACs, DNA methyltransferases, histone methyltransferases, and histone demethylases are expressed postnatally within the organ of Corti, and may be targets for drug inhibition to increase the capacity, speed, and efficiency of reprogramming a supporting cell into a hair cell.
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Program Abstract #312
Tissue Specific Regulation of Igf2r/Airn Imprinting During Gastrulation
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Genomic imprinting results in parent of origin mono-allelic gene expression. Although allele specific DNA methylation and histone modifications have been documented at many imprinted loci, tissue and temporal specific mechanisms in vivo that establish and interpret these epigenetic marks are not fully defined. On mouse chromosome 17, transcription of the paternally expressed long non-coding RNA Airn has been shown to silence the paternal alleles of Igf2r, Slc22a2, and Slc22a3 resulting in reciprocal imprinting at the locus (maternal specific expression of Igf2r, Slc22a2, and Slc22a3). Here we document changes in imprinted Igf2r/Airn in a cell lineage and temporal-specific manner during gastrulation in the mouse. Although we observe imprinted expression in extraembryonic tissues at all stages, we find that Igf2r is biallelic and Airn is not expressed in the epiblast prior to gastrulation. Once gastrulation commences, Igf2r and Airn become reciprocally expressed and imprinted. We examined the DNA methylation and histone modifications at two differentially methylated regions (DMRs) at the locus and find epiblast specific spreading of DNA methylation at DMR2 coincident with the start of gastrulation. Furthermore, we also find that CTCF is only present after gastrulation begins, suggesting CTCF may regulate the Igf2r/Airn locus. Our data present a model similar to the H19/Igf2 locus in which spreading of allele specific DNA methylation prevents binding of CTCF to the paternal allele, permitting paternal Airn expression and silencing of paternal Igf2r. These results indicate that similar to events during preimplantation, a wave of epigenetic alterations occurs as the epiblast differentiates into embryonic lineages. We have therefore initiated transcriptome-wide studies to identify changes in parent of origin allele specific expression during gastrulation and will present these findings as well.

Program Abstract #313
Deciphering the regulatory mechanism of Fgf8 repression by retinoic acid signaling
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Retinoic acid (RA) controls development by acting as a ligand for nuclear RA receptors (RARs) that directly regulate transcription via RA response elements (RAREs). During embryogenesis, Fgf8 plays important roles in caudal development and somitogenesis. RA represses Fgf8 at the anterior end of the caudal progenitor zone to prevent FGF signaling from extending into the developing trunk, thus ensuring normal anterior-posterior body axis extension. Although RA restricts caudal Fgf8 expression, the underlying mechanism is unknown. RA activates transcription by recruiting coactivators to RARs bound at RAREs, resulting in altered histone modification and chromatin remodeling. However, the ability of RA to directly repress transcription is not well understood. Here, chromatin immunoprecipitation analysis of E8.25 mouse embryos shows that a conserved RARE upstream of Fgf8 recruits all three RARs in vivo, and mutation of this RARE abolishes the binding of RARs in gel shift assay. To determine the repressive function of RA, we analyzed the enrichment of histone markers H3K27me3/H3K4me3 near the Fgf8 RARE in head, trunk and caudal progenitor zone of E8.25 wild-type and Raldh2-/- mutant embryos. We also analyzed the relative regional occupancy of Polycomb components Ezh2/Suz12, histone deacetylase (HDAC1) and a ligand-dependent corepressor RIP140 near the Fgf8 RARE. Our findings demonstrate that the repressive histone marker H3K27me3, Polycomb, HDAC1, and RIP140 were recruited to the vicinity of the Fgf8 RARE in an RA-dependent manner. Furthermore, deletion of the Fgf8 RARE in transgenic embryos demonstrated that this element is required for RA repression of caudal Fgf8 transcription. Our findings demonstrate that RA directly represses Fgf8 through a RARE-mediated mechanism, thus providing valuable mechanistic insight into RA-FGF antagonism during embryogenesis.
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Program Abstract #314
The role of Wnt signaling in temporal and spatial patterning of mes-r1 in zebrafish
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The midbrain hindbrain boundary (MHB) is an evolutionarily conserved signaling center in vertebrates which patterns the mesencephalon anteriorly and hindbrain rhombomere 1 posteriorly. MHB development has been proposed to comprise three phases: in the positioning phase, otx2 and gbx1 expression patterns abut each other to position the MHB, in the establishment phase, pax2a, wnt1, and fgf8 are activated to initiate the development of the MHB, and in the maintenance phase, these genes establish a regulatory loop to maintain MHB identity and drive MHB morphogenesis. Because Wnt ligands are expressed spatially and temporally so as to be involved in each phase, we asked how different phases of Wnt signaling affect the MHB gene regulatory network and morphogenesis. To answer this question, we blocked Wnt signaling at specific developmental time points using a heat shock dkk-GFP zebrafish line and assayed the presence of the MHB morphological constriction and neural markers indicative of mes-r1 patterning. Blocking Wnt signaling between 6 and 15 hours post fertilization (hpf), results in the absence of the MHB constriction and corresponding neural marker expression, while blocking Wnt prior to or after this window results in embryos with both a MHB constriction and patterned gene expression in mes/r1. This suggests that 6-15 hpf is a critical window for Wnt activity to drive MHB development. How this time window relates to the three proposed phases in MHB development will be discussed. Overall our results suggest that temporally controlled Wnt signaling differentially affects MHB gene expression and morphogenesis during the proposed three phases of MHB development.

Program Abstract #315
Characterization of murine cranial neural crest cell culture models for investigating pathways of chondrogenic and glial differentiation
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Cranial neural crest cells (CNCC) are a migratory, embryonic cell population with the potential to differentiate into many different cell types, yet the mechanisms underlying CNCC migration and differentiation remain poorly understood. Recently, we have discovered that knockdown of Cabin1, a negative regulator of T-cell receptor-mediated signaling, results in a range of defects in developing zebrafish embryos, including craniofacial deformities and abnormal CNCC migration. In T-cells, Cabin1 is known to both directly and indirectly inhibit the activity of transcription factors that also have established roles in regulating chondrogenic or glial differentiation in cells derived from the neural crest. We hypothesize that Cabin1, through its repression of these transcription factors, inhibits the premature differentiation of the CNCCs during migration. In order to investigate Cabin1 function during CNCC development, we have established a system for culturing CNCCs isolated from embryonic mice expressing YFP under the regulation of the Sox9, Sox10, or Wnt1 gene promoters. We are currently characterizing the cultured cells from the different reporter strains to compare their expression of CNCC markers, and their ability to proliferate and differentiate along the glial and chondrogenic lineages in culture. We will use the cultured cells to investigate how Cabin1 protein-protein and protein-gene interactions change in proliferating and differentiating CNCCs, and how these interactions play a role in the gene regulatory network underlying CNCC development. Optimization of this in vitro approach will facilitate our investigations into the biochemical functions of Cabin1 during CNCC migration and differentiation, and will serve as a complement to our ongoing in vivo studies of CNCC development in zebrafish. UWM Children’s Environmental Health Core Center (2P30ES004184), Greater Milwaukee Foundation, UWM Ruth Walker Grant-in-Aid Award, UWM Office of Undergraduate Research

Program Abstract #316
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 #317
Splicing of a Specific Intron is Required for Protein but not RNA Expression of a Neurofilament Reporter Gene in the Developing Nervous System
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The functional roles of introns, despite their ubiquitous presence in eukaryotes, are poorly understood. Although intron splicing in general is known to be important for promoting the nuclear export, stability, and translation of mRNAs, the splicing of specific introns has not been linked directly with the post-transcriptional control of individual genes. Using an assay of injecting reporter plasmids into Xenopus embryos to identify cis-regulatory elements of the middle neurofilament (nefm) RNA, we discovered that splicing of specifically the last intron of the nefm gene was required for robust reporter protein expression in vivo, regardless of promoter or cell type. Reporter plasmids bearing no introns or other introns, whether from the same or a different gene, produced poor protein expression, despite yielding essentially identical, fully spliced, mature mRNAs having the same 3'UTR. Thus, splicing of an intron in general was insufficient for robust protein expression, and the deficient protein expression seen with these other introns was attributable to neither defective transcription nor premature decay of the RNA. Further analysis revealed that the elements critical for robust protein expression lay within the 3' most region of the last intron of the nefm gene. This region, but not the comparable region of the other introns, contained a consensus sequence for binding hnRNP K, an RNA binding protein required for nefm mRNA translation through its association with the nefm 3'UTR in Xenopus neurons. Co-immunoprecipitation experiments indicated that splicing of the last nefm intron indeed promoted the association of hnRNP K with the nefm 3'UTR reporter RNA in vivo. These findings indicated that splicing of a specific intron may be used to initiate association of the RNA with the ribonucleoproteins that subsequently regulate trafficking of the mature mRNA through the cell. Supported by NSF IOS-1257449.

Program Abstract #318
DBL-1 Target Gene Regulation By SMA-2, SMA-3, and SMA-4
Uday Madaan, Cathy Savage-Dunn
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The DBL-1 ligand, a TGF-b homolog in C. elegans, is necessary for body size regulation, mesodermal patterning, innate immunity, aging, reproductive life span, and male tail sensory ray identity. Transcriptional response to DBL-1 relies on Smads SMA-2, SMA-3, and SMA-4. Although the initial steps of the DBL-1 pathway are known, the mechanisms of DBL-1 target gene regulation are still relatively unknown. Previously experiments in our lab demonstrated that the hypodermis is the main tissue responsible for body size regulation via the DBL-1 pathway. In addition, at least three cuticle collagen genes (col-41, rol-6 and col-141) were shown to be targets of the DBL-1 pathway involved in body size regulation. We tested whether col-41 is a direct or indirect target of the DBL-1 pathway. Via Electrophoretic mobility shift assay (EMSA) we illustrated that col-41 is not a direct target of Smads. This was further confirmed by SMA-3 ChIP-seq data obtained recently (M Kudron and V. Reinke, pers. comm.). In contrast, SMA-3 binding is detected downstream of col-141. Future experiments will test whether col-141 is a direct or indirect target of Smads. A second set of experiments conducted to test target gene regulation by Smads led to an interesting observation. Upon QRT-PCR analysis of dbl-1 and sma-3 mutants, we observed that targets genes of the pathway are largely regulated in opposite directions between the two mutants. This could have two possible implications: 1) In absence of DBL-1, SMA-3 is regulating genes in an opposite manner; 2) DBL-1 can regulate target genes in a SMA-3-independent manner. Further experiments need to be conducted to confirm any speculation beginning with construction of the sma-3;dbl-1 double mutant.

Program Abstract #319
Germline cosuppression requires the nuclear RNAi pathway in C. elegans
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Cosuppression, a phenomenon first described in plants, is a variant of repeat-induced silencing in which the presence of multiple copies of a transgene results in lack of expression from both the introduced transgene and endogenous genes sharing sequence similarity. We have been working with a previously established model of germline cosuppression in C. elegans in which an integrated single copy of full-length gfp, driven by the germline promoter pie-1, is silenced by a repetitive array of truncated gfp sequences integrated into a different chromosome. Previous research on this model found that cosuppression and RNA interference (RNAi) are distinct processes that share molecular machinery, including several mutator (mut) genes and the fertility factor pgl-1. Previous genetic screens designed to uncover additional factors required for cosuppression suggested a role for transcriptional gene silencing at the single copy “endogenous” locus. However, whereas H3K9 methylation is clearly enriched along the repetitive transgene array, work in our lab found no enrichment in the promoter region of the single copy gfp. Moreover, ChIP-qPCR experiments indicated that RNA polymerase II associated with the transcriptional initiation site of the single copy gfp at similar levels in both the presence and absence of the repetitive array. We suspected that RNA transcription might be initiated but elongation blocked, and thus decided to test whether the nuclear RNAi defective (nrde) genes might be involved. We found that nrde-2 and heritable RNAi defective-1 (hrde-1) are essential components of the cosuppression pathway. These results are consistent
Cartilage is an important tissue in vertebrates beginning in the early embryo where it lays down the scaffolding for the skeleton, and continuing through adulthood where it makes up joints and intervertebral disks. The major component of cartilage is a filamentous protein known as Collagen type II, alpha 1 (Col2a1). Mutations in \textit{col2a1} in humans can lead to multiple congenital disorders and could produce novel therapeutic targets. Previously, we identified a 310 bp regulatory region (R2) 1.7 kb upstream of the transcriptional start site that is critical for cartilage, ear, and notochord expression of the \textit{col2a1} gene in zebrafish. Utilizing transgenic zebrafish EGFP reporter analysis, we have now narrowed R2 down to 120 bp that can still reproduce the full \textit{col2a1} mRNA expression seen by \textit{in situ} hybridization. Further characterization allowed us to identify a 60 bp sequence that specifies cartilage and ear but eliminates notochord expression. By employing comparative genomic analysis we have identified three highly conserved putative transcription factor binding sites for Ets, Runx, and Sox families. Generation of \textit{col2a1} R2 deletion EGFP transgenic reporter embryos and transcription factor overexpression suggest that these three transcription factors are sufficient for expression in the ear and cartilage.

Program Abstract #320  
Roles of Brg1 during retinal development and tumorigenesis  
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The roles of the chromatin remodeling factor Brg1 during retinal development and tumorigenesis are poorly understood. We show that conditionally inactivating Brg1 in the retina leads to a dramatic reduction in eye and retinal size. Double labeling, immunostaining, and clonal analysis experiments suggest that reduced retinal size is due to elevated levels of apoptosis and an increase in cell cycle length in retinal progenitor cells. While retinal cell fate is not altered upon loss of Brg1, retinal lamination is severely impacted and is associated with disruption of retinal apical-basal polarity. Analysis of gene expression in Brg1-deficient retinai indicates that Brg1 is required for the expression of genes important for cytoskeleton organization, cell survival and proliferation. We further provide evidence that Brg1 functions as a tumor suppressor gene in the context of Rb:p107 mutation. Our data underscore the importance of epigenetic processes of Brg1 containing SWI/SNF complexes in coordinating retinal progenitor cell proliferation, survival and retinal lamination during development.

Program Abstract #321  
EWS/FLI Utilizes NKX2-2 to Repress Mesenchymal Features of Ewing Sarcoma  
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Ewing sarcoma, the second most common bone cancer in children and adolescents, is caused by chromosomal translocations between EWS and ETS genes. The most common of these, t(11;22)(q24;q12), encodes EWS/FLI, which aberrantly regulates expression of genes to maintain the transformed phenotype. Among these is NKX2-2, a homeodomain transcription factor which is critical for central nervous system and endocrine pancreas development. We used differential RNAi and RNA sequencing to determine changes in gene expression attributable to NKX2-2 depletion that may contribute to pathogenesis. We find that NKX2-2 and EWS/FLI repress a common subset of genes that modulate cell adhesion and migration in Ewing sarcoma. NKX2-2 depletion significantly increases actin stress fiber and focal adhesion assembly in Ewing sarcoma cell lines, phenocopying EWS/FLI depletion. Likewise, both EWS/FLI and NKX2-2 knockdown promote adhesion and migration of Ewing sarcoma cells. By inhibiting these behaviors, and attenuating formation of molecular structures that drive them, NKX2-2 represses mesenchymal features in Ewing sarcoma. In contrast, genes in the cell adhesion/migration signature repressed by EWS/FLI and NKX2-2 are activated by ZEB2. Being highly expressed in Ewing sarcoma, ZEB2 controls the expression of genes that support mesenchymal features and suppress epithelial features. We show that zyxin, a cytoskeletal protein that stabilizes actin stress fibers, is reciprocally regulated by ZEB2 and NKX2-2 or EWS/FLI. NKX2-2 or EWS/FLI depletion causes zyxin accumulation to correlate with enhanced mesenchymal features. We hypothesize that Ewing sarcoma cells exist in a state of developmental tension between epithelial and mesenchymal fates. In this state, they may combine growth and self-renewal features with a capacity to escape the primary tumor and occupy distant niches. This hypothesis offers a framework to interrogate mechanisms for invasion and metastasis in Ewing sarcoma.

Program Abstract #322  
Elucidating the transcriptional regulation of the zebrafish \textit{col2a1a} gene  
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Cartilage is an important tissue in vertebrates beginning in the early embryo where it lays down the scaffolding for the skeleton, and continuing through adulthood where it makes up joints and intervertebral disks. The major component of cartilage is a filamentous protein known as Collagen type II, alpha 1 (Col2a1). Mutations in \textit{col2a1} in humans can lead to multiple congenital disorders and the early onset of joint and retinal deterioration. Our laboratory is interested in understanding the conserved transcriptional regulation of this critical structural gene utilizing the zebrafish, \textit{Danio rerio}. Understanding the genetic network upstream of \textit{col2a1a} could produce novel therapeutic targets. Previously, we identified a 310 bp regulatory region (R2) 1.7 kb upstream of the transcriptional start site that is critical for cartilage, ear, and notochord expression of the \textit{col2a1a} gene in zebrafish. Utilizing transgenic zebrafish EGFP reporter analysis, we have now narrowed R2 down to 120 bp that can still reproduce the full \textit{col2a1a} mRNA expression seen by \textit{in situ} hybridization. Further characterization allowed us to identify a 60 bp sequence that specifies cartilage and ear but eliminates notochord expression. By employing comparative genomic analysis we have identified three highly conserved putative transcription factor binding sites for Ets, Runx, and Sox families. Generation of \textit{col2a1a} R2 deletion EGFP transgenic reporter embryos and transcription factor overexpression suggest that these three transcription factors are sufficient for expression in the ear and cartilage.
TRα mutant frogs. The findings in this study support the hypothesis that TRα plays a significant role in timing and rate of measured mRNA expression levels of TH-responsive genes to test gene de-repression in the dual function model in TRs using F1 metamorphic development in the hind limb and skin. We also found that TRα may act to delay development in the lung.

Sources of Research Support: Weimen Wendel Grant, Univ. of Cincinnati awarded to Jinyoung Choi

Program Abstract #323
ACTH knockout frog model to gain insights into glucocorticoid mediated signaling during development.

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Glucocorticoid (GC) hormones play vital roles in development, mainly HPA axis and tissue maturation (e.g. lungs) and mediating the effect of environmental challenges in utero as well as in adult life. Fetal exposure to excess GCs leads to reduced birth weight, hypertension and later-life cardiovascular and metabolic disorders. This effect can be observed in amphibian metamorphosis, which requires Thyroid hormone and where stress in early developmental stages retards growth and transformation, but stress at later stages accelerates development. The key to understanding these complex developmental endocrine actions is by studying the interaction of developmental hormones with each other in gene expression and subsequent morphological and physiological changes. The main circulating GC in amphibians Corticosterone (CORT, the frog stress hormone) is known to mediate the effect of environment during metamorphosis, likely by binding nuclear receptors to initiate the downstream changes. Since CORT and Aldosterone levels both peak at metamorphic climax, we hypothesize that the hormones and their receptors play redundant roles in stress signaling. We created an Adrenocorticotropic Hormone (ACTH) knockout model using TALENs, with the inability to produce CORT. We are currently conducting genetic, gene expression, and phenotype analyses to determine the role of CORT in metamorphosis. We are also creating knockout models for Glucocorticoid and Mineralocorticoid Receptors – two known nuclear receptors in mammals for GCs that are conserved in amphibians and mammals and will help elucidate on the role of stress signaling in development. Since early life stress plays a role in specifying later-life pathophysiology, these results will increase our understanding of the developmental mechanisms underlying those effects and could pave ways to identifying and treating developmental defects.

Funding Sources: Weimen Wendel Grant, Univ. of Cincinnati awarded to Leena Shewade

Program Abstract #324
The role of thyroid hormone receptor alpha in frog development

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Thyroid hormone (TH) receptor (TR) is a central player required for TH-dependent development in vertebrates. TR mutation in humans causes severe developmental abnormalities, especially in brain development. TH works by binding TRs in the cell nucleus to stimulate production of proteins, leading to diverse developmental changes, such as growth, differentiation, cell death, and remodeling. There are two types of TR, TRα and TRβ, and many studies have examined their roles in development using mammalian animal models. However, it has not been understood yet how TR subtypes play dual functions in different target tissues during development. Based on the expression patterns of two TRs in different tissues during frog metamorphosis, we hypothesized that the absence of TRα will alter the timing of metamorphosis in different TH target tissues consistent with expression levels in TRα in the tissues. Using a recently developed gene disruption technology (Transcription activator-like effector nucleases, TALENs), we created founder TRα mutant frogs, Xenopus tropicalis. The results revealed that mutation on TRα accelerated metamorphic development in the hind limb and skin. We also found that TRα may act to delay development in the lung. We measured mRNA expression levels of TH-responsive genes to test gene de-repression in the dual function model in TRs using F1 TRα mutant frogs. The findings in this study support the hypothesis that TRα plays a significant role in timing and rate of metamorphosis in frogs, showing its tissue-specific manner in TH target tissues/organisms.

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Program Abstract #325
BioTapestry: Modeling Gene Regulatory Networks for Development

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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. BioTapestry was first used to model the sea urchin endomesoderm GRN in 2003, and has since been used across a wide variety of different model systems. It uses a hierarchy of models to present multiple views of the network at different levels of spatial and temporal resolution, and uses a visual representation that is tailored to the presentation of developmental GRNs.

We are continuing to actively develop BioTapestry. The currently available Version 6 introduced several helpful new automatic network layout tools, and we will shortly be releasing Version 7. This upcoming version uses recent web browser technologies such as HTML5 Canvas to allow interactive graphical GRN models to be shared over the web without requiring the end user to have Java installed, since GRNs can now be presented directly inside the web browser. At the same time, this new software architecture continues to support the traditional BioTapestry Editor Java desktop application that is used to build GRN models. We are also now
Program Abstract #326
Tropomyosin regulates myofiber elongation prior to sarcomere assembly
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The myofiber is the basic unit of skeletal muscle in metazoans, and during myogenesis nascent myofibers undergo directed elongation to locate and adhere to tendon cells. The molecular machinery that directs myofiber elongation in vertebrate and invertebrate systems is poorly understood. We performed a genetic screen in Drosophila and identified the RNA binding protein Hoir polloi (Hoip) as a novel regulator of myofiber elongation (Johnson et al., Development 2013). In addition to myofiber elongation defects, hoip mutant embryos fail to express a number of RNAs that encode sarcomeric proteins including muscle-specific Tropomyosin 2 (Tm2). Since Tropomyosins also regulate cytoskeletal dynamics during cell migration, we hypothesized that Tm2 RNA could be a key target of Hoip during myofiber elongation. We generated a series of Tm2 transgenes and found that Tm2 overexpression restored myofiber elongation in hoip embryos in a dose-dependent fashion. Mechanistically, Tm2 stabilizes lamellipodial F-actin at the myofiber leading edge to promote elongation. Since myofiber elongation precedes sarcomere assembly, we were surprised to find that Tm2 overexpression also restored sarcomeric RNA levels and protein expression in hoip embryos. These results raise the exciting possibility that Tropomyosin-mediated cytoskeletal dynamics regulates not only cell morphology but also muscle structural gene expression during myogenesis. Our in vitro studies show Hoip physically interacts with Tm2 mRNAs and promotes Tm2 protein expression via sequences in the coding region. Taken together, our studies demonstrate that Tm2, whose expression is tightly controlled by a post-transcriptional regulatory mechanism, is a powerful regulator of myogenesis and not solely sarcomere function. This work was supported by the American Heart Association.

Program Abstract #327
Reexamining the role of titin in muscle thick filament assembly and maintenance using zebrafish
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The sarcomeres of striated muscle are highly structured, semi-crystalline arrays of interdigitated protein filaments, requiring precise alignment and conformation in order to provide contractile function to muscle cells. Mechanisms of sarcomere function are generally well studied, but how these enormous complexes are patterned and assembled during early muscle development remains poorly understood, and we have been using zebrafish to explore these processes. Two widely-cited hypotheses for how pattern is so precisely determined include the “molecular ruler” model, in which the giant protein titin provides a precise scaffold for myosin thick filament assembly, and the “premyofibril” model, which proposes that thick filament proteins substitute for non-muscle myosins in stress-fiber-like templates or “premyofibrils”. Each model posits a different order of necessity for components of the sarcomere during assembly, but the hypotheses have been difficult to test in vivo. We have begun to examine the order of expression of these components in wild type and in zebrafish embryonic mutants with sarcomere assembly defects, as well as examine knockdowns of candidate assembly proteins and chaperones. The herzschlag mutant produces a truncated titin protein, lacking the entire A-band rod region that is proposed to act as a thick filament scaffold, yet muscle patterning is still initiated, with grossly normal A-band periodicity. Only after the onset of embryonic muscle contraction does this sarcomeric myosin patterning break down, consistent with the previously noted role of titin in maintaining the integrity of mature sarcomeres under tension. This conflicts with the “molecular ruler” hypothesis and supports a titin-independent model of thick filament organization during sarcomerogenesis. Our findings are also consistent with the symptoms of human titin myopathies, which typically exhibit a late onset.

Program Abstract #328
Characterization of a dsRNA-binding protein involved in gastric smooth muscle determination and proliferation
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The gastrointestinal musculature is initially composed of splanchnic mesoderm, which differentiates into multiple cell types, principally smooth muscle cells (SMCs). Using the chick embryo as a model, we sought to understand the molecular processes driving the differentiation of SMCs. This approach could offer insight into the mechanisms altered in human intestinal neoplasia. The process of SMC differentiation is commonly decomposed into two major steps. First (around E5), the gut mesenchyme undergoes determination. Later during development (around E7), the determined SMCs begin to express contractile proteins, thus entering a differentiated state. Using a microarray approach to identify candidate genes in visceral SMC differentiation, we isolated a gene coding for an uncharacterized dsRNA-binding protein (dsRBP). Here, we show that this gene is strongly expressed during the early stages of visceral mesenchyme development and is rapidly down-regulated in the determined smooth muscle. Through these results, we have identified this dsRBP-coding gene as a novel marker of undifferentiated stomach mesenchyme. Using targeted avian retroviral transgenesis, we show that down-regulation of this gene in the developing stomach mesenchyme results in a smaller determined smooth muscle domain, suggesting that it is necessary for correct SMC determination. Furthermore, we show that this gene stimulates in vitro cell proliferation and misexpression in the developing avian stomach mesenchyme leads to an enlarged determined smooth muscle domain. These results encouraged us to investigate the expression of this gene in intestinal neoplasia,
Program Abstract #329
The novel zebrafish mutant sea squirt is a suppressor of heart size in retinoic acid deficient embryos
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Proper heart size is necessary for the normal function of heart during embryonic and postnatal life. Retinoic acid (RA) signaling plays critical roles in different phases of normal heart development, with RA signaling being one of the few signaling pathways necessary to restrict cardiac specification. However knowledge about molecular networks that cross talk with RA in determining appropriate embryonic heart size is not yet well understood. We conducted a F2 modifier screen in zebrafish to identify mutants that suppress the enlarged hearts of RA signaling deficient neckless (nls) embryos. We found a novel nls suppressor mutant called sea squirt (sqi). Importantly in the nls: sqi double mutants, heart size and morphology are largely restored to normal. Alone sqi mutants have mildly dysmorphic hearts. While our preliminary data indicate that there is increased apoptosis in sqi mutants, we did not observe cell death in heart, suggesting that cell death might not be the primary mechanism behind heart size suppression. Next, to decipher the molecular nature of the lesion in sqi, we performed traditional positional cloning in combination with recently developed RNA-seq based mapping (RNA mapper). This approach allowed us to narrow the mutation to a region spanning 3mb on chromosome 1. To identify the gene affected in sqi, we are in the process of examining candidate genes within the region using PCR (qRT-PCR), in situ hybridization (ISH) and rescue studies with mRNA overexpression. Deciphering the underlying molecular and genetic networks by which the sqi mutation suppresses the enlarged heart of RA deficient embryos will shed light on the regulation of heart size in vertebrate embryos and may lead to novel understating of congenital heart defects in humans.

Program Abstract #330
The serotonin receptor Htr2a plays a critical role during atrioventricular canal patterning in zebrafish
Andrew Houk, Grant Miura, Richard Shehane, Paulina Delgado Cuenca, Deborah Yelon
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Organogenesis requires signals to become active in the proper places and at the appropriate times in order to trigger the correct morphogenetic events. During vertebrate heart development, atrioventricular valve formation is triggered by signals that are precisely localized to the junction between the cardiac chambers (also known as the atrioventricular canal or AVC). These signals instruct the local endocardium to form endocardial cushions (ECs) that later remodel into valve leaflets. Studies in zebrafish indicate that canonical Wnt pathway activity at the AVC promotes the expression of genes encoding Bmp signals that induce EC formation. Proper heart development necessitates the restriction of EC induction to the AVC; however, we do not know the roster of molecular players responsible for this confinement.

In a small molecule screen aimed at identifying additional regulators of AVC patterning, we found that ketanserin, a serotonin receptor antagonist, causes ectopic expansion of AVC characteristics, such as bmp4 and notch1b expression, throughout the ventricular chamber. Based on the specificity of ketanserin, we next examined whether loss of function of the serotonin receptor gene htr2a would influence AVC patterning. Strikingly, morpholino-based knockdown of htr2a causes the ectopic expression of bmp4, notch1b, as well as the EC marker DmGrasp, throughout the ventricle. Together, our results suggest that serotonin signaling through Htr2a plays a critical role in restricting EC induction. While serotonin is known to regulate several other aspects of cardiogenesis, such as trabeculation and the remodeling of valve leaflets, it has never been implicated in this aspect of cardiac patterning. Future work will test whether serotonin operates upstream or downstream of the Wnt pathway during AVC patterning and determine how it interfaces with the known AVC patterning network. Work in the Yelon lab is supported by grants from the NIH, AHA and March of Dimes.

Program Abstract #331
Shear stress as a factor affecting embryonic cardiac development in zebrafish
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Shear stress is an important factor in cardiac development; it arises as blood cells pass through the heart tube and contact endocardial tissues. Altered shear stress has been correlated with morphological cardiac deficiencies, although these have never been systematically defined. The zebrafish embryo provides optical access for live imaging of embryonic hearts. To more quantitatively describe the impact of blood flow upon the developing heart, we have developed a program to analyze data obtained from high-speed videos in the heart. Spatiotemporal plots were used to calculate blood velocity and luminal diameter at the atrial inlet and the avj. Volume analysis was used to describe hallmark functional parameters, including flow rate waveforms, fraction of retrograde flow, stroke volume, and cardiac output. We have applied these tools to analyze the efficiency of heart function in embryos with defects in sarcomere-related genes, or other genes essential for cardiac development. Additionally, we are exploring ways of systematically disrupting particular aspects of blood flow in normal embryos. Finally, we are constructing a transgenic line to for ChIP studies to identify genes that respond to the shear stress response factor Klf2.
Program Abstract #332
Hey2 acts to restrict second heart field progenitor contribution to the zebrafish heart
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The vertebrate heart forms from cells derived from two progenitor populations, termed the First Heart Field (FHF) and Second Heart Field (SHF). SHF cells make up a significant part of the vertebrate heart, with defects in SHF development implicated in congenital heart disease. The zebrafish, with externally laid embryos and a rapidly developing heart that is readily imaged, has recently become a new model to study SHF development. We identified a novel transgenic line, nppa:EGFP, which marks SHF progenitors immediately adjacent to the developing heart tube. As nppa:EGFP does not mirror endogenous nppa expression, we hypothesized that the short transgenic promoter was lacking inhibitory regulatory elements. In analyzing potential regulators of the nppa:EGFP transgene, we found that hey2 acts as a novel regulator of FHF and SHF development. hey2 was expressed in SHF regions as early as 15hpf and became localized to the SHF posterior of the ventricle by 24hpf. hey2 inhibition resulted in an increase in SHF progenitors and SHF cell addition, with a concomitant decrease in FHF cells in the heart tube. By transplantation, we found that inhibition of hey2 biases individual cardiac progenitors to a SHF fate. These findings indicate that SHF addition to the zebrafish heart is in part regulated by hey2. Our results suggest that hey2 acts as an early regulator of cardiac development, and further may have the novel property of modulating the balance of FHF and SHF progenitors formed in cardiac mesoderm.

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Program Abstract #333
Dissecting the roles of the proepicardium and Fgf10 in cardiac development
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The heart forms from several multipotent progenitor cell populations and abnormalities within these progenitors, or in their interactions with surrounding cells, lead to congenital heart defects. The proepicardial organ (PEO) is a mesothelial structure that forms at the caudal end of the developing heart and contributes cells that migrate across the pericardial space, attach to the myocardium and migrate over the myocardial surface to form the epicardium. This thin epithelial layer surrounds the mouse heart by E11.5. Some epicardial cells undergo an epithelio-mesenchymal transition and enter the heart, predominantly generating the smooth muscle of the coronary vasculature and interstitial fibroblasts. It is thought that the epicardium also provides critical trophic signals supporting expansion of the ventricular compact zone during late-gestation. Fibroblast growth factors (FGFs) provide critical communication within and between developing heart progenitor cells and surrounding tissues. We reported a spectrum of heart defects found in Fgf3/Fgf10 double mutants. Defects included reduced epicardial cell ensheathment, detachment of epicardial cells from the myocardium, and thinned myocardium, with double mutants dying of heart failure by E11.0. Loss of Fgf10 from the PEO may contribute to these phenotypes. To determine the role of Fgf10 in the PEO, conditional mutagenesis of Fgf10 in an Fgf3 mutant background is underway. Loss of Fgf10 in the PEO causes embryonic lethality between E11.5-E12.5. In order to definitively determine the earliest role of PEO-derived cells in murine cardiac development, we are also using diphtheria toxin-mediated genetic ablation of Tbx18+ proepicardial cells and have found that loss of this lineage causes apparent heart failure and death by E12.5. Together, these data suggest the hypothesis that the PEO and its derivatives play a critical and much earlier role in heart development than previously suspected.

Program Abstract #334
The Apelin Receptor (Aplnr) enhances Nodal signaling for proper cardiac progenitor development.
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The Apelin receptor (Aplnr) has been shown to be essential for proper cardiac progenitor migration during gastrulation. Loss of the Aplnr results in a delay in the ingression of lateral marginal cells and the complete absence of the heart and early cardiac gene expression. How the Aplnr is required at a molecular level for cardiac progenitor migration remains completely unknown. We present evidence to suggest that the Aplnr enhances Nodal signaling to initiate the migration program at the correct time in development. Apelin receptor loss of function embryos exhibit a reduction in a wide range of Nodal target genes while activation of the receptor is capable of boosting their expression. Furthermore, Aplnr deficient embryos are exquisitely sensitive to Nodal receptor inhibitors. In order to directly interrogate the functional consequence of reduced Nodal signaling in the Aplnr cardiac phenotype, we raised the level of Nodal activity in aplnr mutants and found that this was able to provide a near complete rescue. We next sought to determine a mechanism by which lower Nodal signaling levels could translate into a delay in migration. Remarkably, we observe a delay in the expression of mesp family members in Aplnr loss of function embryos at the beginning of gastrulation. The mesp family has previously been implicated in mesodermal migration and we show that they are downstream targets of Nodal. We propose that in the absence of the Aplnr the appropriate Nodal threshold required to initiate the downstream cardiac progenitor “specification/migration” program requires a longer length of time to be attained. This translates into a delay in migration and consequently cardiac progenitors are not capable of reaching their final destination. In this study we demonstrate how the fine tuning of a key signaling pathway is critical for the proper development of the early embryo.
Program Abstract #335
Controlling the pace of cardiac differentiation: Cell adhesion molecule 4 restricts the production of outflow tract progenitor cells
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Heart assembly requires input from two sources of progenitor cells, the first and second heart fields, that differentiate at distinct times and create different cardiac components. Notably, the cardiac outflow tract (OFT) is built through recruitment of second heart field (SHF)-derived cardiomyocytes (CMs) to the arterial pole of the heart. However, the mechanisms responsible for production of an appropriate number of OFT cells from the SHF remain unclear. In zebrafish, we find that inhibition of Fgf signaling depletes OFT progenitors and impairs OFT formation, whereas increased Fgfr activity expands the OFT progenitor pool. Additionally, we show that cell adhesion molecule 4 (cadm4) is negatively regulated by Fgf signaling and is expressed in a region near the developing arterial pole. Loss of cadm4 function causes a dramatic enlargement of OFT: a surplus of SHF-derived progenitors aggregates around the arterial pole and ultimately results in the addition of nearly twice the normal number of OFT CMs. As is the case for the wild-type OFT, formation of this expanded OFT requires Fgf signaling. Conversely, increased expression of cadm4 diminishes the OFT by decreasing the number of OFT progenitors. Furthermore, overexpression of cadm4 overrides the ability of elevated Fgf signaling to enlarge the OFT progenitor pool. Through cell-tracking analyses and proliferation assays, we find a pool of OFT progenitors that reside in a relatively distal portion of the SHF and reveal that cadm4 activity limits the proliferation of these progenitors prior to their deployment to the OFT. Altogether, our data support a model in which levels of cadm4 activity, regulated by Fgf signaling, act to restrict the number of OFT progenitor cells that emerge from the SHF and the duration of their accumulation at the arterial pole. Our data are the first to suggest a role for cell adhesion in restraining SHF deployment to the OFT, perturbation of which could cause congenital OFT defects.

Program Abstract #336
Wnt signaling has distinct and dynamic roles in semilunar and atrioventricular canal valve development
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Heart valve development proceeds through a series of highly coordinated steps by which endocardial cushions (EC) develop into mature, elongated, and stratified valves. However, the cell signals that direct these sequential events in valve development and the mechanisms by which they drive different processes remain largely unknown. The Wnt signaling pathway is frequently activated during embryogenesis to promote differentiation, proliferation, and morphogenesis. Expression and genetic studies suggest that Wnt and its effector, β-catenin, have roles during an epithelial-to-mesenchymal transformation (EMT) at the ECs through postnatal steps of valvulogenesis. However, due to the broad requirements for developmental Wnt signals, parsing individual roles of this critical pathway at different stages of valve formation is challenging using conventional genetic approaches. We have developed a transgenic mouse system that provides chemically-induced inhibition of canonical Wnt signaling by expression of Dkk1, a specific Wnt inhibitor, in a tissue-restricted manner. Using these mice and chemical genetic approaches, we show that Wnt/β-catenin signaling is required for EMT in the proximal outflow tract (pOFT) but not atrioventricular canal (AVC) cushions. Further, Wnt’s role in pOFT EMT is non-cell autonomous, likely reflecting earlier Wnt activity in cardiac progenitor cells or neighboring myocardium. Following EMT, Wnt signaling is activated in cushion mesenchymal cells where it suppresses Wnt to diminish the OFT by decreasing the number of OFT progenitors. Axin2, a negative regulator of Wnt, becomes expressed throughout the valves during later stages of their development, where it suppresses Wnt to prevent valve overgrowth. We suggest that while canonical Wnt signaling is active and dynamically regulated at various stages of valve development, its roles may be less discrete than currently understood.

Program Abstract #337
SOX9 regulates the Mecom/Evi1 locus during valve mesenchyme condensation in heart valve development.
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It has been suggested that cardiac valve diseases arise from underlying developmental malformations that occur during embryogenesis. These developmental valve defects are often the cause of disease later in life. Myxomatous mitral valve disease and calcific aortic valve disease are the most common adult degenerative heart valve diseases and both are associated with a gain or loss of a transcription factor called SOX9, respectively. SOX9 is known to play a crucial role during development of the heart valves and its loss leads to major valve defects and embryonic lethality; however, little is known about the transcriptional targets of SOX9 in heart valves. To identify these targets and elucidate SOX9’s role in the developing valves, we have used ChIP-Seq on embryonic day (E) 12.5 atrioventricular canal (AVC) and limbs (for comparison). Gene Ontology (GO) analysis of common E12.5 AVC and limb target genes revealed regulation of cell cycle as a critical process under the control of SOX9. Of particular interest, we identified the Mecom/Evi1 locus as a common direct target of SOX9. Luciferase assays confirmed that SOX9 positively regulates Evi1 and
immunofluorescence showed that EVI1 is expressed in the condensing valve mesenchyme at E12.5. In a valve-specific knockout mouse of SOX9, Evi1 expression was significantly reduced when compared to wild type. Co-factor analysis of SOX9 peak regions suggested that EVI1 may be a potential cofactor for SOX9. Further analyses will focus on the interaction between SOX9 and EVI1 and their function during mouse heart valve development.

This work was funded by the Heart and Stroke Foundation and Genome BC.

**Program Abstract #338**
The Na-dependent phosphate cotransporter PiT-1 is required for early vascular development.
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Inorganic phosphate (Pi) is required for many cellular processes, and imbalance of phosphate handling is associated with several medical disorders including vascular calcification. Interestingly, embryonic extracellular Pi levels are higher than maternal levels, suggesting that Pi is actively transported into the embryo. However, prior to formation of the placenta it remains unknown if and how Pi is taken up into the developing embryonic tissue. We found that the Na-dependent phosphate transporter, PiT-1/Slc20a1 is localized to the apical membrane of the yolk sac (YS) visceral endoderm (VE). Here we present data supporting that PiT-1 KO results in a YS vasculature angiogenesis defect that is not due to upstream cardiac malfunction, as KO of PiT-1 in the developing heart does not recapitulate the PiT-1 global KO phenotype. Furthermore, we find that a reduction of PiT-1 in the YS mesodermal layer (ME) of the YS at E10.5 also results in viable embryos, supporting that PiT-1 is likely required either in YS VE or in YS ME prior to E10.5. Additionally, gene expression of endodysplasia and autophagy genes is significantly upregulated in PiT-1 KO VE, and maternal immunoglobulin accumulation in the apical vacuole is lost. Together, these data support that normal endocytic trafficking and visceral endoderm function is disrupted in the absence of PiT-1. The angiogenesis that normally occurs in the ME layer during yolk sac vasculogenesis is highly dependent upon the VE. Therefore, we propose the novel idea that PiT-1 is central to the regulation of endodysplasia in the YS VE and required for support of YS angiogenesis. Funding sources: Dr. Giachelli’s lab is supported by NIH grants HL62329, HL081785, and HL114611. Dr. Wallingford is supported by NHLBI T32HL007828.

**Program Abstract #339**
Development of a Zebrafish Port-Wine Stain Model
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Port Wine Stain (PWS) and the associated Sturge-Weber Syndrome (SWS) are congenital defects resulting in hypertrophic and dilated vasculature. PWS and SWS have, as of yet, no cure or viable drug-testing model; our project aims to recreate the PWS and SWS phenotype within zebrafish to be used as an animal model. A single nonsynonymous somatic mutation in the gene encoding a G-protein alpha subunit, GNAQ, is the molecular basis for these disorders. We identified and cloned the zebrafish homolog of GNAQ. The amino acids mutated in PWS (R183Q) and a separate activating mutation in GNAQ that often results in uveal melanoma (Q209L), are conserved between human and zebrafish. We injected in vitro transcribed mRNA of the different GNAQ variants at the one cell stage. Expression of the wild type GNAQ did not affect development however expression of either mutant GNAQ resulted in the embryos lysing before the end of epiboly indicating that both mutants have similar activities. We are currently examining the effects of expression of the mutant forms of GNAQ in cell types associated with angiogenesis to identify which cells require activation of GNAQ to promote the PWS phenotype. The development of a PWS model may facilitate the development of PWS therapies. This research was supported in part by Choose Development!, an NSF (grant IOS-1239422) sponsored program through the SDB.

**Program Abstract #340**
Individual disparate cell decisions define the balance between expansion and endocrine differentiation in the pancreas
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Organogenesis relies on the spatiotemporal balancing of differentiation and proliferation that expands the progenitor pool. In the mouse pancreas, lineage tracing at the population level has shown that the expanding pancreas progenitors can initially give rise to all endocrine, ductal, and acinar cells but become bipotent by embryonic day 13.5, giving rise to endocrine cells and ductal cells. However, the dynamic behaviour of individual progenitors balancing self-renewal and lineage-specific differentiation is enigmatic. Using 3-dimensional live imaging and in vivo clonal analysis, we reveal the contribution of individual cells to this global behaviour and demonstrate 3 modes of progenitor divisions: symmetric renewing, symmetric endocrinogenic, and asymmetric generating a progenitor and an endocrine progenitor. The results of our quantitative analysis are most consistent with stochastic priming of progenitors to endocrine fate at multiple stages of the cell cycle, and provide insights to define control parameters to optimize the generation of β-cells in vitro.

**Program Abstract #341**
Pdx1-driven epithelial morphogenesis contributes to pancreatic cell fate specification
Role of Yap/Taz in pancreas morphogenesis and cell specification

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The mechanisms that regulate organ size during development have remained elusive, despite decades of research addressing this topic. The recently identified, evolutionary conserved, Hippo signaling pathway is shown to play an important role in limiting the organ size by modulating the activity of its transcriptional factors Yap/Taz that controls stem/progenitor cell proliferation and apoptosis. Defects in core components of the Hippo pathway leads to robust organ overgrowth, tumorigenesis and cancer. However, the role of Hippo pathway in early pancreas development, progenitor cell expansion and differentiation of progenitors into β-cells is not known. Our preliminary data shows that, in mice, the loss of Hippo signaling caused by constitutively active Yap, results in an unexpected phenotype which is severe pancreatic hypoplasia and the death of transgenic pups just after birth. This is an opposite phenotype to what is seen in other organs such as the liver and heart tissues. Furthermore, conditional delayed overexpression of Yap postnatally leads to hyperglycemia. Here, we aim to address and validate the importance of Hippo pathway transcriptional factors Yap1 and Taz in developing murine pancreas using genetic approaches including germ-line and conditional deletion and conditional over-expression strategies. This work will provide important insight into the molecular mechanism by which Yap/Taz controls both pancreatic exocrine and endocrine cell specification. Elucidating the developmental mechanisms by which Yap/Taz controls pancreas development will also be beneficial in identifying novel targets that cause human pancreatic diseases. The unique insight into pancreas progenitor cell maintenance and β-cell differentiation gained from this research will contribute to generate mature and functional insulin producing β-cells in vitro for stem cell therapies and innovative therapeutic approaches within regenerative medicine.

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Loss of ΔNp63 impairs thymic epithelial progenitor survival and differentiation

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The thymus provides a unique stromal microenvironment that is essential for T-cell development. The thymic stroma consists primarily of thymic epithelial cells (TECs) that originate from endodermal progenitors in the third pharyngeal pouch (3rd pp). A common epithelial progenitor in the fetal thymus differentiates into robust networks of cortical (cTEC) and medullary (mTEC) cells which provide distinct selection and maturation signals that support the generation of immunocompetent, self-tolerant T-cells. The transcription factor p63 is highly expressed in cTECs and mTECs and global deletion of p63 results in a severely hypoplastic thymic phenotype. Alternative promoter usage generates two N-terminal isoforms, TAp63 containing a transactivation (TA) domain and ΔNp63, an isoform lacking the TA domain. The roles of TAp63 and ΔNp63 in thymus organogenesis have not been determined. We find that deletion of TAp63 in fetal TECs does not affect thymus size or TEC development in young adult mice. In adult skin stem cells, TAp63 regulates senescence and genomic stability. A similar role may be found in the thymus because TAp63 is upregulated in TECs in response to irradiation. In contrast to the undetectable levels of TAp63 in the fetal or steady state adult thymus, we find that ΔNp63 is highly expressed as early as E9.5 and persists in cTECs and mTECs in the postnatal thymus. We used Foxn1 Cre;ΔNp63 fl/fl to delete ΔNp63 in TEC progenitors of the 3rd pp. Loss of ΔNp63 results in a severely hypoplastic thymic phenotype by E14.5. Initial patterning of 3rd pp endoderm into thymus fated and parathyroid fated domains is not disrupted. However, deletion of ΔNp63 causes increased apoptosis in TECs as early as E12.5. In addition, these cells fail to differentiate
The thymus originates from a shared primordium in the 3rd pharyngeal pouch (pp). The dorsal domain gives rise to the parathyroid (PT) and the ventral domain gives rise to the thymus. At E11.5, 3rd pp cells are morphologically indistinguishable, yet are specified to a thymus or PT fate by expression of the transcription factors Foxn1 or Gcm2, respectively. Previous reports suggest that the transcription factor TBX1 promotes thymus development. However, pharyngeal segmentation is impaired in Tbx1 null mice, making the role of TBX1 in thymus organogenesis unclear. To clarify this, we generated a mouse strain in which a stop-floxed Tbx1 allele was knocked into the Rosa26 locus. Foxn1Cre was used to activate Tbx1 expression in the thymus-fated domain of the 3rd pp. Ectopic expression of Tbx1 at E11.5 results in a marked reduction of FOXN1 expressing cells, but does not affect GCM2, leaving a number of 3rd pp cells that express neither fate identifier. However, these cells express wildtype levels of IL-7 and FOXP1, which identify thymus-fate independently of FOXN1. Therefore, ectopic expression of Tbx1 does not repress or reverse thymus fate. FOXN1 is required for thymic epithelial cell proliferation and differentiation. Its loss in the Foxn1Cre; R26Tbx1/+ 3rd pp results in severely hypoplastic thymi throughout ontogeny, likely due to decreased proliferation in the thymic primordia. Also, ectopic Tbx1 expression impairs TEC differentiation, resulting in an accumulation of PLET1+ TEC progenitors. These data demonstrate that extinction of 3rd pp endoderm is a prerequisite for thymus organogenesis.

Current studies focus on molecular pathways that regulate TBX1 in the 3rd pp. Tbx1 expression is down-regulated by miR-17-92 in cardiac progenitor cells. Our preliminary data suggests a similar pathway operates in the 3rd pp as miR-17-92 null mice have expanded TBX1 and reduced FOXN1 expression at E11.5 in the 3rd pp. Funded by NIH grant R01AI107096.

Hypodysplasia and glomerular cysts in a new mouse model of CAKUT generated by ENU mutagenesis

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Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) are responsible for 30–40% of end-stage renal disease in children. This disease group is highly heterogenous such that many of the underlying genetic determinants are still unknown. To identify new determinants of CAKUT, we have performed a recessive ENU mutagenesis screen, which uncovered a novel mouse line that harbors congenital renal hypoplasia and glomerular cysts.

Initial phenotypic characterization has revealed that severely hypodysplastic kidneys are highly disorganized, lacking distinct renal pelvis and medullary regions, with an apparent enlargement of the cortical zone. Conversely, moderately affected kidneys preserve a normal architecture, concomitant with appropriate expression of nephron and early glomerular differentiation markers. Strikingly however, these kidneys develop large glomerular cysts, which are associated with aberrant morphology of Bowman’s capsule and glomerular tufts.

To identify the gene responsible for this renal phenotype, we used a SNP array analysis, which linked the causal mutation to an 18 Mb region in the proximal region of chromosome 7. Whole exome sequencing further revealed potential mutations in two genes located in the 18 Mb interval, namely Lilra5 and Grlf1. Future studies are aimed at elucidating which of these is causative, and understanding its function during kidney development.

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Osrl interacts synergistically with Wt1 to regulate metanephric kidney formation

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The Odd-skipped related 1 (Osrl) and Wilm’s tumor suppressor 1 (Wt1) genes are both expressed in the early developing nephrogenic mesenchyme and each is required for early kidney organogenesis. Here we report that Osrl and Wt1 interact physically and genetically to regulate kidney formation. We show that co-expressed Osrl and Wt1 form protein interaction complexes. We generated Osrl+/Wt1+/ double heterozygous mice and found that most of the Osrl+/Wt1+/ mice had kidney malformations, including bilateral/unilateral renal agenesis and renal hypoplasia. The developmental defects in the Osrl+/Wt1+ mice were detected as early as E10.5, during specification of the metanephric mesenchyme. By E11.5, as the ureteric buds invaded the metanephric mesenchyme and initiated branching morphogenesis, kidney morphogenesis was histomorphologically impaired in the Osrl+/Wt1+ embryos compared with the Osrl−/− or Wt1−/− embryos. Moreover, expression of Gdnf, the major signal for induction of ureteric bud branching, was significantly reduced in Osrl+/Wt1−/− embryos in comparison with the Osrl−/− or Wt1−/− littermates. These results
indicate that Osr1 and Wt1 interact to regulate metanephric mesenchyme specification and metanephric kidney induction. This work was supported in part by NIH/NIDCR grant DE013681.

Program Abstract #347
Modeling Congenital Anomalies of the Kidney and Urinary Tract: a Role for Wnt5a/Ror2 Signaling in Duplex Kidney/Ureter Formation
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Congenital anomalies of the kidney and urinary tract (CAKUT) affect about 1 in 500 births and are a major cause of morbidity in infants. Duplex collecting systems rank among the most common abnormalities of CAKUT, but the molecular basis for this defect is poorly understood. In mice, conditional deletion of Wnt5a in mesoderm results in bilateral duplex kidney and ureter formation. The ureteric buds (UB) in mutants emerge as doublets from the intermediate mesoderm (IM)-derived nephric duct (ND) without expansion of the normal Gdnf expression domain in the surrounding mesenchyme. Wnt5a is normally expressed in a graded manner at the posterior end of the IM, but its expression is down regulated prior to UB outgrowth at E10.5. Furthermore, ablation of Wnt5a in mesoderm by activation of an inducible Cre during early IM development (E7.5) results in duplex UBs; whereas, later ablation (E9.5) yields normal UB outgrowth, demonstrating that Wnt5a functions in IM development well before formation of the metanephros. In mutants, the IM itself is shortened along the anteroposterior axis and widened along the mediolateral axis. Moreover, the posterior ND is duplicated and surrounding Pax2-positive mesenchymal cells persist in the posterior nephrogenic cord in mutants, suggesting that disruption of normal ND patterning prompts the formation of duplex ureters and kidneys. Although Wnt5a can activate β-catenin signaling, TCF-dependent canonical Wnt activity is not affected in Wnt5a mutants. On the other hand, Ror2 homozygous mutants, which infrequently yield duplex collecting systems, show a dramatic increase in incidence with the additional deletion of a single copy of Wnt5a, implicating this receptor in non-canonical Wnt5a signaling during IM development. This work provides the first evidence of a role for Wnt5a/Ror2 signaling in IM extension and kidney morphogenesis and offers new insight into the etiology of CAKUT and possible involvement of Wnt5a/Ror2 mutations.

Program Abstract #348
The Role of Flanking and Paralogous Abdominal-B Hox Genes During Kidney Development
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Hox genes are known to be key patterning regulators during embryogenesis. In mammals there are 39 Hox genes located on four paralogous clusters. They show extensive functional overlap and share cis-regulatory elements. The abdominal-B (abd-b) Hox genes are expressed in the most posterior region of the developing embryo and are necessary for proper kidney development. To better understand the functional overlap of flanking and paralogous Hox genes, we have devised a recombinerineering method that allows the simultaneous frameshift mutation of multiple adjacent genes. This preserves regional enhancers while removing coding function. Our lab has generated mouse lines with frameshift mutations in Hoxa9, Hoxa10, Hoxa11 (Hoxa9,10,11-/-) ; Hoxd9, Hoxd10, Hoxd11 (Hoxd9,10,11-/-) ; and Hoxc9, Hoxc10, Hoxc11 (Hoxc9,10,11-/-). Combinations of these mutant mouse lines are being used to define the overlapping functions of flanking and paralogous Hox genes during kidney development. Hoxa9,10,11-/-; Hoxd9,10,11-/- mice show severely disrupted kidney development. At E11.5 the ureteric bud (UB) is malformed and the metanephric mesenchyme is reduced in size. At E13.5 UB branching is severely reduced and nephrogenesis fails to progress beyond the pre-tubular aggregate. The molecular basis of this phenotype is being further investigated. We also observed that Hoxc9,10,11-/- in combination with either Hoxa9,10,11+/- or Hoxd9,10,11+/- gives a severe cystic kidney phenotype. We are investigating possible underlying mechanisms, including changes in gene expression and cell division polarity. In summary, the results reveal novel Hox mediated kidney phenotypes and serve to better define the overlapping functional relationships of these remarkable genes.

Program Abstract #349
Mosaic Analysis with Double Markers reveals Ret activity is essential to coordinating the behavior of “tip” cells as progenitors of the renal collecting system
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The renal collecting system is formed by branching morphogenesis of a tubular epithelium, the ureteric bud. Despite an increasingly comprehensive understanding of the genetic factors regulating this process, little is known about the cell-level mechanisms that sculpt the growing kidney. We show that the Ret-expressing tip cells of the branching ureteric bud compose a special progenitor population. The entirety of the collecting system is derived from these cells, including mature cell types essential to adult kidney functionality. While these tip cells are self-renewing, we show via genetically induced ablation that they function as a limiting progenitor pool, and kidneys that lose a portion of their Ret-expressing tip cells cannot recover to achieve a normal size. The mosaic deletion of Ret in this domain similarly stunts kidney growth, suggesting Ret is essential to the identity of these cells as progenitors. To further explore this observation, we have mapped the fates of tip cells that lose Ret expression using Mosaic Analysis with Double Markers (MADM). This elegant genetic technique generates fluorescently labeled mutant and wild-type sister cells in a
small number of mitoses. Strikingly, tip cells that lose Ret are near completely excluded from the tip domain, while wild-type cells remain in this more proliferative compartment. We hypothesize that the cell rearrangements observed in these mosaic analyses simulate a normal process essential to renal branching morphogenesis, in which the tight regulation of Ret activity leads some cells to be left behind in the “trunk” domain while others remain at the tips.

Program Abstract #350

Rudhira controls actin organization to regulate ultrafiltration in insect nephrocytes and kidney podocytes.
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Insect nephrocytes and vertebrate kidney podocytes are required for ultrafiltration of body fluids to maintain homeostasis. They have a complex architecture whose integrity is maintained by the interaction of cytoskeletal elements with the slit diaphragm. Disruption of this structure in mammals hinders podocyte function and is the leading cause of several renal disorders. Insect nephrocytes provide a simple model for studying podocyte biology. Nephrocyte slit diaphragm molecules are well conserved in terms of localization and function. However little is known about the cytoskeleton of the nephrocyte cell body and foot processes, largely made up of microtubules and actin respectively. Here we describe a unique pattern of actin organization in the nephrocyte cytoplasm and show that it is regulated by slit diaphragm molecules and Rho GTPases. The conserved nephrocyte-specific cytoskeletal protein Rudhira regulates cardiac function and homeostasis. We show that Rudhira maintains actin organization and thereby regulates ultrafiltration in nephrocytes. We also show mouse Rudhira expression in kidney podocytes and are testing its role in podocyte structure and function. Human Rudhira/BCAS3 is implicated in chronic kidney disease. Hence our analysis of Rudhira function could help devise novel approaches to treat kidney diseases.

Program Abstract #351

Novel insight into the development of left-right asymmetry in the Xenopus liver
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Many internal organs are left-right asymmetric in their anatomical configuration within the vertebrate body cavity, including the liver, whose left and right lobes exhibit differences in both size and anatomical morphology across species. As many as 1 in 10,000 humans are born with defects in left-right asymmetry that often involve severe anomalies in liver laterality or biliary tract function, yet the mechanisms underlying the development of normal and abnormal left-right asymmetries in the liver are virtually unknown. We sought to determine how lobular and biliary left-right asymmetries are established during hepatogenesis in the model frog species Xenopus laevis. Fate-mapping studies show that the left and right lobes of the liver arise from the left and right halves of the embryo respectively, suggesting that there are likely unilateral differences in the development of the left and right contributions to the liver. Consistent with this model, we uncovered asymmetric expression of the left-right determining gene, Pitx2, in the left side of the early hepatic diverticulum, and later in the left liver lobe and left side of the extrahepatic biliary tree. At the cellular level, immunohistochemical analyses indicate dissimilarities in the epithelial morphology of the left and right sides of the early liver diverticulum, including differences in tissue thickness, cell shape, and apicobasal polarity, as well as left-right differences in the localization of several proteins known to be involved in epithelial morphogenesis and extracellular tissue remodeling. These findings reveal new insight into the repertoire of morphogenetic mechanisms that generate laterality in asymmetric organs, and establish the Xenopus liver as a novel model system to study left-right asymmetric organ development.

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Program Abstract #352

YY1 is required in the definitive endoderm for hepatoblast migration and VEGF maintenance
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Yin Yang1 (YY1) is a ubiquitously expressed factor that plays multiple essential roles in early mouse development. In a previous attempt to assess the role of YY1 in the definitive endoderm (DE), we found that YY1 plays an earlier essential role the visceral endoderm (VE) of the yolk sac where it is required to maintain Vascular Endothelial Growth Factor (VEGF). Ex vivo rescue was used to demonstrate that VEGF produced by the VE is required for the mesoderm derived paracrine signal(s) that in turn support maintenance of the VE. Herein, an inducible-conditional strategy is used to bypass the VE requirement and demonstrate that in the DE, YY1 is essential for the liver bud to invade the surrounding mesenchyme. Each phase of liver bud invasion is normally preceded by expression of VEGF from the DE-derived hepatoblasts. Loss of YY1 in the hepatoblasts leads to a reduction of VEGF that coincides with the invasion failure as well as the loss of the critical hepatoblast expressed transcription factor HNF4-alpha. Addition of exogenous VEGF rescues HNF4-alpha expression in YY1 deficient liver buds and supports the hypothesis that a VEGF-dependent mesenchyme derived paracrine signal is necessary for maintenance of hepatoblast specification. Because of the myriad of similarities between the VE and early liver bud, documented in this study and by many others, we propose that the yolk sac is an abundant and easily accessible tissue source that may be used to probe the transcriptional networks and signals supporting early liver development.
Program Abstract #353
A Novel Non-Neuronal role for Acetylcholinesterase in Gut Morphogenesis
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Acetylcholinesterase (AChE) is a highly conserved protein, well-studied for its importance in terminating nervous signaling through degradation of the neurotransmitter, acetylcholine. However, AChE has also been shown to function independently of cholinergic synapses in many taxa, including non-innervated organisms. Such non-neuronal roles are poorly understood, but may modulate cell adhesion and migration. As these processes are essential for many morphogenetic events, we hypothesized that AChE may be essential for normal development. Indeed, in situ AChE enzymatic assays and immunohistochemical staining reveal the presence of AChE within the non-innervated endoderm cells of the embryonic gut, suggestive of a non-neuronal function during intestine morphogenesis. Consistent with this model, both chemical inhibition of AChE by organophosphate compounds and endoderm-targeted morpholino knockdown of AChE resulted in gut elongation and rotation defects. Morpholino-induced gut phenotypes were rescued by co-injection of AChE mRNA, confirming that AChE is specifically required in the endoderm. Additionally, endoderm cells lacking AChE exhibit disrupted polarity, decreased integrin levels, and fail to rearrange to form an organized digestive epithelium, suggesting that AChE is required to maintain cell-ECM adhesion during polarized migration. These data reveal a previously unrecognized non-neuronal role of AChE in coordinating cell rearrangement during large scale morphogenetic events, and increase our understanding of the potential genetic and environmental etiology of congenital human gut defects. Funding for this research was provided by the NIDDK and DoED.

Program Abstract #354
Temporal mapping of enteric development and villus morphogenesis.
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In the intestine, correct patterning of nerves and vasculature is critical for normal digestive function. A hierarchical branching pattern of blood vessels allows for efficient nutrient absorption, while the complex enteric nervous system performs diverse functions, which include monitoring local environments and maintaining gut homeostasis. Further, defects in the enteric nervous system can be debilitating or fatal, as in Hirschsprung’s disease, where incomplete development of the enteric nervous system often requires surgical removal of a portion of the intestine. Despite the well-recognized significance of these systems, the precise mechanisms by which they develop remains unclear. In mice, the gut tube forms from endoderm by embryonic day 9.5. Using a novel whole-mount immunohistochemical protocol, we visualize the pattern of murine neurovascular development in the intestine between embryonic day 10 and birth. Processes of particular interest include the development and remodeling of the enteric vascular plexus, the migration and organization of neural crest-derived cells to form the enteric nervous system, and the morphogenesis and innervation of villi at later stages. These observations provide useful descriptive data for future investigations into the functional mechanisms responsible for the observed processes.

Program Abstract #355
Hoxb5b Controls the Midline Convergence of the Foregut Endoderm
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The zebrafish gut tube gives rise to internal organs and forms from a bilateral monolayer of endoderm cells. Coordinated movement of endoderm cells towards the midline is crucial for generating internal organs in the correct locations. During gastrulation stages the bilateral endodermal cell movements require cues from endoderm, mesoderm and also from the extra-embryonic yolk syncytial layer. Cell tracing analysis suggests that during subsequent segmentation stages, the mediolateral and anteroposterior location of the endoderm cells determine their cell fate choices. However, the signals guiding endoderm cell migration towards the midline are not completely understood. Here we show that the Hoxb5b transcription factor is required for the midline convergence of foregut endoderm. Using a morpholino knockdown approach we have established that down-regulation of hoxb5b throughout the embryo causes a bifurcated gut tube phenotype. Interestingly, we have found that pancreatic cells are specified but fail to differentiate properly in the bifurcated endoderm. To better characterize endoderm cell migration we have used time-lapse microscopy of embryos carrying the endoderm-specific reporter (Tg:sox17GFP). We have found that in hoxb5b-depleted embryos midline convergence of the endoderm halts at 14 hpf, a stage when hoxb5b is expressed in both the endoderm and surrounding mesoderm. To determine in which germ layer Hoxb5b is required for endoderm migration, we are now performing a series of cell transplantation experiments to allow germ layer specific down-regulation of Hoxb5 function. Identification of the germ layer in which Hoxb5b functions to influence endoderm cell movements will be crucial to establishing a full understanding of the migration mechanisms.
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Program Abstract #356
Meis3 is required for the proper specification and efficient migration of enteric neural crest cells to the primitive gut
Rosa Uribe
Parasympathetic gangliogenesis is initiated by Sprouty1/2-regulated Wnt signals from epithelial progenitors. During development, vagal neural crest cells fated to contribute to the enteric nervous system emigrate ventrally from the dorsal aspects of the post-otic neural tube, and, in response to environmental cues, migrate to and along the primitive gut. While much is known about the migration of enteric neural crest along the gut, knowledge of the intrinsic factors that regulate their early migration and specification prior to gut colonization remains elusive. Meis cofactors are TALE-family homeodomain proteins that control accessibility at Hox-regulated promoters. Although previous studies have described meis3 expression in the foregut environment during zebrafish development, no functional roles for Meis3 during enteric neural crest development have been described. Here, we show that meis3 is expressed along vagal neural crest pathways to and around the gut, as well as in a subset of migratory enteric neural crest. Using loss of function analyses, we show that while apoptosis is not significantly increased in Meis3 depleted embryos, there is a reduction in cell number and migratory chain behavior of neural crest cells localized near the gut. Although neural crest and gut specification markers occur normally, the spatial expression of the epibranchial placode and enteric neural crest specification marker phox2bb is altered, suggesting that Meis3 function is required for proper enteric neural crest development and migration prior to gut colonization. Together, these observations support a model in which Meis3 regulates the efficient migration of enteric neural crest into the gut and enhances our knowledge of the early events regarding enteric nervous system development.

Program Abstract #358
Parasympathetic gangliogenesis is initiated by Sprouty1/2-regulated Wnt signals from epithelial progenitors
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Temporal and spatial communication among multiple cell types during embryogenesis initiates and exquisitely coordinates organogenesis. In the initiating submandibular gland (SMG) the parasympathetic ganglion (PSG) forms around the epithelial duct and maintains adjacent ductal keratin 5-positive (K5+) progenitors required for organogenesis. However, the signals that initiate gangliogenesis around the duct are not known. Here, we identify Wnt signals from the epithelial K5+ ductal progenitors that initiate gangliogenesis and association with the duct, which results in subsequent innervation. FGF signaling reduces Wnt expression and inhibits gangliogenesis, depleting K5+ progenitors. Deletion of negative regulators of FGF signaling in vivo identifies Spry1/2 as key modulators of Wnt expression and parasympathetic gangliogenesis. When Spry1/2 are deleted Wnt expression is reduced and there is a striking loss of parasympathetic gangliogenesis. This absence of the PSG results in a loss of epithelial progenitor cells and disrupted gland development. Deletion of a single allele of Fgf10 and addition of a Wnt activator in vivo restores gangliogenesis and reestablishes the association of the PSG with the duct, which rescues epithelial development. These results show that Spry1/2 regulation of Wnt signaling controls gangliogenesis around the duct, which is essential to establish the neuronal-epithelial communication required for progenitor cell maintenance and organogenesis. These findings identify Spry1/2 and Wnt signals as potential targets for initiating and improving gangliogenesis and innervation during organ repair and/or regeneration. This research was supported by the Intramural Research Program of the National Institute of Dental and Craniofacial Research at the National Institutes of Health.

Program Abstract #359
Junctional Protein Cingulin Play a Potential Important Role in Cilia
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Cilium, a hair-like structure protruding from cell surface to environment and functions as a chemical-mechanic sensor, plays a very important role in embryonic development and homeostasis of organs defect in cilium will cause a variety of dysfunction in multiple organs, including polycystic kidney/liver/pancreas, situs inversus, mental retardation, infertility, obesity and diabetes, which are coined as ciliopathy. However the mechanism that how cilia formation and functions are regulated is not well understood. Here we found three cingulin homologous genes, cingulina (cgna), cingulinb (cgnb) and cingulin like1 (cgnl1) are expressed in ciliated organs in zebrafish embryos, like pronephric duct, optic vesicle, lateral line, ear, Kupffer’s vesicle, indicating a close relationship between Cingulin protein and cilia. Further more, knocking down of cgnb caused kidney cysts, as well as cilia defects. And loss of cgnl1 resulted in left-right defect. So we hypothesized that Cingulin might regulate renal functions and left-right patterning via affecting cilia function.

Program Abstract #360
The Role of Primary Cilia in Neural Development and Disease
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Cincinnati Children's Hospital Medical Center, USA

Patients with mutations in genes important for primary cilium function often suffer from cognitive impairment. Mouse mutants in several ciliary genes have forebrain phenotypes. The underlying mechanisms of these phenotypes are not fully understood. We have previously demonstrated loss of Ttc21b leads to decreased retrograde trafficking in the cilium. A null mouse allele shows microcephaly and abnormal patterning of the embryonic neural tissue along both the anterior-posterior and dorsal-ventral axes. However, important aspects of the Ttc21b null forebrain phenotype remain completely unexplored. Here we show abnormal proliferation, migration and differentiation of neural progenitors. We are also using a conditional allele to determine the spatio-temporal requirement for Ttc21b. Surprisingly, a forebrain specific ablation has only minor consequences for cortical development. Our current model holds the Ttc21b null phenotype is due to an early event in patterning the nascent neural ectoderm, prior to definitive forebrain development. The consequences of the loss of Ttc21b then perdures into the neurogenic cortex. We are comparing these results from the Ttc21b mutants to conditional ablations of Kif3a and Ifit88. Using a series of Cre transgenic mouse lines to define the spatiotemporal role of these genes, we note that each gene has unique phenotypes from ablation at specific time points. Taken together, these studies are revealing subtle differences in the role of ciliary proteins in the control of neural patterning and neuroprogenitor proliferation/differentiation. We are also exploring the molecular mechanisms of Ttc21b activity. We demonstrate a genetic interaction with a null allele of Gli3 and rescue of some Ttc21b phenotypes with an allele of Gli3 lacking a putative transactivator domain. We also show a biochemical interaction with Gli3. These are consistent with a model where Gli3 is a cargo protein linked to the ciliary retrograde machinery by TTC21B.

Program Abstract #361
A spatial analysis of hair cell development in the mouse crista
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The inner ear contains three types of sensory organs. The auditory system consists of the organ of Corti, while the vestibular system contains two gravity-sensing maculae and three rotation-sensing cristae. Each of these organs is comprised of mechano-sensory hair cells and their surrounding support cells. Loss of the hair cells from these organs results in hearing loss and balance disorders such as vertigo, for which there is currently have no effective treatment. Recent studies have shown that each of these organs has some regenerative potential through Notch inhibition in adult mice after damage. The degree of hair cell regeneration, however, is low and it is unclear why only a subset of support cells in these organs are competent for regeneration. One possibility is that the regions that retain some regenerative potential were the last regions to mature in these organs, explaining why they still exhibit some properties characteristic of immature organs. In the cochlea and utricle, previous work has characterized both the spatial and temporal development of these organs, however, very little is known about where and how hair cells are born in the cristae. In this study, we wanted to determine how hair cells are added during development and what their locations are in the mature crista in order to relate this information to adult regeneration. To answer this, we administered single BrdU injections to time-mated Swiss Webster mothers at different embryonic stages spanning the known period of hair cell birth in the cristae. We then analyzed the spatial pattern of hair cell birth in the cristae once the mice were one month old. We found that hair cell birth begins in the central region of the cristae and with age shifts towards the periphery or lateral edges. This pattern correlates with the regions that maintain active Notch signaling in the adult and where we have also observed some support cell transdifferentiation in the adult in response to Notch inhibition.

Program Abstract #362
Sonic hedgehog gradient regulates the tonotopic patterning of the vertebrate cochlea
Ji Hyun Ma1, Eun Jin Son2, Harinarayana Ankanreddy1, HongKyung Kim1, Jae Young Choi2, Doris K. Wu3, Jinwoong Bok1,2
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Sonic hedgehog gradient regulates the tonotopic patterning of the vertebrate cochlea
Program Abstract #362
Sonic hedgehog gradient regulates the tonotopic patterning of the vertebrate cochlea
Ji Hyun Ma1, Eun Jin Son2, Harinarayana Ankanreddy1, HongKyung Kim1, Jae Young Choi2, Doris K. Wu3, Jinwoong Bok1,2
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Notch inhibition.
Discrimination of sound frequencies, which is crucial for verbal communication and daily activities, is possible because the peripheral auditory organ, the cochlear duct, is tonotopically organized, such that the base of the cochlear duct is sensitive to high frequency sounds and the apex to low frequencies. Although various structural and physiological characteristics that contribute to the frequency discrimination have been revealed, it is currently unclear how and when the tonotopic axis is established during cochlear development. Our temporal and spatial gene expression analyses using region-specific cochlear markers suggest that positional identity along the tonotopic axis is specified early in the otocyst stages. Sonic hedgehog (Shh) has been shown to provide unique positional information in various tissues such as the spinal cord and limb. Thus, we tested if Shh signaling is also involved in providing positional information to the developing cochlea using mice and chicken models. In the mutant cochlea that failed to mediate high levels of Shh signaling, the apical identity was selectively lost with the basal identity being specified. Conversely, the mutant cochlea that was exposed to high levels of ectopic Shh signaling lost the basal identity and instead acquired the apical identity. Our results also demonstrated that Shh's role in the tonotopic patterning is conserved in the chicken basilar papilla, in which temporal ectopic Shh activation by implanting Shh-beads into the otocyst in ovo induced basal hair cells to adapt more apical characteristics. These results suggest that the Shh gradient provides positional information to the cochlear primordium along its longitudinal axis, which prefigures the tonotopic axis responsible for frequency discrimination in the mature cochlea. This work was supported by the Brain Korea21 PLUS Project for Medical Science, Yonsei Univ.

Program Abstract #363

Maintenance of cochlear sensory progenitor population via FGF-FGFR dependent epithelial-mesenchymal crosstalk
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Cochlear sensory progenitor cell population, which generates hair cells and supporting cells in the organ of Corti, has been formed during development and remains unchanged once formed. Due to lack of regenerative capability of cochlear sensory epithelium in human and mouse, once damaged, cochlear hair cells are lost permanently resulting hearing loss. Therefore, understanding how sensory progenitor cell population is formed is critical to cochlear sensory cell regeneration. Fgf9 and Fgf20 are expressed in nonsensory and sensory epithelium during cochlear progenitor cell formation, respectively. Loss of Fgf9 and Fgf20 results in shortened cochlear length in addition to loss of lateral compartments (outer hair cells and supporting cells) and disrupted pattern formation. Staining of Sox2 shows decreased cochlear progenitor population in Fgf9 and Fgf20 compound mutants. This decrease is due to decrease of sensory progenitors proliferation. Tissue specific deletion of Fgfr1 and Fgfr2 shows that Fgfr1 in sensory epithelium is required only in lateral compartment differentiation and pattern formation, and mesenchymal Fgfr1 and Fgfr2 are required only for proper cochlear length by promoting cochlear progenitor cell proliferation. In addition, ectopic activation of FGF signal in mesenchymal cells is enough to increase of cochlear progenitor cell proliferation and of cochlear length. These data elucidate roles of Fgf9/20-Fgfr1/2 signal axis required for cochlear sensory cell regeneration.
Funding: Hearing Health Foundation, Action On Hearing Loss, NIDCD K99/R00 award

Program Abstract #364

Temporal requirement of TGF-beta and Hedgehog signaling during middle ear ossicle formation
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The vertebrate auditory system consists of outer, middle, and inner ears. Although most of hearing loss observed in humans is originated from the inner ears, malformation of the middle ear ossicles can also cause hearing loss. The middle ear ossicles are originated from neural crest cells (NCCs). However, it is currently unclear how NCCs migrate to the correct location, where they condense and differentiate into bony ossicles. In order to elucidate the molecular mechanisms regulating the initial condensation and differentiation of NCCs into middle ear ossicles, we used Cre/lox system to manipulate signaling pathways specifically in NCCs. In particular, we focused on TGF-β and Hedgehog (Hh) signaling, which are known to be involved in NCC development. We observed that expression domains of Sox9, a marker for NCC condensation, were closely related with the expression domains of Bone morphogenetic protein 4 (Bmp4) and Sonic hedgehog (Shh) in the pharyngeal endoderm. When TGF-β signaling was inactivated in the NCCs using Wnt1Cre; Smad4lox/lox mutants, NCC condensation in the prospective middle ear region failed at embryonic day (E) 10.75. However, NCC-specific inactivation of Hh signaling using Wnt1Cre; SmoM2/lox, mutants, the initial condensation occurred at E10.75 but disappeared at E11.5. On the other hand, ectopic activation of Hh signaling in the NCCs using Wnt1Cre; SmoM2/+ mutants caused led to enlarged condensation of the NCCs in the branchial arch region at E11.5. Interestingly, the resulting middle ear ossicles were fused and dislocated away from inner ear at E15.5. These results suggest that TGF-β signaling emanating from the specific location of pharyngeal endoderm regulate the initial condensation of migrating NCCs, and then Hh signaling is required for further differentiation and possibly for cell survival. This work was supported by the Brain Korea21 PLUS Project for Medical Science, Yonsei Univ.
Program Abstract #366
Mechanisms of FGF signaling in lung development reveal a link between Dicer1 loss and the pathogenesis of pleuropulmonary Blastoma
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Mice lacking Fibroblast Growth Factor (FGF) 9 have hypoplastic lungs and die at birth due to respiratory failure. Investigation of the underlying mechanisms identified an early embryonic feed-forward signaling pathway involving mesenchymal FGF and β-catenin-dependent Wnt signaling that regulates lung mesenchymal growth and differentiation. To further probe the function of FGF9, we developed an inducible mouse model in which FGF9 can be expressed in lung epithelium. Induced expression of FGF9 in embryos results in lung mesenchymal hyperplasia, whereas expression in adults causes the rapid proliferation of progenitor-like cells in distal airway epithelium. Embryonic induction of FGF9 closely models the histopathology of Pleuropulmonary Blastoma (PPB), a syndromic lung cancer that is associated with heritable mutations in Dicer1. PPB is a familial disease presenting in early childhood with multifocal cystic lung lesions. Heterozygosity of Dicer1 predisposes to PPB, and loss/mutation of the wild type allele in lung epithelium is hypothesized to initiate cyst formation. In later stages, the mesenchymal component of PPB cysts progresses to malignant sarcomas. We hypothesized that loss of Dicer1 function in lung epithelium leads to persistent hyperplastic mesenchyme (and subsequent risk for malignancy), implicating a non-cell autonomous mechanism. Here, we show histological and molecular similarity in mice lacking epithelial Dicer1, mice induced to overexpress epithelial FGF9, and human Type I PPB. We further demonstrate that Fgf9 expression is increased in lung epithelium lacking Dicer1 in a micro RNA-dependent manner and that Fgf9 expression is essential for the Dicer1-deficient lung phenotype. These studies identify FGF9 as a biologically active downstream target of Dicer1 in lung epithelium that functions as an initiating factor for PPB.

Program Abstract #367
Hedgehog effectors Gli2 and Gli3 are redundantly required for respiratory epithelium specification
Lu Han, Chris Anglin, Scott Rankin, Aaron Zorn
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Hedgehog effectors Gli2 and Gli3 are redundantly required for respiratory epithelium specification.Embryonic induction of FGF9 closely models the histopathology of Pleuropulmonary Blastoma (PPB), a syndromic lung cancer that is associated with heritable mutations in Dicer1. PPB is a familial disease presenting in early childhood with multifocal cystic lung lesions. Heterozygosity of Dicer1 predisposes to PPB, and loss/mutation of the wild type allele in lung epithelium is hypothesized to initiate cyst formation. In later stages, the mesenchymal component of PPB cysts progresses to malignant sarcomas. We hypothesized that loss of Dicer1 function in lung epithelium leads to persistent hyperplastic mesenchyme (and subsequent risk for malignancy), implicating a non-cell autonomous mechanism. Here, we show histological and molecular similarity in mice lacking epithelial Dicer1, mice induced to overexpress epithelial FGF9, and human Type I PPB. We further demonstrate that Fgf9 expression is increased in lung epithelium lacking Dicer1 in a micro RNA-dependent manner and that Fgf9 expression is essential for the Dicer1-deficient lung phenotype. These studies identify FGF9 as a biologically active downstream target of Dicer1 in lung epithelium that functions as an initiating factor for PPB.

Program Abstract #368
Dissecting Roundabout receptor function in a mouse model of Congenital Diaphragmatic Hernia
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Congenital Diaphragmatic Hernia (CDH) is one of the deadliest birth defects (40-62% mortality rate). Malformation of the diaphragm is commonly believed to be the cause of CDH, as the characteristic symptom is a protrusion of abdominal organs into the thoracic cavity through an aberrant opening in the diaphragm. Using mouse genetic tools, we recently identified a role for Roundabout (Robo) receptor genes in CDH. Our results show that global inactivation of Robo1 and Robo2 (Robo1;2) leads to malformation of the diaphragm and respiratory failure at birth, classical CDH phenotypes. We traced the primary defect to a failure in foregut separation from the dorsal body wall, which leads to improper organ positioning. These phenotypes precede and likely underlie diaphragm malformation. Our findings offer the first demonstration of a clear diaphragm-independent CDH mechanism that leads to organ herniation and lethality at birth. To address the cause for life-long breathing difficulties observed in CDH survivors, we have generated an endoderm-specific knockout of Robo genes. Further studies will be aimed at characterizing the lung phenotype in these mice. As deletion of the chromosomal region containing Robo genes has been documented in human CDH, our study will reveal the disease mechanism that underlies Robo-associated CDH. This work was supported by March of Dimes grant 6-FY10-339 and KB is supported by an NIH training grant.

Program Abstract #369
Endodermal Wnt signaling directs dorsal-ventral patterning of developing trachea
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Tracheomalacia is a condition in which the walls of the upper airways are soft because of lack of cartilage. The etiology of this prevalent congenital defect (1:3000/live births) is unknown. Similarly, mechanisms underlying cartilage formation during
respiratory tract development are unclear. Our goal is to understand how the trachea is patterned during normal development and in congenital airway malacia. We generated a mouse model where Wls, which mediates Wnt ligand secretion, was deleted from endoderm of developing respiratory tract using ShhCre mice. In Wls\textsuperscript{ShhCre} embryos, the trachea was mispatterned: cartilage was near absent while muscle was expanded into the ventral region of the trachea. We hypothesize that Wnt signaling is required for patterning of tracheal mesenchyme. To determine the cause of absent cartilage Wls\textsuperscript{ShhCre} tracheas were stained with Sox9 or PNA lectin. At E11.5 Sox9 expression was dramatically reduced and at E13.5 mesenchymal condensations were absent. To test if expansion of muscle observed in Wls\textsuperscript{ShhCre} tracheas occurred at expenses of cartilage, Sox9 was deleted from tracheal mesenchyme using Dermo1Cre mice. In these embryos, cartilage and tracheal length were reduced without effect in muscle. Similarly, deletion of Sox9 in chondroblast, caused absent cartilage without expansion of muscle into ventral side of trachea. Deletion of Wls in tracheal mesenchyme did not affect cartilage or muscle formation, suggesting that Wnt ligands produced by the mesenchyme are not essential for tracheal patterning. In Wls\textsuperscript{ShhCre} tracheas, mRNA levels of Tbx4, Tbx5, Bmp6, Msx1 and Msx2, which mediate cartilage and muscle patterning, were diminished. We conclude that paracrine Wnt signaling from the airway epithelium patterns tracheal mesenchyme: promotes cartilage and actively represses muscle in ventral side of the trachea by controlling gene expression of key regulators of muscle and cartilage lineage. Partially supported by NIH-NHLBI K01HL115447 to DS.

**Program Abstract #369**

**Hedgehog signaling is required for dental papilla formation in zebrafish teeth**

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Cell signaling by secreted hedgehog proteins is essential for many aspects of vertebrate embryonic development including mammalian tooth formation. However, we lack a comprehensive understanding of the instructional role hedgehog signaling plays at a cellular level during odontogenesis. We have therefore employed laser-scanning confocal microscopy in the zebrafish vertebrate model system to investigate specific consequences of altering hedgehog signaling during tooth development. We have found that reducing hedgehog activity just after the initiation of tooth morphogenesis with the Smoothened inhibitor cyclopamine results in a complete absence of the dental papilla, the embryonic structure that normally gives rise to the odontoblasts that secrete the dentin layer of the mature tooth in all vertebrates. Interestingly, the dental epithelium which gives rise to enamel-secreting ameloblasts forms relatively normally after such treatment, suggesting that the dental papilla is specifically sensitive to hedgehog signaling levels in this developmental window. We are currently examining whether hedgehog signaling normally directs cell proliferation, inhibits apoptosis, and/or guides cell migration in the developing dental papilla. Overall, this work helps give us a more detailed understanding of how cell signaling directs dental organogenesis.

**Program Abstract #370**

**Involvement of region-distinctive expressed Rac1 during palatal shelves elevation**

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Palate development requires growth, elevation and fusion of the palatal shelves during embryogenesis. Palatal shelves elevation is one of the most important mechanisms to avoid cleft palate and is triggered by both external influence and intrinsic force. Various factors contribute to intrinsic force within palatal shelves, including increased extracellular matrix, epithelial covering and migration of palatal mesenchymal cells. During palatal shelves elevation, the bend and groove regions had different functions and morphology. In our study, we found that the bend and groove regions play pivotal roles during palatal shelves elevation, by contributing tension and repulsive force respectively. This may caused by the molecular heterogeneity of Rac1 between these two regions. After overexpression of Rac1 in epithelium of bend region, palatal shelf elevation was faster than control group. However, mesenchymal overexpressed Rac1 within bend region induced failure of palatal shelf elevation. The mesenchymal overexpression of Rac1 reduced epithelial cell proliferation, changed the epithelial cell shape and influenced the migration of mesenchymal cells during palatal shelves elevation. Our results indicated that Rac1 may regulate palatal shelves elevation by control various cellular and molecular event in bend and groove regions of the palatal shelves.

**Program Abstract #371**

**A splice-site mutation in Pibf1 causes bilateral cleft lip and palate in mice**

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Cleft lip and palate is one of the most common human birth defects; however, there are few mouse models that recapitulate the human cleft lip phenotype. We identified mutant mice with bilateral cleft lip and palate in an ENU-induced mutagenesis screen for brain patterning defects. The mutation was located using whole-genome SNP analysis to chromosome 14 between 79-108 Mb. Whole exome sequencing identified a mutation at the exon-intron junction of Pibf1 (also known as CEP90). This mutation results in skipping of exon 9, which has total 126 bp corresponding to 366-407 amino acid residues. OPT and micro CT imaging showed that the mutant displayed deficient premaxillary growth and abnormal nasal turbinate and septum. In addition, the upper incisors did not
develop in the mutants. Histological analysis revealed abnormal vomeronasal organ morphology, and fusion of the elongated nasal septum to one side of the oral cavity was often found. Pibf1 has been shown to localize in the basal body of primary cilia and to be required for ciliogenesis in vitro. Consistent with the previous studies in cell lines, we found that primary mouse embryonic fibroblasts from the mutant have reduced number of cilia. Furthermore, whole-mount immunostaining revealed that the mutant embryo has fewer cilia in the upper lip during early embryogenesis. These data confirmed the importance of Pibf1 for ciliogenesis in vivo, and also suggest a role for cilial-mediated signaling during lip formation. Given the known importance of primary cilia for sonic hedgehog signaling, we are investigating whether this pathway is specifically perturbed in these mutant mice. This work was supported by NIH grants R01 HD36404 and MH081187.

Program Abstract #372
Novel mechanisms in frontal bone ossification
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Craniosynostosis is the most common congenital anomaly of the human skull vault. It affects approximately 1/2000 children. The skull vault develops from paired flat bones that are joined together by a flexible suture. The flexible suture allows for birth and postnatal growth of the brain. Craniosynostosis results from ectopic ossification in the suture, and prevents appropriate growth of the skull vault. In humans, most (80%) of craniosynostosis cases have an unknown genetic and molecular etiology. The development of the skull and suture requires controlled cell migration and differentiation in a time and location dependent manner, both processes that may be under the control of the non-canonical Wnt/planar cell polarity (PCP) signaling pathway. We have identified an ENU-mutagenized mouse line, that has damaging mutations in the core PCP component: Prickle1 and its downstream effector ROCK1 (rho-associated coiled-coil kinase 1). Both Prickle1 and Rock1 are expressed in the developing frontal bones. We hypothesize that Prickle1 prevents precocious ossification in the skull vault. The mutants develop ectopic ossification between the frontal bones, an area that should never ossify, and is reminiscent of metopic craniosynostosis. We have inhibited ROCK function in embryonic calvarial osteoblasts, and observed accelerated ossification. We conclude that Prickle function, and therefore PCP signaling may be a novel mechanism contributing to the development of metopic craniosynostosis.

Program Abstract #373
Modifier Gene Discovery Using a Novel ENU Mutagenesis Approach
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The discovery of modifier genes in human and mouse has seen slow progress. In mice, this is largely due to the reliance on the variability inherent in genetic backgrounds; identifying the causal variant even in highly resolved mapped loci has proved challenging. We have developed a fast and cost-effective method for genetic mapping using Next Generation Sequencing that combines single nucleotide polymorphism discovery, mutation localization, and potential identification of causal sequence variants. Importantly, this approach can potentially be applied to mutagenized mice that have not been outcrossed, using the ENU-induced variants as SNP markers. This will enable the possibility of doing both primary screens and modifier screens on fully inbred lines. We have begun a test of this approach using sensitized strains that cause a variety of skeletal defects, as these can be readily assessed in late gestation. We selected the following C57BL/6 congenic strains: a “knock-in” strain carrying the Pfeiffer syndrome P250R mutation in Fgfr1, a loss-of-function allele of the SHH transcriptional effector Gli2, a strain with loss of the osteoblast-specific isoform of Runx2, and a spontaneous loss-of-function allele of Pibf1. The Pfeiffer model exhibits variable craniosynostosis, while the Gli2 mutation can be modified by prenatal ethanol exposure, leading to holoprosencephaly phenotypes. The Runx2 line could lead to identification of modifiers of bone growth and mineralization, and the Pibf1 mutant’s cleft lip, cleft palate, and ciliopathy-like phenotype could reveal modifiers of craniofacial development and function of the primary cilium. In the simplest cases, whole-genome sequencing will reveal areas of homozygosity shared between mutants with a consistent “modified” phenotype, and allow us to narrow our focus to the induced variants included in these homozygous regions. We anticipate that we may ascertain more complex genetic interactions as well. NIH R01 HD36404

Program Abstract #374
Adrenergic signaling regulates long 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. Longitudinal vertebrate growth occurs at the growth plates (GP) located at both ends of the long bones. Chondrocytes, the cells within the GP, go through subsequent stages of differentiation from the ends towards the center of the bone: resting, proliferative, and hypertrophic chondrocytes (HTC). Regulation of HTC number and size determines bone length, as HTCs provide the scaffold that will be later replaced by bone. While most growth regulation occurs intrinsically within the GP, animals also need to adapt their growth rate to environmental changes, such as varying nutrient levels and disease outbreaks, but the mechanisms by which they do so are mostly unknown. We hypothesize that this regulation occurs in part through the nervous system, along with its known role in the regulation of bone mass. For this project we focused on the effects of the sympathetic nervous system, responsible for the fight-or-flight response, on long bone growth. We characterized the innervation of the long bones at different developmental stages and analyzed...
the expression of adrenergic receptors (necessary for sympathetic signaling) in chondrocytes, and observed notable changes during development, suggesting the existence of a susceptibility window to sympathetic signaling in chondrocytes. Lastly, we tested the effect of pharmacologically increasing or decreasing adrenergic signaling on chondrocyte function, and found that bone growth and expression of some chondrocyte differentiation markers were impaired. We are currently trying to confirm that bone innervation is the in vivo source of adrenergic stimulus to the GP. This study expands the repertoire of extrinsic factors controlling the GP and suggests that some pharmacological treatments are contraindicated during early life. Supported by NSF grant IOS-1239422, HFSP fellowship LT000521/2012-L and NIH T34 Grant #GM007684

Program Abstract #375
The Dynamics of BMP Signaling in Digit Differentiation and Polydactyly
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Mutations in the Bone Morphogenetic Protein (BMP) pathway are associated with a range of defects in digit formation. Standard genetic methods have provided limited resolution into the underlying mechanisms because redundant BMPs are required for multiple stages of limb development. We generated an inducible allele of the BMP inhibitor Gremlin and used this to define the temporal period when BMPs regulate digit formation. During this period, inhibiting BMPs causes increased proliferation as well as elevated, persistent expression of FGFs. These changes are followed by persistent expression of undifferentiated chondrocytes at the anterior and posterior margins that ultimately resolve into polydactylos digits. These effects are mirrored in micromass cultures, where the addition of FGFs inhibits BMP-directed chondrocyte differentiation. Collectively, our results provide new insights into the timing and mechanisms underlying BMP signaling during digit morphogenesis.
This work was supported by National Institutes of Health #R01HD073151 to S.A.V.

Program Abstract #377
RNA-Seq analysis of tendon development
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Tendons are fibrous connective tissues that transmit force generated by muscle contraction to bone and are thus essential for the proper function of the musculoskeletal system. However, there is currently very limited understanding of the molecular mechanisms regulating tendon development. We report here whole transcriptome expression profiling analysis of tendon development in mice using fluorescence-activated cell sorting (FACS) and RNA-seq. We identified many genes whose expression is highly enriched in the early developing tendon cells using the Scn-GFP transgenic mice and characterized their expression patterns by whole mount and section in situ hybridization analyses. Furthermore, we analyzed differential gene expression profiles of the developing limb tendons in Mkx-/- mutant and control embryos to gain new insight into the molecular regulation of tendon fibrillogenesis. This work was supported by NIH grant R01AR056943.

Program Abstract #378
c-myc regulates cell proliferation during lens development
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Myc protooncogenes are important for the balance between cell proliferation, growth and differentiation in various developing organs, and its deregulation may lead to tumorigenesis or non-functional tissues. The precise coordination of cell cycle dynamics is crucial for the formation of a functional, transparent lens. In order to understand c-myc roles during lens development in vivo, we investigated the consequences of c-myc targeted deletion in the mouse lens precursor cells.
We show that c-myc gene is highly expressed during early stages of mouse lens development. Targeted deletion of c-myc gene from head surface ectoderm dramatically affected eye organogenesis, resulting in severe reduction of eye and lens size and in dysgenesis of the eye anterior segment. The c-myc gene content was relevant for the phenotype severity. Defective development of c-myc-null lens was not caused by increased cell death and the initiation of important terminal differentiation events, such as crystallin synthesis and denucleation were undisturbed. However, fiber cell degeneration was evident during postnatal stages of lens development. Interestingly, c-myc loss led to reduced cell proliferation in the lens, associated with ectopic localization of Prox1 and p27kip1 proteins within epithelial progenitor cells. These proteins are crucial for cell cycle arrest of lens epithelial progenitors and have c-myc binding sites in their promoters. Additionally, a sharp decrease in the mRNA and protein content of the transcription factor Foxe3 was observed after c-myc inactivation. These data represent the first description of the physiological roles played by a Myc family member during mouse lens development. Our findings support the conclusion that c-myc regulates the proliferation of lens epithelial cells in vivo, modulating the expression of cell cycle regulators in the developing lens and contributing for the proper balance between cell proliferation, cell cycle exit and cell differentiation.
miR-137 regulates mammary gland morphogenesis and breast carcinogenesis

Discontinued-2 (Dab2) is a widely expressed protein that was discovered as a mitogen responsive phosphoprotein typically lost at premalignant stages of tumorigenesis. Subsequently, Dab2 was identified as an adaptor protein that functions in receptor mediated endocytosis, by orchestrating the recruitment of clathrin and cargo into foci prior to internalization as a clathrin coated vesicle. We have discovered a profound induction of Dab2 proteins during pregnancy and lactation within all the epithelia of the murine mammary gland. To study the physiological function of Dab2, we have constructed mice genetically mosaic for Dab2 in order to bypass an early embryonic lethality. We have found that epithelial branching morphogenesis, gross milk composition and nurturing mammary involution was consistently retarded in Dab2 null tissue concomitant with augmented phosphorylation and activation of Erk1/2, a pro-survival signal. Our data suggest that the Dab2 induction suppresses TGF-beta-induced Erk1/2 activation that may counter cell survival during apoptotic clearance in the mammary gland. Supported by NIH RO1 CA095071, CA79716 and CA75389.

miR-137 regulates mammary gland morphogenesis and breast carcinogenesis

Breast cancer is one of the most frequently occurring cancers and a major cause of death in women. MicroRNAs, small non-coding RNAs which control gene expression are differentially expressed in breast cancer cells and have been implicated in cancer formation, tumor invasion and metastasis. The embryonic mouse mammary gland has been used as model for breast cancer as initial stages in development involve mesenchymal invasion by an epithelial bud. We investigated expression profiles of miRNAs and protein-coding genes in the developing mouse mammary gland. Many miRNAs are differentially expressed with miR 137 expressed at particularly high levels and also genes already known to be expressed in both embryonic gland and breast cancer. When miR137 was over-expressed in the embryo, rather than forming a mammary bud, the epithelium became thickened and genes normally associated with mammary gland formation and genes such as Tbx3 and Lefl were not expressed. This suggests that miR137 modulates gland formation and invasion during development. MCF10a cells from human adult mammary epithelium also express high levels of miR-137 but MDA-MB-231 cells, a human breast cancer cell line, lower levels. When miR-137 was expressed in MDA-MB-231 cells, their ability to form tumours in adult mice was reduced and associated with changes in epithelial cell behavior, angiogenesis and tumour suppressor gene expression. These data are consistent with miR-137 modulating epithelial cell behavior in the adult breast and suggests that miR-137 is a potential therapeutic target for breast cancer. This study also confirms the usefulness of the embryonic mouse mammary gland as a model for breast cancer.

Drosophila melanogaster: an emerging model system in lipid metabolism research

During development, Drosophila undergoes major changes in its physiology to transform the larval feeding into the adult reproduction stage. The major energy resources are carbohydrates (sugar) and fat - especially triacylglycerols (TAGs). Fat is mainly stored in the fat body, which undergoes vivid changes during development. In this study, we are interested in the molecular changes of the fat body during the early and the later stage of the adult fly. Freshly hatched adult flies have a different fat storage than those who are at least some days old. The fat cells (adipocytes) are less connected right after the hatching and Nile Red staining showed differences in the ratio of neutral lipids to phospholipids during the first days of adult development. To further understand the underlying changes, we harvested fat bodies and adipocytes from freshly hatched (less than 6 hours post hatching (hph)) and mature (seven days post hatching (dph)) flies. We extracted total and subsequently semi-quantitatively analyzed the lipids on a quadruple TOF mass spectrometer (Synapt). The outcome of the measurements was processed with CoMeT® Software and downstream statistics with MetaboAnalyst 2.0. Principal component analysis showed a clear separation between the young and the older flies, indicating the major changes. We were able to identify over 100 lipids among which many were differentially regulated. ANOVA showed that ceramides are highly and significantly regulated. For instance, long chain unsaturated ceramides are significantly increased in early adulthood. This is an interesting observation since ceramides were found to be important for the
regulation of lipid uptake and storage. In summary, we found that many lipids are changing in the early adult developmental stage involving signaling lipids which leads to the hypothesis that the restructuring of the fat body cells needs precise fine tuning.

**Program Abstract #382**

**Fibroblast growth factor signaling influences ovary morphogenesis in Drosophila**

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The *thisbe* (*ths*) gene encodes a *Drosophila* fibroblast growth factor (FGF) ligand. We found that *ths* mutant females are viable but sterile. Upon dissection, we observed ovaries exhibited abnormal morphology. Specifically, the epithelial sheath, an outer layer of muscle tissue, was absent. Epithelial sheaths are formed in the developing gonad during the pupal stage as a result of migration of apical cells. Therefore, we examined the role of FGF signaling in the developing gonad. We found that the *heartless* (*htl*) gene, encoding a FGF receptor, is expressed in the apical cells as they migrate within the pupal ovary. In *ths* mutants, apical cell migration was incomplete. Furthermore, when the *ths* gene was downregulated or upregulated, the ovaries exhibited under- or over-proliferation of apical cells. Collectively, these results suggest FGF regulates proliferation of apical cells to support their migration.

*htl* expression was also identified in the gonad earlier at the larval stage, preparation, suggesting earlier additional roles for this signaling pathway. We found that a different FGF ligand, *pyramus* (*pyr*) gene, is expressed in primordial germ cells (PGC); while *ths* is expressed within somatic cells. When either *pyr* or *ths* was downregulated at the larval stage, excessive interstitial cells were identified. Interstitial cells are somatic cells, which have been shown previously to regulate PGC number. Fewer PGCs were present upon ectopic expression of either FGF ligand. Currently, we are testing the hypothesis that FGF signaling directly impacts germ line-soma homeostasis at the larval stage. In summary, this study demonstrates that FGF signaling supports multiple roles during *Drosophila* ovary morphogenesis both in regulating epithelial sheet formation as well as interstitial cell/PGC number. It is possible that FGF signaling influences morphogenesis of vertebrate ovaries as well.

**Program Abstract #383**

**MAB-3 and DMD-3, two DM-domain transcription factors, direct the development of the somatic gonad in *C. elegans* males.**

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Organogenesis is a complex and poorly understood process. We are utilizing the development of the *C. elegans* male somatic gonad as a model to uncover conserved genetic and cell biological pathways that control organ formation in animals. During male gonadogenesis the migratory route of a “leader cell” known as the linker cell determines the shape of the male gonad. Linker cell migration terminates at the hindgut, which subsequently engulfs the linker cell. We are studying the function of two conserved DM-domain containing transcription factors, DMD-3 and MAB-3, in directing the late stages of male somatic gonad development. We have found that both DMD-3 and MAB-3 are expressed in tissues involved in male gonad formation, including the linker cell. Furthermore, DMD-3 expression in the linker cell is dependent on NHR-67, a nuclear hormone receptor that directs linker cell migration. Consistent with the expression pattern, multiple aspects of somatic gonad development are defective in *mab-3;mdm-3* double mutant males. Specifically, linker cells in *mab-3;mdm-3* double mutant L4 males exhibit an aberrant morphology during the last leg of their migratory routes. Strikingly, this defect causes the linker cell to arrest before it completes its journey to the hindgut in ~60% of males examined. Furthermore, linker cells that do successfully migrate to the hindgut fail to be engulfed. Mutant hindgut cells also fail to undergo male-specific cell shape changes during the later stages of proctodeum development. We are currently undertaking experiments to identify the downstream mediators of MAB-3 / DMD-3 activity and to determine if DMD-3 is functioning cell-autonomously within the linker cell and/or hindgut to direct migration and engulfment. Taken together, these results demonstrate a critical role for DMD-3 and MAB-3 in directing the late stages of male somatic gonad development.

**Program Abstract #384**

**Elucidating the mechanism by which mechanical force stabilizes Cell-ECM adhesion during development.**

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Cells in multicellular organisms are arranged in complex-three dimensional shapes. To achieve such complexity, cells must form adhesive contacts with the extracellular matrix (ECM). During early embryonic development Cell-ECM adhesions are typically dynamic and transient but later on, as tissue architecture is consolidated, they become stable and long lasting. We are interested in the mechanisms that regulate this developmental transition. The principal mediators of Cell-ECM adhesion are the integrin family of transmembrane adhesion receptors. Integrin-based adhesions undergo assembly and disassembly, or turnover; this provides important means of regulating the duration and strength of Cell-ECM attachment. We are particularly interested in how the turnover of Cell-ECM adhesion is controlled by mechanical force experienced by cells. To investigate the turnover of the integrin adhesion complex *in vivo* we perform FRAP on the myotendinous junctions (MTJs) in live embryos and use conditional mutants to alter the force acting on the MTJs. Using this approach in combination with mathematical modeling we elucidate the kinetics of adhesion complex turnover. Our analysis has uncovered a role for mechanical force in regulating integrin turnover and in stabilizing cell
characterized regulators of cell and tissue growth in response to nutrients. Although the signaling networks that link nutrient availability to TOR are well defined, the effectors via which TOR controls cellular metabolism to drive growth are less clear. Here we show that control of mRNA translation via transcription of rRNA and tRNA is a key determinant of TOR-dependent body growth, size and development via stimulation of tRNA and rRNA synthesis during larval development; TOR signaling stimulates tRNA synthesis by inhibiting Maf1, a pol III repressor, and stimulates tRNA synthesis via activation of TIF-IA, a pol I transcription factor. Both actions are required for cell and tissue growth. We also show that TOR-dependent control of rRNA and tRNA synthesis exerts non cell-autonomous effects on overall body growth and development. We find that levels of tRNA synthesis in the larval fat and rRNA synthesis in larval muscle control overall body growth and development. In both cases, these effects involve endocrine signaling to the brain to promote the expression and release of several Drosophila insulin-like peptides, thereby leading to enhanced systemic insulin signaling and increased body growth. Finally, we find that increases in tRNA and rRNA synthesis alone are sufficient to drive body growth. In the cases of tRNA, we show that simply increasing levels of just the initiator tRNA – tRNAiMet – can accelerate development and increase final body size.

Program Abstract #385
Examing cell cycle gene regulation in different states of G0
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States of cellular withdrawal from the cell cycle or G0 can range from readily reversible to permanently postmitotic. We are interested in how different states of G0 are controlled during development and why some are more reversible than others. We have focused our studies on a developmentally controlled G0 that occurs when cells in the Drosophila eye and wing terminally differentiate and become postmitotic. We find that this postmitotic state is actively maintained, even in the face of strongly mitogenic signals. Yet, this state appears distinct from senescence, a permanent G0 induced by aberrant oncogenic signaling. Interestingly, we find there are two kinds of postmitotic states in these Drosophila tissues, a flexible G0 state where cells can still be induced to re-enter the cell cycle under specific manipulations and one we call "robust" where cells become very strongly refractory to re-entering the cell cycle. We have begun studying how chromatin dynamics, histone modifications and regulation of cell cycle gene expression all influence the proper timing and flexibility of G0 in vivo. We will also present new projects aimed at studying how G0 flexibility may be controlled in states of regeneration and compensatory proliferation as well as certain types of cancer, where quiescent non-dividing cells evade chemotherapy, only to seed new tumors at a later date.

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Program Abstract #386
Nutrient/TOR kinase signaling controls body growth, size and development via stimulation of tRNA and rRNA synthesis and enhanced insulin signalling.
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Nutrient availability is a key determinant of animal growth and body size. The TOR kinase signaling pathway is one of the best-characterized regulators of cell and tissue growth in response to nutrients. Although the signaling networks that link nutrient availability to TOR are well defined, the effectors via which TOR controls cellular metabolism to drive growth are less clear. Here we show that control of mRNA translation via transcription of rRNA and tRNA is a key determinant of TOR-dependent body growth in Drosophila. We present three main findings. We first show that TOR signaling exerts tight regulation of rRNA and tRNA synthesis during larval development; TOR signaling stimulates tRNA synthesis by inhibiting Maf1, a pol III repressor, and stimulates tRNA synthesis via activation of TIF-IA, a pol I transcription factor. Both actions are required for cell and tissue growth. We also show that TOR-dependent control of rRNA and tRNA synthesis exerts non cell-autonomous effects on overall body growth and development. We find that levels of tRNA synthesis in the larval fat and rRNA synthesis in larval muscle control overall body growth and development. In both cases, these effects involve endocrine signaling to the brain to promote the expression and release of several Drosophila insulin-like peptides, thereby leading to enhanced systemic insulin signaling and increased body growth. Finally, we find that increases in tRNA and rRNA synthesis alone are sufficient to drive body growth. In the cases of tRNA, we show that simply increasing levels of just the initiator tRNA – tRNAiMet – can accelerate development and increase final body size. Together, our findings suggest a new model of growth control in which tRNA and rRNA synthesis are key metabolic outputs of the TOR pathway in the control of metabolism and body size. Given the universal function of tRNA and rRNA in protein synthesis, we suggest this mechanism may operate in all animals.

Program Abstract #387
Comparation of cell polarity formation between repair of porcine tubular necrosis and embryogenesis
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Objective: Cell polarity is a fundamental feature of renal tubular cells’ physiological functions. This study was aimed to compare cell polarity formation between repair process of adult porcine renal tubular necrosis and renal embryogenesis. Methods: Acute kidney injury was established with miniature pigs of 12 weeks old by injecting gentamycin [80 mg/(kg.d)] for 10 days. Specimens were collected on day 11, 17, and 24 after injecting gentamycin, and kidney specimens were also collected from fetal pigs of 42-day gestation. The cell polarity was examined with Na+/K+-ATPase staining, and PCNA was stained for counting proliferating cells. Results: (1) Tubular cell necrosis and detachment and its debris in lumen were observed on day 11 after gentamycin injection. No Na+/K+-ATPase or PCNA staining were observed on exposed tubular basement membrane at the same time. On day 17, PCNA-positive tubular cells appeared on basement membrane, and Na+/K+-ATPase was localized at tubular cell membrane and cytoplasm, but no obvious polarity. On day 24, kidney tubules restore more normal renal structure, and PCNA-positive tubular cells also
showed polar distribution of Na⁺/K⁺-ATPase on lateral-basal membrane. (2) Development of embryonic renal tubes included tubular precursors developed into early renal tubules, and then into mature tubules, with PCNA positive in both tubules. The polarized distribution of Na⁺/K⁺-ATPase on cell membrane and in cytoplasm changed to the lateral-basal membrane of tubular cells, demonstrating that Na⁺/K⁺-ATPase expression changes in the no-polar and polar distribution. Conclusion: Polarization of the cell during repair process of cranial tubular necrosis was similar to that of embryogenesis, indicating that at least the process of repair/regeneration of tubular necrosis may also be similar to that of embryonic renal tubules development.

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Program Abstract #388
Understanding Dlx-mediated effects on cell proliferation
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Dlx5 and Dlx6 are members of the Distal-less homeobox (Dlx) gene family that is conserved across evolutionarily diverse species and that, in vertebrates, encodes transcription factors essential for craniofacial, limb, and neural development. Dlx5 and Dlx6 are generally co-expressed and have redundant functions during craniofacial patterning and differentiation of mineralizing cell types in the skeleton. Forced expression of Dlx5 or Dlx6 in multipotent or progenitor cells promotes their differentiation and suppresses proliferation in vitro and in vivo. During the transition from proliferation to differentiation, Dlx5 and Dlx6 are known to activate lineage-specific genes in bone and cartilage progenitors, likely via direct interactions with cis-regulatory regions. Conversely, studies involving cancer cell lines have shown that elevated levels of Dlx5 can promote proliferation, perhaps via direct transcriptional activation of cell cycle regulatory genes like c-Myc. Whether a similar effect on cell cycle regulators accounts for the anti-proliferative effects of Dlx5 or Dlx6 during normal development is unknown. We have shown that Dlx5 and Dlx6 inhibit cell growth in multipotent progenitor cells and primary limb bud cells. Time-pulse EdU-incorporation assays indicate that Dlx5 and Dlx6 increase the length of the cell cycle and flow cytometry experiments indicate a higher proportion of Dlx-expressing cells are in G1, compared to controls. We have also investigated whether the proximal c-Myc promoter is responsive to Dlx5 and Dlx6 in non-tumourigenic cells capable of undergoing Dlx-mediated differentiation. Dlx5 and Dlx6 both modestly upregulate c-Myc reporter transcription in such cells. Interestingly, in HEK293T cells, where we observed no effect on cell growth we also observed Dlx-mediated repression of the proximal c-Myc promoter.

Program Abstract #389
Ciliary localization of Gli2 is critical for its activation by Hedgehog signaling
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Hedgehog (Hh) signaling, which is essential for development and physiology, requires the primary cilium in mammals. Gli2, the primary effector of Hh signaling, localizes to the cilium, but the importance of this ciliary localization in Hh signaling-induced Gli2 activation remains unclear. Our working model is that Hh signaling activates Gli2 by antagonizing the inhibitory function of Suppressor of Fused (Sufu) in the cilium, which requires the ciliary localization of Gli2. To test this model, we replaced endogenous Gli2 with Gli2CLDKI, a Gli2 variant not localizing to the cilium, in a Gli2CLDKI mouse allele. We observed greatly decreased Hh pathway activities and abnormal ventral spinal cord patterning in Gli2CLDKI single and Gli2CLDKI;Gli3 double mutants, suggesting that the non-ciliary Gli2CL variant was not properly activated in development. Subsequently, we show that neither a small molecule agonist of Smo in vitro, nor loss of Ptch1 in vivo, could activate Gli2CL, further implicating the ciliary localization of Gli2 in its Hh pathway-induced activation. Finally, if the lack of ciliary localization prevents Hh pathway induced de-repression of Gli2CL by Sufu, we predicted that removing Sufu should activate Gli2CL. Indeed, we found that the Hh pathway was highly activated in Gli2CLDKI,Sufu double mutants. In summary, our results provide strong evidence that Gli2 ciliary localization is critical for the activation of Hh signaling pathway. This work was supported by NSF grants IOS-0949877 and IOS-1257540.

Program Abstract #390
Effects of IGF1-RUNX2 pathway activation on traction force and focal adhesion distributions in single suture craniosynostosis
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Craniosynostosis – premature fusing of one or more calvarial sutures – restricts the natural growth and alters the development of infant calvaria, leading to increased intracranial pressure and abnormal calvarial shape. A genetically variable subgroup of children with single suture craniosynostosis has been identified, which demonstrates activation of the IGF1-RUNX2 pathway. This work identifies changes in the cellular phenotype of osteoblasts in this biologically relevant subgroup, to better understand causes of craniosynostosis. Here, we focus on the cellular biomechanics, including observed traction forces and focal adhesion distributions of cells demonstrating high levels of IGF1 compared to controls, i.e. non-diseased cells. The cellular traction forces are quantified by seeding cells on micropost arrays, which display a linear increase in deformation with increased traction force. The distribution of focal adhesion proteins, including vinculin, paxillin, talin, FAK and zyxin, and the β3 integrin are also compared between diseased...
and control cells. We observe an increase in traction forces and clustering of focal adhesion proteins in the diseased cells compared to controls, supporting the hypothesis of increased bone formation related to IGF1-RUNX2 pathway activation. This work was supported by the National Institutes of Health, Grant R01 DE018227.

Program Abstract #391
Dual antagonistic signaling during C. elegans vulval fate patterning promote developmental fidelity
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EGF induces six C. elegans vulval precursor cells (VPCs) to form a highly reproducible 3°-3°-2°-1°-2°-3° fate pattern; 1° and 2° cells enact specialized proliferative programs while 3° cells remain unspecialized. In a sequential induction process EGF Receptor activates Ras and the canonical Raf-MEK-ERK effector cascade to induce a single 1° cell, which subsequently expresses DSL ligands that laterally induce Notch-dependent 2° fate in the two neighboring VPCs. Multiple mechanisms mediate mutual antagonism between 1° and 2° fates. We previously published that Ras switches effectors during vulval patterning by, in presumptive 2° cells, engaging the noncanonical effector RalGEF. Ras thereby mediates an EGF 2°-promoting signal in support of the Notch 2°-promoting signal, and Ras-RalGEF-Ral mediates an EGF patterning gradient that overlays EGF -> Notch sequential induction. RalGEF activates the small GTPase Ral to promote 2° fate. Surprisingly, we found that RalGEF has an additional, Ras-independent function that antagonizes its Ral-dependent 2°-promoting function. Previous cell culture studies suggest that RalGEF scaffolds PDK and Akt in the PI3K signaling cascade. The PI3K-PDK-Akt cascade promotes 1° fate, and genetic epistasis analysis suggests that RalGEF also scaffolds PDK and Akt in vulval patterning. Thus, RalGEF mediates opposing and counterbalancing Ras and Akt outputs, such that RalGEF deletion does not alter the balance of 1° and 2° fates. Furthermore, preliminary data suggest that the putative Ral effector, a MAP4 kinase activating a p38 MAP kinase cascade, similarly mediates two opposing and counterbalancing outputs. RalGEF deletion increases the patterning error rate 15-fold, but does not confer sensitivity to environmental insults. We speculate that multiple levels of antagonistic “signaling duality,” e.g. by EGFR, Ras, RalGEF and MAP4K, collectively impose cell fate patterning fidelity. This work was supported by NIH grant GM085309.

Program Abstract #392
The COP9 signalosome restricts EGFR signaling through regulation of Capicua degradation in the developing fly
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Post-translational modifications can alter protein activity. Several signaling pathways rely on such alterations of one or several of their components for normal signal transduction. The Epidermal Growth Factor Receptor (EGFR) pathway, for instance, uses a kinase cascade to activate Mitogen-Activated Protein Kinase (MAPK) by phosphorylation. Phosphorylated MAPK can translocate into the nucleus and activate the transcription of target genes by phosphorylating transcription factors and altering their activity or stability. In a genetic screen in the Drosophila eye, we found two mutations in the csn1b gene that induce ectopic activation of EGFR signaling, as demonstrated by ectopic expression of argos (aos), an EGFR target gene. csn1b encodes a subunit of the COP9 signalosome (CSN), a complex that removes the Ubiquitin-like Nedd8 modification from substrates. The main substrates for the CSN are Cullsins (scaffold subunits of E3 ubiquitin ligases), which are active in their neddylated state, but also prone to auto-ubiquitination and degradation. The role of the CSN in EGFR signaling is unknown. Other components of the complex, CSN4, CSN5, CSN6, CSN7 and CSN8 also negatively regulate EGFR signaling, as mutations or RNAi targeting these genes results in increased aos expression. This effect is dependent on MAPK. Capicua (Cic) is a transcription factor that negatively regulates EGFR signaling: its direct phosphorylation by activated MAPK prevents it from binding to the promoter of EGFR target genes such as aos by promoting either its degradation or its re-localization to the cytoplasm. We have found that Cic protein levels are reduced in csn1b, csn4 and csn5 mutant cells. Cullin1, on the other hand, promotes Cic degradation in the wing imaginal disc. We are testing whether neddylation of Cullin1 by the CSN promotes Cic activity andubiquitination to inhibit EGFR signaling. Project funded by NIH grant EY13777

Program Abstract #393
tRNA modification and TOR kinase: something new to consider
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Transfer RNAs (tRNAs) are the most highly modified class of RNA species, and these modifications are found in every organism examined. Threonylcarbamoyl-adenosine (t6A) is a universal modification occurring at position 37 in tRNAs that pair A-starting codons. In yeast it is synthetized by Sua5 and Kae1 and it is required for proper codon recognition and translation accuracy, thus mutants for sua5 or kae1 show a slow-growth phenotype. We have studied Drosophila mutants for the kae1 ortholog; these animals present an impaired growth phenotype, very similar to mutants for components of the insulin/TOR kinase pathway. Considering this, we studied the activation of this signaling pathway in these mutants. We observed no changes in insulin secretion, measured by immunofluorescence against dILP2 in insulin-producing cells. Additionally, we established that cell growth was impaired cell-
autonomously and detecting S6K phosphorylation we also determined that TORC1 activity was decreased in kae1 mutants. Interestingly, TORC2 activity, evidenced by Akt (Ser505) phosphorylation, was also diminished. Our results reveal a previously unknown aspect of TOR kinase regulation, which is related with tRNA modification and perhaps with the accuracy of translation machinery itself. In addition, our results suggest an interplay between TOR complexes in the control of cell growth.

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Program Abstract #394

Src42A Modulates Tumor Invasion and Cell Death via Ben/dUev1a-JNK Signaling in Drosophila

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Loss of the cell polarity gene could cooperate with oncogenic Ras to drive tumor growth and invasion, which critically depends on the JNK signaling pathway in Drosophila. By performing a genetic screen, we have identified Src42A, the ortholog of mammalian Src, as a key modulator of both RasV12/lgl-/- triggered tumor metastasis and loss of cell polarity gene induced cell invasion. Our genetic evidence further established Bendless (Ben)/dUev1a E2 complex as an essential regulator downstream of Src42A. Reducing Ben/dUev1a activity suppressed Src42A overexpression induced cell invasion and cell death, while ectopic expression of Ben/dUev1a restored src42A loss thorax cleft defect. Furthermore, we showed Ben/dUev1a expression cells could migrate invasively in wing disc epithelia along with increased MMP1 secretion. Moreover, when RasV12 is co-expressed, Ben/dUev1a expressing cells promoted tumor overgrowth and caused invasion into the ventral nerve cord. Our data provides new mechanistic insights into in vivo role of Src42A in regulating tumor progression and highlights the importance of Src42A- Ben/dUev1a- JNK signaling in regulating tumor invasion and cell death in Drosophila.

Program Abstract #395

Identification of motifs that tune the rate of Aux/IAA degradation

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Regulated turnover of diverse proteins by E3 ubiquitin ligases is critical for cell signaling, yet we know remarkably little about what controls degradation rates. These gaps in knowledge impede full understanding of both normal cellular function and disease states. One of the best characterized E3s acts in plants. The receptor for the plant hormone auxin acts within an SCF-type E3 to degrade Aux/IAA repressor proteins. The auxin pathway has unique advantages for studying E3 function, including: auxin receptors (TIR1/AFBs) are themselves F-box proteins; substrate (Aux/IAA) degradation is small-molecule-triggered rather than requiring substrate phosphorylation; and evolution has provided many sequence variants with distinct properties for both F-boxes and substrates. Recent studies indicate that degradation rates within the Aux/IAA family vary widely and that sequences outside of the known degron (the minimum region required for auxin-induced degradation) accelerate or decelerate degradation in a substrate-specific manner. We have used synthetic auxin degradation systems in yeast and in plants to identify and characterize two motifs outside the Aux/IAA degron that were required to maintain wild-type auxin-induced Aux/IAA degradation rates. These sequences, which we named “rate motifs”, are conserved in phylogenetically-distant members of the Arabidopsis thaliana Aux/IAA family, as well as in putative Brassica rapa Aux/IAA orthologs. Some rate motifs appear to exert their influence on Aux/IAA degradation rate by altering the strength of interaction between an Aux/IAA and the TIR1 auxin receptor. Other rate motifs do not influence interaction strength, suggesting instead that they act on another control point during substrate turnover.

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Program Abstract #396

Recapitulation of the auxin response pathway in yeast

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Auxin influences nearly every aspect of plant growth and development through a relatively simple pathway that pivots on the relief of transcriptional repression. Auxin triggers degradation of Aux/IAA repressors, thereby activating ARF transcription factors and triggering a global change in gene expression. The large size of the ARF and Aux/IAA gene families suggest that local differences in Aux/IAA-ARF composition could contribute to distinct signaling dynamics. However, characterization of individual Aux/IAA-ARF auxin response modules has been confounded by the ubiquity of auxin in plants, feedback, interactions with other auxin response components and interference from other signaling pathways. Here, we recapitulated the entire Arabidopsis thaliana forward auxin signal transduction pathway in Saccharomyces cerevisiae to assess if the composition of different modules was sufficient to tune the dynamic response. Sensitivity analysis guided by a small mathematical model revealed the centrality of Aux/IAA co-repressors in driving response dynamics. In addition, we found that when multiple Aux/IAAs were co-expressed, one
Gtpbp2 destabilizes Axin and is required for embryonic Wnt signalling

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The canonical Wnt pathway is a core metazoan signaling pathway responsible for directing axial polarity and differentiation. Wnt signals transduced by stabilized β-catenin inform cells whether to proliferate, differentiate, or remain quiescent. Mutations in Wnt signaling components are common in many cancers and other diseases, however Wnt-pathway directed therapeutics remain limited. Here we identify a GTP-binding protein 2 (Gtpbp2), a largely unstudied GTPase distantly related to the elongation factor eEF1a, as essential for normal response to Wnt signaling in Xenopus embryos. We demonstrate that Gtpbp2 is required for axis formation and induction of organizer gene expression. Gtpbp2 interacts with members of the β-catenin destruction complex, including Gsk3b and Axin, as well as inactivators of the destruction complex, such as Disheveled. Epistatic analyses suggest Gtpbp2 is involved in Gsk3b complex inactivation, being required for transduction of signals from Dishevelled and a kinase-dead mutant of Gsk3b, but not for a phospho-stable form of β-catenin. Overexpression of Axin, the scaffolding and rate-limiting component for Gsk3b-mediated destruction of β-catenin, has similar effects. Our loss of function experiments demonstrate Gtpbp2 is a negative regulator of Axin protein levels, suggesting a model in which Gtpbp2 regulation of Wnt signaling and axial polarity occurs by maintaining low levels of Axin. This study establishes Gtpbp2 as essential for canonical Wnt signaling and vertebrate embryonic development, and identifies Gtpbp2 as a potential drug target for the treatment of diseases driven by aberrant Wnt signaling.
Program Abstract #400
Investigation of Wnt Secretion
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Wnt glycolipoprotein secretion and activity require post-translational lipidation mediated by Porcupine and the function of Wntless/Gpr177/Evi in association with the retromer complex that involve retrograde transport from the plasma membrane to the Golgi and ER. At least some Wnt appears to be associated with exosomes released from expressing cells. Porcupine and Wntless each encode multiple splice variants expressed in specific tissues, and not all Wnts can be secreted by all cells. We are characterizing and manipulating the expression of these Wnt-specific secretory components in various eukaryotic cells while monitoring the Wnt-secreting properties of those cells. In particular, we are interested in understanding whether specific Porcupine and/or Wntless splice variants are required for the efficient secretion of different Wnt proteins and the association of those Wnts with exosome or free extracellular pools.

Program Abstract #401
Reelin regulates Schwann cell migration by activating the Rho GTPase Rac1
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ApoER2 is an endocytic and signaling receptor, member of the low-density lipoprotein family. ApoER2 and its ligand, Reelin, play a relevant role in the central nervous system. Reelin guides migration of neurons during development. Reelin binds to ApoER2 inducing the recruitment of Dab1, which is phosphorylated by the SRC-family kinase Fyn. Subsequently, ApoER2/Reelin activate PI3K, Akt and GSK3. In consequence, cell migration related proteins are activated, such as LimK1 and small GTPases, Rap1 and Cdc42. The present study was designed to investigate the role of ApoER2/Reelin during cell migration of Schwann cells as this process is critical for development and regeneration of peripheral nervous system. Previously, we have observed over-expression of ApoER2 in sciatic nerve after injury. We used primary culture schwann cells or spontaneously immortalized Schwann cell lines (IFRS1) treated or not with Reelin. We demonstrated that Schwann cells express the full length sequence, the proline-rich and O-glycosylation splicing variants of ApoER2. In addition, these cells express the GTPases Rac1 and Cdc42 and the polarity protein Par3. ApoER2 signaling in response to Reelin is active in Schwann cells. Additionally, in a wound healing assay Reelin stimulates cell migration. In order to explore the possible molecular mechanism involved we performed pull-down of active Rac1 from lysates of cells stimulated with Reelin. We observed that Reelin activates Rac1 in a time dependent manner. Finally, immunoprecipitations experiments show that ApoER2 associate with Par3 and Par3 directly with Tiam, a specific GEF for Rac1. Taken together, our experiments suggest that Reelin could regulate cell migration activating Rac. We hypothesize that the interactions between ApoER2/Par3/Tiam have a function in the Rac1 activation. Ongoing experiments are being carried out to study this signaling pathway during cell migration of Shwann cells. Supported by FONDECYT Post-doctoral #3130373

Program Abstract #402
Mutation of the Kif5Aa tail domain leads to mitochondrial deficits and axonal degeneration of peripheral sensory neurons in larval zebrafish
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Defective axonal transport has been linked to neurodegenerative and neurodevelopmental disorders. Mutations disrupting specific Kinesin motors (Kifs) involved in axonal transport lead to central and peripheral nervous system defects. Human mutations in Kif5A, a Kinesin-1 heavy chain family member, cause peripheral motor and/or sensory dysfunction classified as either Hereditary Spastic Paraplegia or Charcot Marie Tooth Type II depending on which modality predominates. Global Kif5A mouse knockouts are perinatal lethal; thus, the relevant cargo and basis of sensitivity of subsets neurons to Kif5A disruption remain unclear. The zebrafish genome has two kif5A genes, kif5Aa and kif5Ab. Here we show that homozygous loss of the C-terminal tail of Kif5Aa, but not Kif5Ab, causes striking sensory neuron defects including degeneration of posterior lateral line nerve and cutaneous nerve axons. Electron microscopy and transgenic approaches show a drastic reduction in axonal mitochondria, while presynaptic vesicles and lysosomes remain present. Mitochondria deficits are rescued cell autonomously by overexpression (OE) of wild-type Kif5Aa but not OE of other Kinesin-1 family members thought to be functionally redundant with kif5Aa or other motors implicated in mitochondrial transport, suggesting Kif5Aa is specifically dedicated to mitochondrial transport in these cells. Compound loss of kif5Aa and cfd enhances sensory neuron defects via a mechanism that is independent of mitochondria transport. The neurologic syndrome observed in kif5Aa mutants resembles the human disorder, including motor spasms, uncoordinated movements, increased seizure susceptibility, peripheral neuropathy, and partial blindness. Our results show that specific motors fulfill transport in discrete neuronal subsets, implicate mitochondrial transport as a key factor in human disease and development, and underscore the importance of evaluating functional redundancy between Kinesin motors in vivo.

Program Abstract #403
Studying the mechanisms of mesenchymal to epithelial transitions in lateral line primordium
Uma Neelathi
The Posterior Lateral Line Primodium primodium (PLLp) is a group of approximately 100 cells, originating from the ectodermal placode, which migrates along the horizontal myoseptum depositing neuromasts at regular intervals. These neuromasts mature and form the lateral line sensory system. There is a progressive change in the organization of cells in the PLLp; in the leading domain cells are mesenchymal in nature with a flattened morphology, while cells in the trailing domain acquire a columnar morphology and form center oriented epithelial rosettes. Thus the PLLp serves as an excellent in vivo model for studying the mesenchymal to epithelial transitions. Cdh-1 and Cdh-2 are present all over the primodium except a few cells in the leading domain and recent studies have hinted that these cadherins might not have much role in the morphological transitions going on in the PLLp. However, certain transcription factors (TF) specifically expressed in mesenchymal cells, might facilitate migratory behavior and have to be down regulated as cells become more epithelial and are eventually deposited from the trailing end. These include Zeb, Sip, Snail, Slug, Scratch and twist families. Currently we are investigating their potential role in facilitating these morphological transitions within the PLLp.

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Program Abstract #404
Role of Lhx9 in the development of amacrine cell subtype and its dendritic stratification
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Amacrine cells are the most diverse group of retinal neurons and perform a wide variety of visual functions. Understanding the molecular basis of genesis of individual amacrine cell subtypes helps elucidate their visual functions. We have identified a LIM-homeodomain transcription factor – LHX9 that is expressed in developing and mature amacrine cells. We have developed a lineage tracing strategy by constructing an Lhx9-GFP-CreER (Lhx9(CRE)) mouse line and crossing to a reporter line, to map the fate of Lhx9 expressing cells. LHX9 expressing cells are mostly GABAergic and our lineage tracing studies suggest a role for Lhx9 in specifying individual identity of amacrine cell subtype from progenitor cells. We also find that in retinas lacking Lhx9, amacrine cells strongly expressing nitric oxide synthase are abolished and the laminar stratification at the inner plexiform layer is significantly altered. Overall, our studies indicate a significant role of Lhx9 in specifying amacrine cell subtype identity and dendritic stratification.

Program Abstract #405
Notch signaling differentially regulates Atoh7 and Neurog2 in the distal mouse retina
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Notch signaling regulates basic helix-loop-helix (bHLH) factors as an evolutionarily conserved developmental module, but tissue-specific mechanisms remain incompletely elucidated. In the embryonic mouse retina, bHLH genes Atoh7 and Neurog2 have distinct functions, with Atoh7 regulating retinal competence and Neurog2 required for progression of neurogenesis. These transcription factors are extensively coexpressed, suggesting similar regulation. Here, we directly compare Atoh7 and Neurog2 regulation at the earliest stages of retinal neurogenesis in a broad spectrum of Notch pathway mutants. Notch1 and Rbpj normally block Atoh7 and Neurog2 expression. However, the combined activities of Notch1, Notch3 and Rbpj regulate Neurog2 patterning in the distal retina. Downstream of the Notch complex, we found that the Hes1 repressor mediates Atoh7 suppression, but Hes1, Hes3 and Hes5 do not regulate Neurog2 expression. We also tested Notch regulation of Jag1 and Pax6 in the distal retina, to establish the appropriate context for Neurog2 patterning. We found that Notch1;Notch3, and Rbpj block coexpression of Jag1 and Neurog2, while specifically stimulating Pax6 within an adjacent domain. Our data suggest that Notch signaling controls the overall tempo of retinogenesis, by integrating cell fate specification, the wave of neurogenesis, and the developmental status of cells ahead of this wave.

Program Abstract #406
Transcription Factor Profiling: A Tool for Analyzing Ventral Interneuron Specification in the Zebrafish Spinal Cord
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Ventral spinal interneurons are essential for locomotion in vertebrates. 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 TF in the zebrafish genome. Our microarray data are revealing, for the first time, the complete TF expression profiles for the different ventral interneurons examined. Furthermore, since different populations share critical functional characteristics, such as a specific axon trajectory or neurotransmitter phenotype, we are identifying TFs that are candidates for specifying that trait by comparing the TF expression
profiles of populations with opposing phenotypes. Our expression data is validated using in situ hybridization on whole-mount zebrafish embryos. We are now establishing a functional validation pipeline using morpholinos and 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.

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Program Abstract #407
**prdm12b specifies the p1 progenitor domain of the ventral neural tube and reveals a role for V1 interneurons in swim movements**
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The dorsoventral (DV) axis of the neural tube is divided into 11 domains, each expressing a unique transcription factor code and giving rise to a unique cell type. For example, V1 interneurons (IN) arise from the p1 domain while motor neurons and oligodendrocytes share origins in the pMN domain. Perturbation in DV patterning leads to miss-specification, or loss, of particular cell types. We discovered a relatively uncharacterized gene, *prdm12b*, with expression limited to the p1 domain. Prdm12b is a putative transcription factor belonging to a class of genes characterized by a Pr containing domain and multiple zinc fingers. To determine the role of Prdm12b in DV patterning, we used antisense morpholino oligonucleotides (MO) designed to block the translation of Prdm12b. We find that loss of *prdm12b* function leads to loss of V1 INs, identified by *eng1b* expression. Loss of V1 INs also leads to deficits in touch evoked escape responses, as these INs play inhibitory roles in locomotion circuits. Specifically, control larval embryos touched on the head arch their bodies into a single c-bend, bringing the head adjacent to the tail, thus orienting the head away from the stimulus, and then swim away in a stereotypical manner. MO-injected embryos exhibit more c-bends, longer durations of response, and swim shorter distances than control MO-injected embryos. Additionally, the left-right alternation of body bends appears delayed. Loss of *prdm12b* function also leads to misexpression of the DV markers *nkx6.1* and *pax3*, expressed respectively ventrally and dorsally to *prdm12b*, as well as to a reduction of *olig2* expression in the pMN domain of the hindbrain. The hindbrain reduction of *olig2* leads to an early reduction in oligodendrocyte lineage cells. Taken together, *prdm12b* regulates DV patterning, is required for V1 IN specification, and plays an important role in escape responses. This work was supported by NIH grant HD065081 to CGS.

Program Abstract #408
**Sequential posteriorization by FGF signaling in the Ciona anterior neural plate**
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In early cleavage stages of ascidian embryogenesis, anterior neural tissue is induced in two pairs of animal (a-line) blastomeres by FGF signaling from the vegetal A-line blastomeres. During successive rounds of A-P oriented cell division, the posterior a-line daughter cells remain in contact with this vegetal source of FGF. Previous work in our lab has shown that FGF signaling at the 76-cell stage is required to establish the anterior neural boundary in the nascent anterior neural plate of *Ciona intestinalis*. Here, we investigate the role of FGF signaling in the next stage of neural plate patterning. We show that FGF signaling at the 110-cell stage is required for proper patterning of the mid-gastrula neural plate. At this stage, the neural plate is arranged in six rows of six cells each, with FGF emanating from the second most caudal row (Row 2). Inhibition of FGF signaling at the onset of the 6-row stage results in normal anterior neural boundary formation, but an expansion of Row 4 (anterior-most brain) gene markers at the expense of Row 3 (e.g., loss of *Mitf*+ pigmented sensory cells). These results are consistent with a model whereby FGF signaling mediates repression of anterior genes at sequential steps of neural patterning. We have begun to investigate the transcriptional effectors of this FGF-dependent fate choice, and our results suggest distinct roles for two different ETS family members.

Program Abstract #409
**Sox21 regulates the progression of neuronal differentiation in a dose-dependent manner**
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Neurogenesis, the progression from neural progenitor to committed neuron, is a tightly regulated process that is fundamental for development of the central nervous system. Members of the SoxB transcription factor family play critical roles in this process. Whereas the SoxB1 transcriptional activators are required for induction and maintenance of a proliferating neural progenitor population, the closely related SoxB2 proteins function as repressors and are proposed to inhibit SoxB1 targets to control the progression of neuronal differentiation. To determine the mechanism of action of the SoxB2 proteins, we characterized the function of SoxB2 protein Sox21 during primary neurogenesis in the African clawed frog *Xenopus laevis*. Our gain of function assays showed that Sox21 expands the neural progenitor domain and prohibits neuronal differentiation by interacting with proneural protein Neurogenin to inhibit its induction of neuron formation. Our loss of function assays demonstrated that Sox21 is required for neural progenitor induction by noggin and is consequently required for neuron formation in embryos and in ectodermal explants.

Together our gain and loss of function data suggest that Sox21 plays more than one role in neurogenesis, and like other Sox proteins
the level of Sox21 expression determines how it functions. Here, a high level of Sox21 expands progenitors and represses differentiation, but a threshold level is required for proper neuron formation. Since Sox protein target specificity and function are dependent on partner protein interactions, we propose that the role of Sox21 is dependent on interactions with partner proteins based on its level of expression.

This work was supported in part by NIH grants NS048918 and NS078741.

Program Abstract #410
Characterizing the roles of Sox2 and Sox3 in sensory/neural patterning during zebrafish inner ear development
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Vertebrate Sox2 and Sox3 are closely related members of the SoxB1 transcription factor family involved in early neural development and stem cell maintenance. Previous studies suggest that functional diversity of SoxB1 factors depends on their level of expression and availability of other cofactors. We are exploring these parameters in the context of inner ear development. Zebrafish sox2 and sox3 are expressed in partially overlapping domains in the otic placode and in the floor of the otic vesicle. We hypothesize that Sox2 and Sox3 help promote sensory vs. neurogenic development in abutting spatial domains within the otic vesicle. Expression of sox2 normally marks developing sensory epithelia in a medial domain, and knockdown of sox2 reduces production and maintenance of hair cells. In contrast, although expression of sox3 marks both sensory and neural precursors, knocking down sox3 reduces neurogenesis but does not affect hair cell production. High-level misexpression of either Sox2 or Sox3 greatly expands both sensory and neurogenic domains. The medial factor Pax2a is specifically required for Sox2/3 to expand the sensory epithelia, but not the neurogenic domain, identifying at least one cofactor that influences SoxB1 function. Additionally, the concentration of Sox2 (but not Sox3) critically affects function: moderate misexpression of Sox2 inhibits neurogenesis while promoting sensory development, revealing a fundamental difference between Sox2 and Sox3. Thus both the level of expression and availability of regionally expressed cofactors diversify the functions of Sox2 and Sox3 and helps coordinate sensory and neural patterning in the inner ear. Supported by NIDCD grant R01-DC03806.

Program Abstract #411
Candidate co-factors for vertebrate Six family transcription factors are required for otic development
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Several members of the Six family of transcription factors play important roles in vertebrate craniofacial development. It is well documented that Six protein transcriptional activity can be modified by co-factor proteins. Two Six genes (Six1, Six5) and one cofactor gene (Eya1) are involved in the human craniofacial syndromes BOS (Branchio-Otic Syndrome)andBOR(Branchio-Otic-Renal Syndrome). However, mutations in Six and Eya genes only account for about half of the BOS/BOR patients. To discover potential new causative genes, we identified over 30 Xenopus genes with high sequence identity to proteins previously shown to interact with the Drosophila Six homologue, SO, and we determined that many of these are expressed at some stage of craniofacial development (Neilson et al., 2010, Dev Dyn 239: 3446). We now show that 4 of the novel candidate co-factors interact with vertebrate Six1 protein. When their endogenous expression is reduced in the embryo, there is a loss of otic placode genes and an expansion of neural plate genes. When their endogenous expression in increased in the embryo, Six1 expression is reduced. These results suggest that these Xenopus proteins, which have homologues in humans, are previously uncharacterized Six-interacting partners with potential important roles in vertebrate craniofacial development and congenital syndromes.

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Program Abstract #412
Restriction of Cartilage Condensations by Jagged1 Differentiates the Upper from the Lower Facial Skeleton
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The diversity of vertebrate body shapes is determined by the precise positioning and shaping of hundreds of cartilages and bones. Such patterning relies in part on the selection of cartilage, bone, and other fates from embryonic fields of mesenchymal cells. By studying how the facial skeletal develops in zebrafish, we have uncovered a critical patterning role for Jagged-Notch signaling in the local restriction of cartilage fates and expansion of dermal bone fates. The lower face, which is derived from ventral mesenchymal cells of neural crest origin, consists of a large cartilage and a small dermal bone. In contrast, the upper/dorsal face is composed of a stubby cartilage and a large dermal bone, which are prefigured by a limited barx1+ precartilaginous condensation and a large field of runx2b+ dermal bone progenitors. In the dorsal arches of either jag1b or notch2; notch3 mutants, we find that barx1+ condensations are expanded and runx2b+ progenitors are reduced, with the dorsal cartilage of jag1b mutants partially rescued by loss of barx1. In the lower/ventral face, we find that Endothelin1 (Edn1) signaling prevents Jag1-mediated restriction of cartilage condensations. edn1 mutants lose ventral expression of the barx1 condensation marker and upregulate both jag1b and the earlier prrx1a/b progenitor markers, with loss of either Jag1 or Prrx1a/b rescuing cartilage formation in the lower face. Interestingly, we find that the stapes and incus middle ear bones of mice and humans, which similarly derive from the upper/dorsal face, also depend on Jagged1 and Notch2 function within neural-crest-derived mesenchymal cells. In contrast, the jawless lamprey, which has a largely symmetrical upper and lower face, lacks dorsal-arch-specific Jagged1 expression. We therefore propose that Jagged-Notch
signaling is an evolutionary add-on that modifies the upper face of jawed vertebrates by promoting dermal bone at the expense of cartilage.

Program Abstract #413
Identify target genes regulated by Osr2 transcription factor during palate development
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Osr2 is a transcriptional factor required for normal palate development. To identify the target genes and molecular networks regulated by Osr2 in palate development, we carried out RNA-seq analysis to get the gene expression profiles in the palatal regions of Osr2 mutant embryos and compared them with their littermate controls. We also manually dissected developing palate shelves and conducted ChIP-seq to identify the genome-wide binding sites for Osr2. Preliminary analysis of the RNA-seq data identified about 500 genes differentially expressed in the Osr2 mutant vs. control palate, such as Igf2, Dlx5, FoxF1, FoxF2 and Bmp2. Meanwhile, ChIP-seq data identified about 3000 peaks with p-value less than 10^-6, among which we found Fox, Tcfap, Runx motifs in addition to the Osr motif. More importantly, by integrating RNA-seq and ChIP-seq data, we identified about 200 potential target genes of Osr2, out of which there are 33 genes are associated with abnormal craniofacial morphology, such as Satb2, Dlx5, Shox2, Meox2, Cited2 and FoxF2. These data suggest that Osr2 plays important roles in palate development by directly binding and regulating the expression of many important palate development genes. Further experiments such as in situ hybridization, luciferase reporter and enhancer activity assays are undergoing. Our results would shed light on the transcriptional regulatory network involving Osr2 in palate development. This work is supported by National Institutes of Health (NIH)/National Institute of Dental and Craniofacial Research (NIDCR) grants DE013681 and DE015207 to R.J.

Program Abstract #414
Retinoic acid regulates size, pattern and alignment of neural and mesodermal tissues at the head-trunk transition
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At the head-trunk transition, alignment of hindbrain and spinal cord territories to occipital and cervical structures is critical for coherent organization of neural and skeletal systems. Changes in neural or mesodermal tissue configuration arising from defects in territory size specification, patterning or relative axial placement can severely compromise system integration and function. Here we show that neural and mesodermal tissue coordination at the zebrafish head-trunk transition critically depends on two novel activities of the signaling factor Retinoic Acid (RA) specifying the size and axial position of the hindbrain territory relative to mesodermal structures. These activities are each independent but coordinated with RA’s well-established function in hindbrain patterning. Using neural and mesodermal landmarks we demonstrate that RA function in aligning neural and mesodermal tissues temporally precedes the specification of hindbrain and spinal cord territories and the activation of hox transcription. Using cell transplantation assays we show that RA activity in the neuroepithelium is direct for hindbrain patterning but indirect for hindbrain territory size specification. This indirect function is independent of FGF and dependent on Wnts. Importantly, RA specifies and patterns the hindbrain territory by antagonizing the activity of the spinal cord specification gene cdx4; loss of Cdx4 rescues the defects associated with the loss of RA, including the reduction in hindbrain size and the loss of posterior rhombomeres. We propose that at the head-trunk transition, RA coordinates the specification, alignment and patterning of neural and mesodermal tissues essential for neural and skeletal system’s functional organization.

Program Abstract #415
Tbx6, Ripply1, and Mesp-b in dermomyotome development
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The dermomyotome is a transient embryonic tissue that generates the embryonic myotome and the myogenic stem cells responsible for muscle growth and repair throughout life. The development of the dermomyotome and the morphogenesis of the myotome take place within the somites, epithelial segments of the paraxial mesoderm. We have recently shown that in zebrafish the T-box transcription factor tbx6 is required for the formation of the Pax7-expressing central dermomyotome. We have now begun to characterize the molecular mechanism of Tbx6 action with respect to dermomyotome development. In mouse and zebrafish somite formation, Tbx6 regulates, and/or is regulated by, the bHLH transcription factor mesp-b and the transcriptional co-repressor ripply1. We have analyzed the interactions between these genes and their respective proteins in dermomyotome development, upstream of the expression of pax3/7. Using a combination of gain and loss of function approaches we show that Mesp-b is required for the formation of central dermomyotome cells, and suggest that Mesp-b functions by temporarily inhibiting the differentiation of primary fast myotome and by promoting the specification of dermomyotome fate in the pre-somitic mesoderm. We further show that Ripply1 negatively regulates Tbx6 protein levels, which restricts the expression of mesp-b and other Tbx6 targets to the pre-somitic
mesoderm. This negative feedback loop is required in order to initiate the timed maturation of dermomyotome and primary myotome. Based on our findings, we propose a gene regulatory model that provides an explanation for the spatial and temporal link between the segmentation of the paraxial mesoderm into somites and the specification of dermomyotome and myotome progenitors within each of those somites. Our data provide the first insights into the genetic basis for the specification of the dermomyotome.

**Program Abstract #416**

**Characterization of the TBX5 interactome provides mechanistic insight into Holt-Oram Syndrome**

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A series of clinical studies has provided direct evidence of a role for members of the T-box transcription factor family in heart development and human disease. Specifically, mutations in Tbx5 have been associated with Holt-Oram Syndrome (HOS), an autosomal dominant disorder characterized by a wide array of cardiac abnormalities, including ventral and atrial septal defects, and conduction abnormalities. Furthermore, a combination of TBX5 and other cardiac factors have been shown to convert mouse mesoderm and fibroblasts into functioning cardiomyocytes, demonstrating the need for understanding TBX5 protein interactions. Despite the crucial role for TBX5 in development and disease, little is known about the mechanisms by which TBX5 functions. To this end, we have introduced a tagged version of Tbx5 into the mouse by homologous recombination and taken a directed proteomics approach to determine the composition of the cardiac TBX5 transcription complex. This has led to the identification of a panel of proteins that function in complex with TBX5 in the adult heart. From these proteins, we have isolated a complete Nucleosome Remodeling and Deacetylase (NuRD) complex. We have verified the interaction of TBX5 with the central catalytic protein Mi-2B though reciprocal co-immunoprecipitations. We also show that the interaction between TBX5 and the NuRD complex is required for atrio-ventricular septation. This validates both the TBX5-NuRD interaction as well as its role in Holt-Oram Syndrome. We have mapped the interaction domains of TBX5 and Mi-2B and show that HOS mutations S261C, V263M, and K266R located within the Mi-2B interaction domain leads to the ablation of the TBX5-NuRD interaction, providing an additional connection between this protein-protein interaction and human disease. We are currently in the process of determining the molecular mechanism of the TBX5-NuRD complex in the mouse heart.

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**Program Abstract #417**

**Musculoskeletal patterning by Hox: analysis of Hox function in stromal fibroblasts**

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The mammalian Hox genes have long been appreciated to have a role in patterning the axial and appendicular skeleton. In the limb, Hox9-13 genes function regionally along the proximal-distal axis and loss-of-function paralogous mutations result in regional malformations of the skeletal elements. Development of the musculoskeletal system is a complex process whereby muscle, tendon, and bone tissues are coordinately patterned and precisely connected to allow for physiologically relevant movement. Significant knowledge exists on the differentiation of individual tissues, but less is understood regarding how they are integrated into a functional unit. A growing body of evidence supports a role for connective tissue stromal cells in patterning the musculoskeletal system. Using a Hox11eGFP knock-in allele, we recently published that Hox11 genes are expressed in the mesenchymal stroma and not in differentiated cartilage, bone, or muscle. This leads to the hypothesis that Hox genes function in the connective tissue to mediate patterning of the musculoskeletal system. To investigate the nature of Hox gene function, we have established cell lines of Hox11eGFP+ control and mutant limb fibroblasts from embryonic day 12.5 forelimb buds. These cell lines provide a system to assess differences in cellular behavior between control and mutant cell populations. During exponential growth, there is no difference in the proliferation profile of control and mutant fibroblasts. However, as monolayer cultures reach confluence, Hox11 mutant fibroblasts demonstrate an apparent defect in contact inhibition of proliferation. Micromass analysis from demonstrates that Hox11GFP+ cells do not localize to collagen-producing nodules normally in Hox11 mutant cultures. Finally, cellular migration does not appear to be affected by loss of Hox function. The mechanisms of cellular behavioral differences are under active investigation.

Funding: NIH-RO1 AR061402

**Program Abstract #418**

**The Iroquois transcription factors Irx7 and Irx5a promote the zebrafish hyoid joint by arresting chondrocytes at an early state of maturation**

Amjad Askary, Xinjun He, Lindsey Mork, Andrew McMahon, Gage Crump

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Joints provide essential mobility to the vertebrate skeleton, and arthritic degeneration of joints is the leading cause of disability in humans. An early event in joint development is the creation of an “interzone” between two prospective skeletal elements. Interzone cells are maintained as fibroblasts and/or articular chondrocytes while flanking cells mature into hypertrophic chondrocytes. Whereas a number of studies highlight the importance of BMPs and Gdf5/6 in balancing hypertrophic and articular cartilage,
respectively, much less is known about their downstream targets during joint development. Here we show that two Iroquois transcription factors, Irx7 and Irx5a, are negative targets of BMPs that arrest the further differentiation of early chondrocytes in the hyoid joint of zebrafish. Iroquois transcription factors are expressed in joints of amniote limbs, and a large deletion encompassing Irx3, Irx5, and Irx6 is associated with digit fusions in mice. Interestingly, an additional Irx gene – irx7 – exists only in fishes, where it is expressed exclusively within the hyoid joint. We find that, like the jaw joint marker nkx3.2, both irx7 and irx5a become restricted to the hyoid joint by the combined actions of Endothelin1 signaling and Hand2 and Hox transcription factors. We also generated irx7 and irx5a mutants, using TALEN technology, and find that these display specific losses of the hyoid joint and adjacent symplectic cartilage. Conversely, mosaic Irx7 misexpression inhibits chondrocyte differentiation in a cell-autonomous manner. Whereas wild-type joint cells express SoxE transcription factors but not the later chondrocyte marker col2a1a, irx7 mutant joint cells upregulate col2a1a. We showed that Irx7 and Irx5a arrest chondrocyte differentiation by directly binding to col2a1a enhancer and repressing its expression. This suggests that Irx genes promote joints by specifying a low level of col2a1a in early-chondrocyte-like interzone cells.

Program Abstract #419
Modulation of FGF signaling influences anterior-posterior patterning during zeugopod development
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Fibroblast growth factor (FGF) signalling appears essential for the regulation of limb development, but a full complexity of this regulation remains unclear. Here, we used new generation of FGFR inhibitors to analyze patterning of the chicken limb. Microinjection of the inhibitor PD173074 into the wing bud resulted in shorter and thinner wing when using lower concentration. Skeletal analysis revealed a partial reduction or full absence of the radius but not ulna. The phenotype of hindlimbs resembles the wing phenotype with a more severe effect on the anterior skeleton (tibia) than posterior bone (fibula). To analyze possible differences at the cellular level, we cut the wing buds into anterior and posterior parts at the time of collection and established micromass cultures from both areas separately. The basal level of chondrogenesis was different in the micromass cultures prepared from the anterior and posterior parts of limb buds with less of proteoglycan production by the anterior cells. Treatment with PD173074 demonstrated the inhibition of chondrogenesis in comparison to control cultures. We found that PD173074 caused early inhibition of cartilaginous nodule formation, evidenced by lack of sulphated proteoglycan and peanut agglutinin expression. Furthermore, the effect of FGFR inhibition on limb development observed here was unlikely mediated by excessive cell death as inhibitor did not caused massive apoptosis in the anterior area in contrast to posterior mesenchyme. More likely, FGFR inhibition decreased both the proliferation and adhesion of mesenchymal chondroprogenitors as the expression pattern of N-cadherin around the core was disrupted. In conclusion, we showed that the anterior limb cells are more sensitive to FGFR inhibitors and we propose that FGFR signalling contributes to the regulation of the anterior-posterior patterning of zeugopod during chicken limb development. This study was supported by the Grant Agency of the Czech Republic (14-31540S).

Program Abstract #420
Sp6 and Sp8 transcription factors control AER formation and dorsoventral patterning in limb development
Marian Ros1,2, Endika Haro1, Irene Delgado1, Marisa Junco1, Yoshihiko Yamada1, Ahmed Mansouri4, Kerby C Oberg5
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The apical ectodermal ridge (AER) is critical for the outgrowth and patterning of the vertebrate limb. The formation and maintenance of the AER is a complex process that relies on integrated interactions among the Fgf, Wnt, and Bmp signaling pathways within the ectoderm and between the ectoderm and mesoderm of the early limb bud. The transcription factors Sp6 and Sp8 are expressed in the limb bud ectoderm and function downstream of WNT/bcatenin signaling in AER induction and maintenance. However, AER induction occurs in the absence of either these two factors even though its maturation and/or maintenance is defective. To gain further insights into the role Sp6 and Sp8 play in limb development, we have simultaneously removed them from the limb ectoderm using a variety of loss-of-function alleles. Our results show the absolute requirement of Sp6 and Sp8 for limb development as in their complete absence, or substantial reduction, no limbs form. We also show that the progressive reduction in Sp6 and Sp8 gene products leads to predictable morphology, from syndactyly, to split hand/foot malformation, oligodactyly, truncation and finally amelia. In the complete absence or significant reduction of Sp6;Sp8, initial budding occurs but neither Fgf8 nor En1 are activated and the emergent limb bud regresses causing tetra-amelia. In the presence of a single functional allele of Sp8 (Sp6–/–;Sp8+/+) the resulting phenotype is split-hand/foot malformation with double dorsal distal tips due to the formation of an irregular AER that is not maintained in the center of the bud and to abnormal expression of Wnt7a. Our study supports a model in which Sp6 and Sp8 work together and in a dose-dependent manner as necessary mediators of Wnt/bcatenin signalling in the induction of Fgf8 and
downstream of Bmp signaling in the induction of Enl establishing, therefore, the link between proximo-distal and dorso-ventral patterning.

**Program Abstract #421**
**The Measure of a Morphogen - SHH Quantitation, and Roles in Pre-axial Polydactyly and Evolutionary Digit Loss**
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The expression of Sonic hedgehog (Shh) in the developing limb bud is tightly regulated via feedback loops and a cis-regulatory enhancer called the Zone of Polarising Activity regulatory sequence (ZRS). Mutations within this enhancer are known to be responsible for some pre-axial polydactylies (PPD). We have previously mapped the naturally occurring Polydactyly (Po) locus (found in Silkie chickens) to the ZRS. Extensive genetic analysis shows that unequal allelic SHH expression increases in limb growth and ectopic transcription factor binding all play a part in Silkie PPD.

Certain ratite species exhibit vestigial forelimbs, with a single formed digit. Using comparative genomics and murine/chicken LacZ reporter systems, we present a candidate ZRS base change associated with forelimb digit loss in the emu and cassowary ratite species.

Whilst there have been extensive studies into the formation and patterning of digits in the vertebrate limb bud via a gradient of SHH protein, none have yet answered a fundamental question: just how much SHH is present in the developing limb bud? Using absolute protein analysis we have successfully achieved absolute quantification of SHH protein in the limb buds of mammalian and avian species. Combined with novel data regarding cell number in developing limbs, we find a partial correlation between digit number and total SHH protein. Furthermore we have quantified cell number and SHH protein in the developing chick limb at multiple stages of embryonic development and find a sudden increase at the end of the patterning phase (HH21), prior to exponential growth. Finally, we show in an absolute quantifiable manner that SHH does indeed form a gradient in the limb bud.

**Program Abstract #422**
**Investigating the transcriptional response of forelimb and hindlimb cells to the Sonic Hedgehog morphogen gradient**
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The diffusible morphogen Sonic Hedgehog (Shh) is critical in establishing anterior-posterior pattern in vertebrate limbs. Whilst it has been demonstrated that differences in concentration and the duration of Shh exposure can determine digit identity, how different Shh concentrations and length of exposure are integrated, at a transcriptional level, to produce a digit pattern is not understood. It is also generally assumed that Shh acts equivalently in both forelimbs and hindlimbs, despite the phenotypic differences that exist between the respective limbs of Shh–signalling mutants. To study how limb cells interpret the Shh morphogen gradient over time, we have developed an ex vivo assay to study both dose and temporal transcriptional response profiles of forelimb and hindlimb cells exposed to defined doses of exogenous Shh over fixed periods of time. We have used RNAseq and qPCR analysis to determine expression profiles of key Shh targets and uncover novel Shh targets in limb cells. This work was supported by the Medical Research Council (UK).

**Program Abstract #423**
**Not too high, not too low: a sweet spot in timing and level of Shh pathway activity regulates two distinct progenitor populations in limb development**
Olena Zhulyn¹,²
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The embryonic limb is one of the best understood models of pattern formation in vertebrates. Patterning along the anterior-posterior (AP) axis is often studied in the context of positional information – the idea that cell fate is specified through signaling molecules, called morphogens, in a dose-dependent manner. In the limb, the AP pattern is regulated the morphogen Sonic hedgehog (Shh) which is secreted from an organizing center called the zone of polarizing activity (ZPA) in the posterior of the limb and regulates the activities of the Gli-family of transcription factors. Shh expression is absent during the earliest phase of limb development. During this phase the nascent limb is pre-patterned into anterior and posterior regions through the mutual antagonism of transcription factors Hand2 and Gli3. In two recently published studies, we discovered that precocious pathway activation during the pre-patterning phase interferes with Gli3-dependent specification of anterior progenitors, defined by the novel transcription factors Irx3 and Irx5, and disrupts the induction and maintenance of critical organizing centers, the ZPA and the apical ectodermal ridge (Zhulyn et al., 2014; Li et al., 2014). In contrast, failure to activate Shh in a timely manner, results in a loss of posterior progenitors and concurrent disintegration of signaling centers. Our findings indicate that the limb is comprised of at least two distinct progenitor pools and their induction and controlled expansion depends on a sweet-spot in the level and timing of Shh pathway activity. Based on our findings, we propose a two-population model, which describes limb patterning as a dynamic interplay between two distinct progenitor populations, providing a complimentary framework to positional information which allows us to better understand limb patterning and development.

**Program Abstract #424**
**Genetic analysis of Pitx2 function in zebrafish**

138
Yongchang Ji, Jeffrey Amack  
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Pitx2 is a homeodomain transcription factor that is highly conserved in vertebrates. Mutations in Pitx2 have been identified in patients with Axenfeld-Rieger syndrome (ARS), characterized by eye, craniofacial and tooth malformations. In addition, Pitx2 is a target of a conserved Nodal signaling pathway that mediates left-right patterning of vertebrate embryos. In zebrafish, the pitx2 gene encodes two isoforms-Pitx2a and Pitx2c—that have different N-termini but share the same C-terminal homeodomain. Both isoforms are expressed in specific patterns throughout embryogenesis, which include mesendoderm cells during gastrulation, asymmetric domains during left-right patterning and head and craniofacial regions during later development. To gain new insight into Pitx2 function we have used a genome editing (TALEN) approach to create mutations in zebrafish pitx2. We have recovered three different indel alleles that result in a frameshift and premature stop codon predicted to disrupt the homeodomain of both Pitx2a and Pitx2c such that these proteins cannot bind DNA. Analysis of F1 embryos revealed the expected mendelian ratio of genotypes. Surprisingly, no gross phenotypes were observed in these embryos for the first several days of development and we did not detect abnormal asymmetric patterning of the heart, gut or brain. At 8 days post-fertilization (dpf) homozygous mutants (pitx2<sup>shd/hd</sup>) have reduced body size and poor motility. Morphology of the liver in mutants resembled starved larvae in previous studies, suggesting insufficient nutrition. Consistent with this idea, adding glucose to the larval media improved development of the mutants. Analysis of pharyngeal tooth development at 9 dpf indicated teeth are reduced or absent in pitx2<sup>shd/hd</sup> mutants, which is reminiscent of ARS phenotypes and may be linked to malnutrition. These results suggest pitx2<sup>shd/hd</sup> mutants provide a novel model for ARS that can be exploited to understand molecular mechanisms of Pitx2 function.

Program Abstract #425
Defining Novel Gene Regulatory Networks in the Arabidopsis Root
Colleen Drapek  
Duke Univ., USA

A cell’s trajectory from stem cell to differentiation, while often portrayed as a linear progression, is best described as a network that produces a mature state through several pathways acting in tandem. There are few examples that describe gene regulatory network changes during the entire trajectory of cell differentiation. The goal of my project is to define a gene regulatory network that describes differentiation from stem cell to differentiated cell in the Arabidopsis thaliana root. The root is a powerful model for identifying basic principles of differentiation. Because plant cells do not migrate, entire lineages from stem cell to mature progeny are spatially confined. Furthermore, the root displays indeterminate growth, facilitating the study of many different developmental stages at a single time. One cell type of the root, the endodermis, is particularly suitable for generating a regulatory network because the molecular components required for its formation and terminal differentiation are established. These components constitute the foundation of a gene regulatory network that describes progression to differentiation. I am combining two approaches to connect stem cell specification to terminal differentiation at the level of gene expression. First, I identified novel regulators of endodermal differentiation using a forward genetics screen and am characterizing their role in root development. Second, using a yeast-one-hybrid approach, I will build a predictive gene regulatory network upstream of these differentiation regulators. Lastly, whole transcriptome analysis of wild-type and mutant endodermal cells will be used to understand how novel regulators direct gene expression. Completion of these studies will further our understanding of basic principles of cell fate determination in all multicellular organisms. Project funded by NIH T32 HD40372-12.

Program Abstract #426
Identification of lineage-specific regulators by automated lineage tracing in C. elegans
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Caenorhabditis elegans develops through an invariant lineage, but how this lineage is decoded into robust cell fates and behavior is not known. We developed confocal image analysis methods for automated cell lineage tracing, allowing quantification of expression dynamics and identification of mutant developmental defects at single cell resolution. We used these methods to describe the expression of the more than 200 TFs in every embryonic cell. This identified a large set of lineage-specific TFs that are 1) regulated by the Wnt signaling pathway, 2) expressed in multiple partially overlapping lineages and 3) expressed in cells fated to adopt multiple terminal fates. These lineage-specific TFs are highly conserved and most are essential, suggesting they are important developmental regulators. We defined the functional importance of these factors in a model sublineage “ABpxp,” which produces diverse cell types whose regulation is poorly understood. We identified six lineage-specific TFs expressed early in this lineage’s development. ceh-13/Hox, nob-1/Otx, ceh-27/Nkx and nhr-67/Tlx are expressed in sublineages of ABpxp and mutations in these genes causes lethality. In contrast, ceh-36/Otx and unc-30/Ptx are expressed throughout ABpxp and are 100% lethal only as a double mutant, thus these factors likely act redundantly. We compared cell-tracking data between mutants and wild-type and found defects in cell cycle, death, position and fate in the single mutants, with dramatic increases in defects in the ABpxp lineage of ceh-36,unc-30 double mutants. Analysis of these defects identified roles for these factors in specifying not only cell fates, but also processes such as lateralization (ceh-36, unc-30) and anterior-posterior migration (nob-1). Our data suggests a model where these lineage-specific TFs function to interpret lineage history and ensure robust cell behavior. This work was funded in part by NIH and the Pennsylvania Dept. of Health.
Program Abstract #427
The dADX proteins: The Drosophila orthologs of the amino-terminus of the ATRX vertebrate protein.
Brenda Araceli López Falcón Piza, Silvia Meyer Nava, Benjamin Hernández Rodríguez, Daniel Montero Barrera, Adam Andrés Campos, Enrique Rudiño, Martha Vázquez, Mario Zurita, Viviana Valadez Graham
Institute of Biotechnology, UNAM, México

The human ATRX gene encodes a chromatin remodeling protein that has two important domains, an helicase/ATPase domain and a domain composed of two zinc fingers called the ADD domain. The ADD domain binds to histone tails and has been proposed to mediate hATRX binding to chromatin. The putative ATRX homolog in Drosophila (dATRX/XNP) has a conserved helicase/ATPase domain but lacks the ADD domain. We performed a bioinformatic search in the data bank of the Drosophila genome and found that the annotated gene: CG8290 (which we named adx (which stands for "amigo de xnp", friend of xnp in spanish) encodes four proteins that share a common region in the amino terminal end that contains an ADD domain highly conserved with the ADD domain of the hATRX protein. 3D modeling of the domain shows that the structure and aminoacids which mediate the histone tail contacts are highly conserved. These isoforms (dATRXa, b, c and d) are generated by alternative splicing and are expressed throughout the development of D. melanogaster. We determined using pull-down and CoIP assays that they interact physically with dATRX_1/XNP_1 and HP1a. Furthermore co-immunostaining of polythene chromosomes with specific antibodies show that they co-localize mainly in the chromocenter, with dATRX_1/XNP_1 and HP1a, although euchromatic localization can also be seen through the chromosome arms. ChIP experiments demonstrate that these proteins are present in vivo in the same heterochromatic regions. To determine if the dATRX/XNP and adx alleles interact genetically, we performed crosses between them, hypomorph mutations in both genes result in the generation of melanotic masses in the organism. The ADXb, c and d isoforms have extra domains which suggest newly acquired functions. These results strongly support that in Drosophila the ATRX gene suffered an evolutionary split and that the adx encoded proteins participate with dATRX_1/XNP_1 in some cellular functions such as heterochromatin maintenance.

Program Abstract #428
Carotid body glomus cells develop from autonomic neurons and share a differentiation pathway with adrenal chromaffin cells
Dorit Hockman1,4, Perrine Barraud1, Tomoki Otani1, Adam Hunt1, Patrik Ernfors2, Marthe J. Howard3, Jeffrey Reese4, Elisabeth Sock5, Clare V. Baker1
1Univ. of Cambridge, Dept. Physiology, Development & Neuroscience, Cambridge, UK; 2Karolinska Institute, Division of Molecular Neurobiology, Stockholm, Sweden; 3Univ. of Toledo, Dept. Neurosciences and Program in Neurosciences & Neurodegenerative Diseases, Toledo, OH, USA; 4Vanderbilt Univ. Medical Center, Depts. of Pediatrics, Cell and Developmental Biology, Nashville, TN, USA; 5Universität Erlangen-Nürnberg, Institut für Biochemie, Erlangen, Germany; 6Univ. of Oxford, Weatherall Institute of Molecular Medicine, Oxford, UK

In amniotes, the serotonergic chemoreceptor cells that trigger the ventilatory reflex response to hypoxia are concentrated in the carotid body, which develops as a bulge from the wall of the third pharyngeal arch artery (future carotid artery). In neonatal mammals, chromaffin cells in the adrenal glands (above the kidneys) are also transiently hypoxia-sensitive. Adrenal chromaffin cells differentiate from trunk neural crest-derived sympahtoadrenal progenitors, which migrate from primary sympathetic ganglia near the dorsal aorta into the adrenal gland primordium and down-regulate pan-neuronal markers. Carotid body glomus cells develop from vagal neural crest cells that migrate into the third pharyngeal arch. By analysing sympathoadrenal pathway gene expression during chicken carotid body development, and mouse mutants for a subset of these genes, we show that the molecular basis of carotid body glomus cell development strikingly resembles that of adrenal chromaffin cells. Furthermore, fate-mapping and immunostaining data support the previously proposed hypothesis that carotid body glomus cells are ‘émigrés’ from nearby ganglia: we identified a small population of neural crest-derived neurons in the nodose ganglion (the nearest ganglion to the chicken carotid body, whose neurons are usually considered entirely placode-derived), ‘bridges’ of neurons between the nodose ganglion and the carotid body, and loss of the pan-neuronal marker HuC/D in the carotid body concomitant with up-regulation of serotonin. Overall, our data suggest that, like adrenal chromaffin cells, carotid body glomus cells arise from autonomic neural crest-derived neurons migrating from nearby ganglia, which down-regulate pan-neuronal markers as they adopt a glomus cell fate.

Program Abstract #429
The diencephalic glial bridge represents a heterogeneous population of astroglial cells that support commissure formation in the zebrafish forebrain
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Connections between the two hemispheres of the central nervous system are enabled by commissures, which form when pathfinding growth cones cross the embryonic midline. Little is known about the cellular behaviors exhibited between commissural axons and the cells of the midline growth substrate in the vertebrate forebrain. We take advantage of the simple system of the zebrafish...
Integrin alpha6 Function Is Necessary For Neuronal Migrations and Neurovascular Formations In Zebrafish Hindbrain

Vinoth Sittaramane¹, Kayla Smith¹, Ogonna Ifeadike¹, Clarissa Henry³, Anand Chandrasekhar²

Targeting of Cochlear Inner Hair Cells by Type I Spiral Ganglion Neurons is Controlled by Sema3F/Nrp2 Signaling

Thomas Coate, Kevin Isgrig, Matthew Kelley

Targeting of Cochlear Inner Hair Cells by Type I Spiral Ganglion Neurons is Controlled by Sema3F/Nrp2 Signaling

Thomas Coate, Kevin Isgrig, Matthew Kelley

NIH/NIDCD, USA

The transition of Drosophila third instar larvae from feeding, photo-phobic foragers to non-feeding, photo-neutral wanderers is a classic behavioral switch that precedes pupariation. The neuronal network responsible for this behavior has recently begun to be defined. Previous genetic analyses have identified signaling components for food and light sensory inputs and neuropeptide hormonal outputs as being critical for the forager-to-wanderer transition. Trio is a Rho-Guanine Nucleotide Exchange Factor integrated into a variety of signaling networks including those governing axon pathfinding in early development. Sequoia is a pan-neuronally expressed zinc-finger transcription factor that governs dendrite and axon outgrowth. Using pre-pupal lethality as an endpoint, we screened for dominant second-site enhancers of a weakly lethal trio mutant background. In these screens, an allele of sequoia was identified. While these mutants have no obvious disruption of embryonic central nervous system architecture and survive to third instar larvae similar to controls, they retain forager behavior and thus fail to pupariate at high frequency. In an attempt to understand this dosage-sensitive genetic interaction between Trio and Sequoia, we have begun to identify the CNS tissue(s) critically sensitized. Towards that end, rescue experiments driving Trio expression with the GAL4/UAS system have been initiated. Results ranging from pan-neural expression to expression in specific larval brain regions will be presented.

This work was supported by NSF grants IBN-0344053 and IBN-0990239 to E.C.L., NIH grant 1R15HD059924-01 to E.C.L., funds from the Denison Univ. Research Foundation, and Denison’s Anderson Endowment Program and Bowman Summer Scholarship Program.

Program Abstract #430
A dosage-sensitive genetic interaction between the Trio GEF and Sequoia transcription factor influences a Drosophila larval behavior
Eric Liebl
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The mouse cochlea provides an excellent model for investigating molecular mechanisms associated with sensory organ innervation. To establish appropriate hearing function in the cochlea, type I spiral ganglion neurons (SGNs) must synapse with the mechanosensory inner hair cells (IHCs), whereas type II SGNs must synapse with outer hair cells (OHCs). In addition, low-spontaneous rate (low-SR) SGNs target the medial side of IHCs, whereas high-SR SGNs target the lateral side of IHCs. To better understand cochlear innervation, we developed a live imaging model in which sparse numbers of SGNs express tdTomato, while all hair cells express GFP. Using this we have determined that supernumerary type I SGNs project to the OHCs around E15, but then retract to the IHCs through P0. These data support the hypothesis that cues within the hair cell environment are necessary for correct SGN subtype specificity into different hair cell regions.

Cells within the OHC region express the axon guidance factor Semaphorin-3F (Sema3F) while the SGNs express its receptor, Neurexin-2 (Nrp2). To characterize the role of these factors with high optical resolution, we combined Nrp2 mutants with our imaging model and documented the projection of each labeled SGN. Nrp2 deletion leads to the ectopic projection of type I SGNs into the OHC region, but does not alter the arrangement of low- and high-SR type I SGNs. The loss of Nrp2 also leads to increased numbers of OHC ribbon synapses and changes in auditory brainstem response thresholds, suggesting this receptor is necessary for the establishment of normal hearing function. In addition, treating cultured cochlear cells with Sema3F causes SGN branches to collapse, suggesting Sema3F normally acts as a chemorepellent. These data support a model whereby Sema3F within the cochlear epithelium activates Nrp2 on type I SGNs to sequester them at the IHC. We are currently examining how processing of the co-receptor Plexin-A3 dictates differential targeting by SGNs. Funding by NIDCD.

Program Abstract #432
Integrin alpha6 Function Is Necessary For Neuronal Migrations and Neurovascular Formations In Zebrafish Hindbrain

Vinoth Sittaramane¹, Kayla Smith¹, Ogonna Ifeadike¹, Clarissa Henry³, Anand Chandrasekhar²

Program Abstract #431
Targeting of Cochlear Inner Hair Cells by Type I Spiral Ganglion Neurons is Controlled by Sema3F/Nrp2 Signaling

Thomas Coate, Kevin Isgrig, Matthew Kelley

NIH/NIDCD, USA

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Integrin alpha6 Function Is Necessary For Neuronal Migrations and Neurovascular Formations In Zebrafish Hindbrain

Vinoth Sittaramane¹, Kayla Smith¹, Ogonna Ifeadike¹, Clarissa Henry³, Anand Chandrasekhar²
Early nervous system development involves massive neuron migration and neurovascular formations resulting in defined assembly of functional neural and vascular circuits. Any disturbances in this process could lead to a broad range of human neural deficits including lissencephaly, developmental epilepsies, motor dysfunctions, stroke and learning disorders. Cell-cell and cell-extracellular matrix interactions organized by integrin signaling play a vital role in regulating neuronal migrations and neurovascular development. Here we have identified roles for integrin alpha 6 (Itga6) in neuronal migrations and neurovascular formations in zebrafish hindbrain. Itga6 is ubiquitously in the hindbrain and knockdown of itga6 generated defects with a majority of facial branchiomotor (FBM) neurons failing to migrate normally. We performed genetic mosaic analysis and our results suggest that itga6 functions cell autonomously in the FBM neurons to regulate their migration. Furthermore, we found that itga6 interacts genetically with laminin g1 (lanc1) for neuronal migration, suggesting that Itga6-laminin signaling is critical for FBMN migration. Itga6 has been shown to form heterodimers with integrin beta1 to function as a laminin-binding receptor. Knockdown of itgb1 also results in FBM neuron migration defects suggesting that integrin a6b1-laminin interactions may regulate motor neuron migration in the zebrafish hindbrain. Single nucleotide polymorphisms (SNPs) in integrin alpha 6 (ITGA6) have been closely linked with neurologic symptoms of intracerebral hemorrhaging in humans. Therefore, we examined itga6 deficient zebrafish hindbrains for neurovascular development. Itga6 deficient zebrafish embryos display extensive neurovascular defects: truncation and dilation of the vasculature, loss of vascular network, and neurovascular hemorrhaging. Taken together, we are demonstrating a role for neuronal migrations and neurovascular formations in zebrafish hindbrain.

Program Abstract #433
Stl is an ADAMTS extracellular protease required for proper neuronal migration in the Drosophila embryo
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The three-dimensional extracellular matrix (ECM) is a major substrate through which cells migrate. The ECM comprises a variety of fibrillar proteins, adhesion molecules, growth factors, growth factor receptors, and integrins. Proper migration of cells through the ECM requires modification of the ECM. Among the extracellular enzymes that could modify the ECM to allow for proper cell migration is the ADAMTS family of secreted extracellular proteases. The Drosophila genome encodes three ADAMTS genes, including stl, which is expressed in the peripheral nervous system (PNS) during embryogenesis. The absence of stl displays mis-migration defects in certain PNS neurons. Surprisingly, these neurons seem to migrate past their wild-type counterparts, suggesting a role for this extracellular protease in inhibiting migration along a certain path, instead of permitting migration. To explore this unusual defect further, I am testing potential interacting partners of Stl in PNS migration. I also plan on over-expressing stl in these same cells to try to rescue the mis-migration defect, as well as see the effect of too much of this protease on neuronal migration. I hope to learn if stl functions as an inhibitor or repulsive cue in cell migration. Through these studies, I expect to gain insight into how this ADAMTS family of extracellular proteases contributes to cell migration and tissue morphogenesis during normal development.

Program Abstract #434
The Role of Planar Cell Polarity in Directed Cell Migration
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During development, neurons must migrate from their birthplace to their functional destinations in the brain in order to create working neural networks. In zebrafish and other vertebrates, the facial branchiomotor neurons (FBMNs) undergo a well-characterized migration in the plane of the hindbrain neuroepithelium from rhombomere 4 to rhombomere 6. Rather than identifying chemotropic signals or receptors, forward genetic screens in the zebrafish for FBMN migration mutants have identified mutations in almost all of the core components of the Planar Cell Polarity (PCP) pathway (Vangl2, Fz3a, Pk1b, Celsr3 and Scrib). The PCP pathway is a cell-contact dependent mechanism for generating polarity in the plane of an epithelium, but how PCP controls highly dynamic processes like neuronal migration is not understood. FBMNs contact a variety of tissues during their migration including the segmented hindbrain neuroepithelium, the floor plate, as well as other migrating FBMNs. Where PCP signaling is required for FBMN migration is controversial. Most studies have identified a cell-non-autonomous role for PCP, but which cells promote FBMN migration, and how they use the PCP pathway to do so, is unknown. Furthermore a cell-autonomous role for PCP signaling within the FBMNs has been demonstrated in some studies but refuted in others. Using the Gal4/UAS system and PCP-specific dominant-negative forms of Dishevelled (DN-Dvl) to systematically disrupt PCP in a cell-type and rhombomere-specific manner, we present evidence of both a cell-autonomous requirement for PCP within FBMNs and a non-cell autonomous requirement specifically in the rhombomere 4 environment in which the FBMNs arise. Our findings suggest a model in which PCP signaling between the planar polarized neuroepithelial environment and the nascent FBMNs initiates their directional migration.
NIH RO1 NS082567 and the UW DBTG

Program Abstract #435
Regulation of Motor Neuron Migration by the Planar Cell Polarity Pathway
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The planar cell polarity (PCP) pathway is a cell contact-mediated mechanism for transmitting polarity information between adjacent cells. Although the PCP pathway was first described and is best understood in stable epithelial tissues, PCP “core components” are also essential for a number of cell migratory events. How PCP controls cell migration is not understood in any system. We use the migration of facial branchiomotor neurons (FMNs) in the plane of the zebrafish hindbrain to address this question. FMNs originate in rhombomere (r)4 and migrate posteriorly to r6 and r7, in contact with neural progenitors and the medial floorplate. Work in our lab and others has shown that FNM migration requires the function of PCP core components including the transmembrane proteins Fz3a and Vangl2 both in FMNs and in cells in the migratory environment, but how PCP functions in these tissues to promote migration is not known. Using the Gal4/UAS system to express PCP core component fusion proteins in FMNs and their neuroepithelial environment we have found that Vangl2 localizes to the tips of a subset of FMN filopodia and asymmetrically to the anterior apical membrane of floorplate cells. Since cell migration results from the contact-dependent stabilization of cellular protrusions, we have examined the membrane dynamics of single FMNs undergoing migration in wildtype and PCP mutant embryos using high-resolution single-cell timelapse microscopy in vivo. We have found that wildtype FMNs in vivo are highly protrusive, elaborating both lamellipodia and filopodia. Using chimeras we have uncovered opposing functions for the PCP components Fzd3a and Vangl2 in regulating FNM protrusive activity: within FMNs, Fzd3a is required to stabilize filopodia while Vangl2 has an antagonistic destabilizing role. Current work is focused on determining how PCP signaling in the environment influences filopodial dynamics. Grant support: NIH ROI NS082567 & the UW Developmental Biology Training Grant.

Program Abstract #436
Ephrin-B2 expression in the vascular endothelium is necessary and sufficient for normal neural crest guidance
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Previous studies have implicated a role for ephrin-B2 in the guidance of cranial and trunk neural crest cells (NCC). Ephrin-B2, which is encoded by the efnb2 gene, is a member of the Eph/ephrin family of cell signaling molecules. It has been hypothesized that ephrin-B2 expression in the branchial arch ectoderm and pouch endoderm drives segregation of cranial neural crest and branchial arch morphogenesis, whereas expression of ephrin-B2 in the caudal half of somites has been proposed to drive segregation of the trunk neural crest. Detailed examination of these processes has been hindered, however, by embryonic lethality of efnb2null mutant embryos due to a requirement in angiogenic remodeling. To further elucidate the developmental roles for ephrin-B2, we have generated a conditional rescue allele of efnb2 that allows rescue of ephrin-B2 specifically in the vascular endothelium (VE), but is otherwise ephrin-B2 deficient. Embryos harboring this allele in homozygosity completely lack vascular phenotypes, allowing us to investigate the role of ephrin-B2 in neural crest migration. Surprisingly, we find that expression of ephrin-B2 specifically in the VE is sufficient for normal NCC migration and that conversely, embryos in which ephrin-B2 is lost specifically in the VE exhibit NCC migration defects. Together these data indicate that ephrin-B2 expression in the VE is necessary and sufficient for normal NCC migration. Further, by utilizing additional mouse models to independently perturb vascular development, we find that NCC phenotypes are not likely to be a consequence of perturbed signaling between the VE and NCC, but rather a consequence of compromised embryonic viability attributable to defective angiogenesis.
This work is supported by R00DE020855 to J.O.B.

Program Abstract #437
The Role of MicroRNA-206 in the Distribution of HNK-1 Positive Cells in Xenopus laevis
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Neural crest cells are an important migratory cell population in vertebrates that give rise to various cell lineages such as craniofacial cartilage, peripheral neurons, enteric neurons and melanocytes. Improper migration of neural crest cells can have detrimental effects to the developing organism. Peripheral neurons are of particular importance because they connect the central nervous system to the limbs and organs of vertebrates. We sought to investigate how peripheral neurons are affected by the disruption in somite formation mediated by knockdown of the muscle-specific microRNA, miR-206, in Xenopus laevis. MicroRNAs are small, non-coding sequences of RNA that regulate genes post-transcriptionally by binding specific messenger RNAs and blocking their translation. MiR-206 is a microRNA that is exclusively expressed in the skeletal muscle tissue of vertebrates. Preliminary data from our lab has shown that knockdown of miR-206 via morpholino causes a disruption in somite morphogenesis that leads to a loss of movement observed among tadpoles. Here, we use HNK-1, an antibody that binds to migrating neural crest cells, peripheral neurons and motor neurons, to investigate the distribution of these neuronal populations in miR-206 morphants. We show that the distances of neuronal projections are perturbed or altered in the morphant embryos in comparison to wild type embryos. Also, the migratory distance of the neural crest cells around the neural tube seems to be stunted in morphant embryos. These alterations appear to be both dependent on how well organized the somites are as well as their size, which appears to be influenced by the expression of miR-206. Therefore, these results reveal the important role that the morphology of the somite plays on the distribution and projections of neurons from both the central and peripheral nervous system in the frog, Xenopus laevis.
*NIH MARC funded our travel to SDB and CSUPERB funded this research
Program Abstract #438
Loss of Robo receptor Enhances Trunk Neural Crest Cell Migration
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Neural crest cells are a multipotent cell lineage that delaminates from the dorsal neural tube of developing vertebrate embryos and subsequently undergo an epithelial to mesenchymal transition enabling stationary cells to actively migrate to distant areas. Mechanisms controlling migration of neural crest cells are not fully understood. Slit2 is a chemorepellent guidance molecule that stimulates the motility of trunk neural crest cells and repels them from the developing gut. Slit2 is known to be a tumor suppressor molecule. Recently we found that Slit molecules are capable of impairing neural crest cell migration and suggested that it played a role in pre-migratory neural crest. The present study looked further into whether Slit/Robo interactions have a role in the process of neural crest delamination via gain-of-function (GOF) by electroporating Slit2 and loss-of-function (LOF) experiments by electroporating dominant negative Robo. We found that forced Slit or Robo expression in neural crest cells significantly impaired proper migration while LOF knockdown favored earlier migration of neural crest cells. Slit2 crest microarray showed that in addition to a large set of molecules known to be important in maintaining cells in a non-motile, epithelial phenotype: Ankyrin, DSCAM-L1. A good number of these molecules are also known to be downstream of Slit-Robo signaling, but we also found new molecules previously not associated with Robo signaling: Claudins, Shh, HoxA, ezrin, merlin, Delta, glial genes, Bcl2-A1, Nkx2.6, Otx2, and olfactory receptors. Also among the genes regulated by Slit2 GOF in our microarray were a wide range of cell division ones: EzH1, Nothch1 and cdk5. Our findings reveal for the first time a new role for Slit2 in neural crest cell emigration and provide evidence for the ability of Slits to affect timely migration of neural crest cells in a Robo-dependent manner. Funding was by NIH/NINDS AREA grant 1R15-NS060099-01 to MEdB.

Program Abstract #439
Challenging the Cell Induced Gradient Model of Neural Crest Migration
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The neural crest are an excellent model to study long distance cell migration during embryonic development. Neural crest cells are sculpted into multicellular streams near the dorsal neural tube, but it is largely unclear what mechanisms direct cells through distinct microenvironments to reach distal targets. Previously, we discovered a chemotactic role for vascular endothelial growth factor (VEGF) and developed a cell-induced gradient model for cranial neural crest migration. In this model, lead neural crest cells respond to VEGF and transfer guidance information to trailing cells. Here, we test this model by challenging the VEGF chemotactic profile both in the chick embryo neural crest microenvironment and in silico using our agent-based computational model. We present results in which we place ectopic sources of VEGF into different locations within or adjacent to a typical cranial neural crest stream. We analyze changes to neural crest cell behaviors and gene expression profiles using time-lapse imaging and LCM/RT-qPCR subregions and compare experimental results to computational model predictions. We will discuss our results that further support our cell induced gradient model of neural crest migration and dynamic nature of cells to respond to local changes in microenvironmental signals.
This research was funded by NIH Grant 1R01HD057922 (PMK) and the Stowers Institute for Medical Research.

Program Abstract #440
Investigating the novel role of cdon in Prdm1a-regulated neural crest migration
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Neural crest cells are a population of embryonic progenitor cells that originate in the dorsal neural tube and then undergo an epithelial-to-mesenchymal transition (EMT) and migrate away from the neural tube to populate various developing tissues and differentiate into their final fate. Previous work in our lab has demonstrated the requirement of the transcription factor Prdm1a in specifying neural crest cells during early zebrafish embryogenesis. Here, we demonstrate a separate role for Prdm1a in regulating neural crest EMT and migration following specification. We hypothesize that Prdm1a regulates genes that are required for neural crest cell migration and have determined a novel role of one of these downstream targets, cdon, in Prdm1a-mediated neural crest migration. cdon is expressed early in developing premigratory neural crest cells and is required cell-autonomously for directed migration of the neural crest cells within the trunk. Loss of cdon results in aberrant migration of trunk neural crest cells and failure in formation of ventral trunk derivatives including melanocytes. These studies demonstrate a role for Prdm1a as a regulator of neural crest migration genes, including the novel neural crest migratory regulator cdon, in addition to its previously recognized role in neural crest specification.
This work is funded by a pre-doctoral NRSA fellowship from the NIDCR (F31DE022237) to D.R. Powell.

Program Abstract #441
protocadherin10a is required for migration of neural crest-derived melanocyte precursors
Neural crest derived melanocyte precursors form at the neural plate border, undergo an EMT and migrate along discrete pathways to reach their final destination in the skin. This process requires cells to interact with each other and the extracellular environment via cell-cell and cell-matrix adhesion. Protocadherins are similar to classic cadherins in that they function in cell adhesion and axon guidance in various developing systems. Specifically, protocadherin10a (pcdh10a) has been shown to regulate olfactory axon guidance in the mouse and is implicated in several types of human cancers. Here, we have determined the role of pcdh10a in zebrafish neural crest and melanocyte precursor development. pcdh10a is expressed in a subset of migrating neural crest cells and knockdown of pcdh10a results in a decrease in the number of neural crest-derived melanocyte precursors. Strikingly, pcdh10a Morpholino injected embryos and pcdh10a TALEN mutants develop melanin-expressing melanocytes that stall along ventral pathway adjacent to the notochord and fail to reach their final position in the skin. Live cell imaging analysis suggests that the melanocyte precursors do not migrate in a directed fashion and have diminished contact inhibition of locomotion, resulting in differentiation of melanocytes in ectopic locations. We hypothesize that pcdh10a functions in zebrafish neural crest derived melanocyte precursor migration by regulating actin and N-cadherin and thereby promoting contact inhibition of locomotion.

Program Abstract #442
Molecular cloning of the white mutant axolotl: correspondence to endothelin-3 and the feasibility of genetic and genomic analyses for studying development and regeneration in the salamander.
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Salamanders have long been used for studying fundamental aspects of morphogenesis and patterning as well as mechanisms of regeneration, and, in the Mexican axolotl Ambystoma mexicanum, several mutants are known to affect neural crest development and pigmentation. One of these, “white” (d/d), was isolated a century ago for its striking lack of pigment cells in the adult. The few pigment cells in the earlier larva are clustered dorsally rather than being spread across the trunk, owing to a defect in neural crest migration. Transplantation studies in the 1980s showed that the white gene product acts through the epidermis yet the identity of this factor has been a mystery, owing to the relative paucity of genetic and genomic resources for axolotl, as well as a genome that is ten-fold larger than that of human. Here, we identify the white locus as an A. mexicanum orthologue of endothelin 3 (edn3), which encodes a small signaling peptide that promotes proliferation, differentiation, and survival of neural crest and pigment cells in other vertebrates. By positional cloning and whole-chromosome de novo sequencing we mapped white to the vicinity of edn3. We find that white mutants express lower levels of full-length edn3 transcript, yet higher levels of a shorter, incorrectly spliced transcript. We further show that transgenic expression of full-length edn3 by the epidermis is sufficient to rescue the white mutant phenotype. Taken together, these analyses indicate the correspondence of edn3 and the white locus, and showcase the renewed utility of this classic model organism in the post-genomic era.

Program Abstract #443
Proteolytic control of Cranial neural crest cell migration.
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ADAMs are transmembrane metalloproteases that control multiple signaling pathways (Notch, Ephrin, EGF, TNF) as well as cell adhesion by cleaving ligands, receptors and cell adhesion molecules from the cell surface. There are over 30 ADAMs but most of our knowledge comes from studies of ADAM10 and 17. Our group has investigated the role of lesser known ADAM proteins (ADAM9, 13, 19) during early embryogenesis and shown that they are important for notochord and somite patterning as well as neural crest cell induction and migration. In addition, our group has uncovered a novel mechanism by which ADAMs control gene expression that involves the cleavage and nuclear translocation of their cytoplasmic domain. ADAM13 activity is highly controlled during neural crest cell migration. First, the protein binds to the Wnt receptor Frz4 and this interaction regulates ADAM13 proteolytic activity, including the self-cleavage that leads to the generation of the cytoplasmic domain in vivo. Loss of Frz4 inhibits CNC migration and induces a premature cleavage of ADAM13, resulting in a decrease of the surface level and a corresponding increase of the nuclear form. In parallel, ADAM13 is phosphorylated by GSK3 and subsequently by Polo-like kinase, and both phosphorylations are critical for the nuclear activity of the cytoplasmic domain of ADAM13, while having no effect of proteolysis. Blocking either kinase inhibits CNC migration, while the corresponding phosphomimetic mutations of ADAM13 rescue migration. In addition, migration can be rescued by one of the transcriptional targets of ADAM13, Calpain-8. Using protein prediction tools and mass spec analysis we identified multiple proteins that appear to bind preferentially to the phosphorylated forms of ADAM13 and could account for the nuclear function.

Program Abstract #444
Myosin 10 motor protein plays an essential role in craniofacial development
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Craniofacial development is the process of laying early cartilage and bone patterns in the anterior region of the embryo, which ultimately results in shaping the structure of the face and head of an organism. It is also an extensively evolving trait that helps in
adapting to the changing morphological and functional requirements of a species. Further, Craniofacial abnormalities in humans, such as cleft lip and palate, are among the most common of all birth defects. Therefore, investigating the molecular mechanisms involved in craniofacial development would help us understand both the evolutionary and genetic disease processes. Craniofacial cartilage and bone structures are almost entirely derived from neural crest cells. Neural crest are a pluripotent migratory stream of cells that originate from the early developing brain and settle in final positions that give rise to the future skull and face. Several motor proteins are implicated in the migration of these neural crest cells. We have identified and isolated zebrafish myosin 10 mutants with defective craniofacial development. Currently, we are characterizing the role of myosin 10 in craniofacial development using various staining techniques. Alcian blue staining was used to identify specific defects within the cartilage, specifically ceratobranchial arches 3-5 appeared to be distorted or completely missing in myosin 10 deficient embryos. Using alizarin red staining techniques, pharyngeal tooth development was also examined. Tooth development occurs on the fifth ceratobranchial arch in a three crown clustered manner. However, in myosin 10 deficient zebrafish, pharyngeal crown protrusion was significantly hindered, showing only one developing crown within the tooth in most morphant embryos. We plan on using immunohistochemical staining techniques to identify the specification and position of migrating neural crest cells to establish a link between myosin 10 and neural crest cell migration during early development.

**Program Abstract #445**

**Cell intercalation and migration mediated by actin contractility contribute to fusion of the mammalian secondary palate**

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Fusion of two distinct embryonic prominences to form a single continuous structure occurs in numerous developmental contexts and often requires the removal of an intervening epithelium. During fusion of the mammalian secondary palatal shelves, the medial epithelial seam (MES) is removed to form a continuous secondary palate with mesenchymal confluence, but the cellular mechanisms remain incompletely understood. This problem has been studied at great length, leading to three main hypotheses for how it occurs. Whereas considerable investigation has been made into epithelial to mesenchymal transition (EMT) and programmed cell death of the intervening epithelium as potential cellular mechanisms, the contribution of migration of the intervening epithelium has not been directly evaluated. By combining confocal live imaging in *ex vivo* culture with functional molecular genetics in mice, we now directly observe dynamic actomyosin contractility-driven cell behaviors in the fusion of the secondary palate. First, we identify integration of a disorganized multi-layered epithelium into a shared single layer epithelium and an essential early step in secondary palate fusion. Second, we provide direct evidence of cell migration in removal of the MES. Both integration and migration of the MES requires an actin contractility pathway involving Rho Kinase (ROCK), Myosin light chain kinase (MLCK) and culminating with activation of Non-muscle myosin IIA (NMIIA). Together, these data provide direct support for cell migration contributing to removal of the MES, and begin to indicate a molecular pathway controlling this action.

**Program Abstract #446**

**Activation of IGF1-RUNX2 pathway demonstrates changes in cellular phenotype in single suture craniosynostosis.**

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Craniosynostosis is defined as the premature fusion of the calvarial sutures. This developmental anomaly causes abnormal skull shape in children, leading to increased intracranial pressure, alteration in the craniofacial growth and orbital deformation. Although the molecular pathogenesis of the rare syndromic forms of craniosynostosis have been identified, the etiology of the more common single suture craniosynostosis (SSC) forms still remains elusive. In this study, we identify changes in cellular phenotype of osteoblasts derived from fetal calvarial sutures demonstrating activation of IGF1-RUNX2 pathway. Pathway analysis implicated an IGF1-RUNX2 subgroup defined by SSC-specific expression of extracellular matrix (ECM) and focal adhesion transcripts when comparing SCC cases to controls. We found that cases within the IGF1-RUNX2 subgroup demonstrated increased proliferation and alkaline phosphatase activity. Osteoblasts were seeded onto elastomeric microposts arrays in order to characterize the contractile force and focal adhesion area produced by these cells. Cases ranked by increasing IGF1-RUNX2 expression revealed enhanced formation of focal adhesions, elevated cytoskeletal traction force and reduced migration velocity compared to controls. Therefore, these results suggest that the ECM-mediated focal adhesion subset within the IGF1-RUNX2 subgroup can provide valuable context for phenotype-genotype correlations in SCC pathogenesis.

**Program Abstract #447**

**Pickle1 Mutant Mice Exhibit Congenital Heart Defects and Multiple Other Birth Defects due to Disruption of Planar Cell Polarity**

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The planar cell polarity signaling (PCP) pathway regulates cell polarity, and collective cell movement pivotal to many embryonic
patterning and developmental processes. Not surprisingly, PCP signaling is highly conserved in evolution, and mutations of PCP components can disrupt development from Drosophila to man. Several mouse studies show mutations in PCP core proteins can lead to an array of developmental anomalies, including congenital heart defects (CHD), craniofacial anomalies, abnormal limb development, cochlea stereocilia misalignment, and ciliogenesis defects, all hallmarks of phenotypes associated with cilia defects, also known as ciliopathies. In the course of a mouse mutagenesis screen for mutations causing CHD, we recovered a novel mutant, named *Beetlejuice* (*Bj*), which was identified by exome sequencing analysis to have a missense mutation in *Prickle1* (*C161F*), a PCP effector. *Bj* mutant mice show many phenotypes consistent with PCP disruption, including malalignment of the great arteries associated with double outlet right ventricle or overriding aorta, skeletal defects including foreshortened limbs, cleft palate and facial clefting, and abnormal patterning of the cochlea. These collectively are reminiscent of defects often found in ciliopathies. Analysis of mouse embryonic fibroblasts (MEFs) derived from *Bj* mutant embryos showed a ciliogenesis defect. Analysis using a MEF wound closure assay further showed *Bj* mutant cells are unable to establish cell polarity and engage in directional cell migration. Quantitative analysis of the *Bj* embryonic heart revealed foreshortening of the outflow tract likely accounts for the outflow tract malalignment defect. Analysis of skeletal preps revealed shortening of the long bones in both the fore and hindlimbs. Together these findings suggest an intrinsic defect in cell polarity may account for the many different phenotypes observed in with the disruption of *Prickle1* function in the *Bj* mutant. Funded by HL098180.

Program Abstract #448

**DDR2 coordinates signaling between the cardiac gene regulatory network and cell trafficking to promote directed migration of precardiac cells.**

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During development cell fate specification by gene regulatory networks (GRNs) is translated into complex morphogenetic cell behaviors. To understand how transcriptional network inputs are converted into diverse biomolecular cell processes we use the migration of *Ciona intestinalis* cardiogenic precursors, termed Trunk Ventral Cells (TVCs), as a model of developmental cell behavior. TVC migration from the tail to the ventral trunk is induced by the FoxF transcription factor, a component of the precardiac GRN. We have identified the collagen binding receptor tyrosine kinase, DDR2, as a transcriptional target of FoxF. Our work indicates that DDR2 coordinates multiple subcellular processes necessary for successful TVC migration. 4D imaging and computational analysis of migrating TVCs shows that DDR2 is required for the persistence of TVC migration and maintenance of leader/trailer position during cell movement. Disruption of DDR2 activity in the TVCs results in shorter cell migration paths, changes in cell morphology, and uncoordinated migration leading to cell tumbling. To identify molecular pathways that function downstream of DDR2 we tested the polarity, adhesion, and trafficking pathways in the TVCs for changes under dominant negative DDR2 conditions. We find that disruption of DDR2 changes the number, size, and distribution of endocytic and recycling vesicles marked by specific Rab proteins. Further, we identify changes in localization of Rab effectors, implying that DDR2 may be required for localized Rab activity. Our data also suggests that DDR2 is required for normal actin turnover, possibly contributing to maintenance of cell shape and motility. Our work identifies DDR2 as a signaling hub that coordinates multiple cell behaviors downstream of the cardiac gene network during precardiac cell migration. This works is funded by NIH grant 1F32GM108369 - 01A1 to Y.B.

Program Abstract #449

**ATP-binding cassette transporters mediate small micromere migration and left/right coelomic pouch segregation in the purple sea urchin, *Strongylocentrotus purpuratus***

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One function of ATP-binding cassette (ABC) transporters is to establish morphogenetic gradients in embryos. Sea urchin small micromeres (Smics) are primordial germ cells that undergo a 65% reduction in ABC-transporter activity at formation, presumably to concentrate morphogenetic signals necessary for segregation between the two coelomic pouches (CPs). An average of five Smics are found in the left coelomic pouch (CP), while embryos grown in the presence of ABC-transporter inhibitors have random left/right Smic distributions. To test how ABC transporters control segregation, we fused Sp-ABCB1a to Sp-nanos UTRs and selectively overexpressed it in Smics. In these embryos a dose-dependent increase in the number of Smics in the left CP was observed, while targeted expression of membrane markers and null mutants of ABCB1a did not alter Smic distribution. These results suggest expression of ABCB1a in Smics disrupts their left/right migration, possibly by dampening the detection of chemoaffectants. Smics in gastrulae are embedded in the tip of the archenteron, but remain motile and develop cortical blebs and filopodia. Smics in prism larvae move in the plane of the blastoderm towards the coelomic pouches. Using this information, we are currently investigating whether ABC-transporter activity disrupts Smic migration by altering bleb and filopodia dynamics. Preliminary results indicate Smics expressing ABCB1a in *vitro* extend smaller blebs and fewer filopodia than Smics expressing non-transporter membrane proteins. Collectively, our findings suggest that ABC-transporter mediated secretion of morphogens controls Smic migration necessary for left/right patterning. We speculate that Smic left/right patterning is linked to ABC-transporter mediated Smic migration dynamics. The US-Environmental Protection Agency and the National Institutes of Health supported this work.
Program Abstract #450
Characterizing the role of CDC-42 in cell invasion through basement membrane
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Basement membrane is the dense and highly cross-linked barrier that surrounds most tissues. Cell invasion through this specialized extracellular matrix is essential for developmental, physiological, and disease processes. Anchor cell (AC) invasion into the vulval epithelium during C. elegans uterine-vulval attachment is a genetically and visually tractable model to understand how cells transmigrate basement membrane in vivo. To identify genes required for AC invasion, we performed a sensitized genome-wide RNAi screen. This screen implicated cdc-42 in AC invasion. CDC-42 is a RhoGTPase activated in response to extracellular signals in many contexts, including processes important for AC invasion like cell polarity and cytoskeletal organization. Animals null for CDC-42 have highly penetrant AC invasion defects and a significant delay in initial breach of the basement membrane. Genetic and site of action studies indicate that CDC-42 acts within the AC downstream of the vulval cue. The vulval cue, an unknown signal secreted from the underlying vulval cells, acts in a molecularly unknown manner to activate and control the timing of invasion. A genetically encoded sensor for activated CDC-42 shows punctate localization, similar to highly dynamic F-actin based punctate structures called invadopodia that breach the basement membrane and occur in the AC approximately three hours prior to invasion. Interestingly, CDC-42 is overexpressed in many cancers and has been associated with the formation of invadopodia. Given the known role of the vulval cue in dictating the timing of invasion and the delay in breach in cdc-42 mutants, we hypothesize that CDC-42 might activate the invadopodia at the time of invasion. This work was funded by grant from the National Institutes of Health to DRS (R01GM079320, R01GM100083).

Program Abstract #451
PMR-1/SPCA is important for cell migration during gastrulation in C. elegans
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In C. elegans, three distinctive cell migration events occur that are crucial for final cell positioning during embryonic development. The first involves ingestion of cells from the surface of the embryo into the blastocoel cavity during gastrulation. The second is the movement of cells along the surface of the embryo following ingression. The third involves the epiboly of epidermal cells to enclose the embryo in a single cell layer of epidermis. Using a genetic screen to identify conditional mutants with morphogenesis defects, we identified loss-of-function alleles of the pmr-1 gene. PMR-1 is a Secretory Protein Calcium ATPase (SPCA); mutations in the human SPCA ortholog result in Hailey-Hailey disease, a dominantly inherited skin disorder. In C. elegans, phenotype analysis indicates pmr-1 is crucial for migration of cells along the embryo surface following ingression. By analyzing the migration of individual cells during development, we determined rates of ventral, anterior, and posterior surface cell migrations are significantly slower in pmr-1 mutants compared to controls. Temperature-shift experiments indicate normal pmr-1 gene activity is required during this stage of development. The effect on migration appears direct, as ingestion and epiboly, as well as cell fate determination, cell death, cell attachment, and polarity, are all normal. Genetic interaction studies indicate embryonic lethality rates in pmr-1 mutants are enhanced or suppressed by altering the activity of calcium channels itr-1/inositol triphosphate receptor and unc-68/Ryanodine receptor orthologs, suggesting calcium dynamics are critically important for cell migration at this stage of embryogenesis. These results identify a new gene, PMR-1/SPCA, as important for the second stage of cell migration during embryonic development in C. elegans. This research may also shed light on defects observed in Hailey-Hailey patients. Funding: NSF MRI Grant & Grinnell College.

Program Abstract #452
Developmental Pathways Regulating Melanoma Cytoskeletal Dynamics in vivo
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Communication between cells and their microenvironment is critical for development and disease. During cancer progression, interactions between tumor cells and immune cells play a large role in the initiation of metastasis. In malignant melanoma, it is unclear how a melanocyte transitions from a premalignant nevus to an invasive tumor cell, and which components in the tumor microenvironment regulate this switch. A major limitation to understanding this switch in vivo is the lack of genetically tractable model systems that are amenable to high-resolution imaging. We will overcome this obstacle by visualizing tumor cell motility directly in zebrafish. Using human-in-fish xenotransplantation approaches, we found that human melanoma cells metastasize from the site of injection. To visualize the interactions between melanoma cells and immune cells that regulate the initial steps of metastasis, we injected melanoma cells expressing LifeAct-eGFP into larvae expressing cell type-specific reporters. Live imaging of the primary tumor revealed that tumor cells respond to contact with host macrophages by extending more actin-rich protrusions than those tumor cells not in contact with macrophages. Preliminary data suggests that melanoma cells and cells in the tumor microenvironment communicate via Notch signaling during metastasis. We are currently testing the hypothesis that Notch signaling...
between melanoma cells and macrophages regulate tumor cell cytoskeletal dynamics to trigger malignant melanoma cell exit from the primary tumor. This work was funded by NIH F32CA159663 to MRJ.

**Program Abstract #453**

**MT-1 MMP protein levels affect how this multifunctional protease changes the behaviour of breast cancer cells**

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The matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) are the primary regulators of extracellular matrix (ECM) remodeling. However, it is now known that MMPs and TIMPs are multifunctional proteins with ECM independent functions such as activation of latent growth factors, shedding of cell surface proteins and induction of cell signaling cascades. Membrane Type-1 (MT-1) MMP and TIMP-2 are unique members of the MMP/TIMP family as they possess functions independent of proteolytic activity or MMP inhibition. Specifically, MT-1 MMP and TIMP-2 can interact to activate proMMP-2 and signal to the nucleus through the MAPK/ERK pathway to increase migration and proliferation of breast cancer cells. Current work assumes that the functions of MT-1 MMP are related and linear, meaning that an increase in MT-1 MMP will result in a proportional increase in proMMP-2 activation, migration and cell signaling. In the work shown here, we demonstrate that the level of MT-1 MMP expression is critical as to how MT-1 MMP functions to change the behavior of breast cancer cells. In this study, we have created MCF-7 breast cancer cell lines, which are naturally deficient in MT-1 MMP, that stably express different levels of MT-1 MMP. We show that MCF-7 cells expressing high levels of MT-1 MMP activate proMMP-2, but only cells expressing middle and low levels of MT-1 MMP efficiently interact with TIMP-2 to enhance proMMP-2 activation and increase migration. Understanding how MT-1 MMP levels affect the changes in cell behavior mediated by this multifunctional protease will be an important step to creating a model of MT-1 MMP function in order to comprehensively understand its role in development and disease. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC).

**Program Abstract #454**

**Toddler/Apela regulates germ layer migration during vertebrate gastrulation**

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Gastrulation specifies and shapes the germ layers resulting in generation of the body axis and proper organization of organ precursor cells. While germ layer induction and later convergence and extension movements have been studied in detail, the mechanisms that link these two processes to promote internalization and migration of mesendoderm remain unclear. We discovered a novel peptide, Toddler/Apela, which is highly conserved throughout vertebrates and essential in zebrafish. Using in situ hybridization, we show that reduced or excess Toddler causes defects in internalization and subsequent migration of endodermal and mesodermal germ layers. Toddler mutants resemble loss of the GPCR Apelin Receptors (Aplnr) and expression of Apelin, the only previously known ligand for Aplnr, rescues toddler mutants. Our results suggest that Toddler signals through Aplnr to promote internalization and cell migration after germ layer specification and before dorsal convergence and extension.

**Program Abstract #455**

**p120 Catenin is an Essential Protein in the Development of Vertebrate Embryos**

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During gastrulation zebrafish embryonic mesoderm cells migrate towards the dorsal axis. The migrating cells converge, extend, and intercalate to create a longer, narrower dorsal axis containing the notochord and somites (Myers et al 2002). p120 catenin binds the cytoplasmic tail of E-cadherin to stabilize and strengthen adhesion. When in the cytosol p120 catenin regulates small Rho GTPases to mediate actin dynamics (Anastasiadis and Reynolds, 2001). Our previous studies (Hsu et al. 2012) showed that knockdown of p120 catenin by an antisense RNA splice-site morpholino resulted in widening and shortening of the anterior-posterior axis of the embryo. Thus, p120 catenin is necessary for cell migration to the dorsal axis. We also showed that Cdc42 and Rac GTPases are downstream of p120 catenin as they rescue its knockdown. The regulatory domain of p120 catenin contains tyrosine and serine/threonine residues that are phosphorylated. Currently we are modifying these sites to phenylalanine or alanine residues that lack the ability to be phosphorylated or to glutamic or aspartic acid residues as charged phosphomimetic residues. By coinjecting mRNAs of these modified forms of p120 catenin with the splice-site morpholino, we are determining which phosphorylation sites are essential for axis extension. The tyrosine at position 228 in p120 catenin is not required for cell motility. Phenylalanine mutants rescue the morpholino knockdown phenotype. Serine residues at positions 268, 269 and 288 are currently being investigated.

**Program Abstract #456**

**Structure function analysis of Tre1 GPCR**

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In many organisms primordial germ cells (PGCs) are born in one location and must interpret a variety of guidance cues in order to reach their final destination in the somatic gonad. I study how PGCs see and interpret environmental cues in the complex three-
dimensional environment of the embryo over time. It has previously been shown that the G protein coupled receptor Tre1 is essential for the polarization and initiation of active migration of Drosophila PGCs out of the midgut primordium (1). I took two approaches to dissect Tre1 and its signaling pathway. First, in a dominant modifier screen I found 23 putative interactors. Secondly, I did a structure function analysis by manipulating known sequence motifs within Tre1 GPCR and by generating chimeric receptors with a highly related GPCR, Moody, which does not function in PGCs. Misexpression of moody in PGCs leads to a failure to exit the midgut. In absence of tre1 however, moody expression is able to rescue germ cell motility, but not directionality. This suggests that moody and tre1 share some downstream components but cannot interpret identical cues. Moody-Tre1 chimeric proteins showed that Tre1 transmembrane region but not the C-terminal cytoplasmic domain mediates signaling responses. Next, I mutated the highly conserved DRY (2) and NPxxY (3) domains within the transmembrane domains. These experiments suggest that both domains are required for proper PGC migration. I am now using tissue culture assays to address how these two domains affect downstream signaling.

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Program Abstract #457

miR-219 regulates neural precursor differentiation by direct inhibition of apical Par polarity proteins

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During development neural precursors (NPs) both divide, to expand the cell population, and produce many different kinds of neurons and glia. This balance appears to be regulated by Par complex proteins, which polarize neural precursors and can thereby direct daughter cells for different fates. How Par complex proteins are regulated to appropriately polarize NPs remains unknown. In recent years, regulation of gene function by microRNAs has emerged as an important mechanism during development. Using bioinformatics we identified the polarity genes pard3 and prkci as candidate targets for microRNA219 (miR-219). miR-219-deficient zebrafish embryos have a deficit of oligodendrocytes, the myelinating glial cells of the CNS. Because a disruption in polarity could affect the types of cell divisions that NPs undergo, thus altering the balance of cell types that arise, we hypothesized that neural precursor maintenance is regulated by modulation of polarity cues through miR-219. We found that miR-219 inhibited expression of pard3 and prkci mRNAs via target sites in the 3’ untranslated region and that blocking access to these sites using Target Protector function retained apical protein expression, remained in the cell cycle and failed to differentiate. In addition to the previously reported OPC deficit we also found a decrease in late born neurons supporting the hypothesis that negative regulation of Par protein expression by miR-219 promotes cell-cycle exit and differentiation. These data provide evidence for a new mechanism of NP regulation, in which miR-219 regulates Pard3 and Prkci levels, thereby regulating the transition of dividing neural precursors to differentiated neurons and glia.

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Program Abstract #458

Requirement of Prdm13 in specification of inhibitory dorsal interneurons

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Specification of a balanced inhibitory/excitatory population of dorsal spinal cord interneurons is essential for correct interpretation of stimuli from the environment. Ptf1a is essential for inhibitory interneuron specification and antagonizing the excitatory interneuron program specified by the proneural bHLH factor Ascl1. While the importance of Ptf1a in this process has been well characterized, the downstream effector capable of antagonizing the excitatory program remained unknown until the identification of Prdm13. Prdm13 is a zinc-finger transcription factor with restricted expression in the central nervous system. At mouse embryonic day 10.5, it is expressed in the proliferating progenitor population of the dorsal neural tube. Prdm13 is a downstream target Ptf1a, and in chick functions as a transcriptional repressor capable of antagonizing the neuronal sub-type specification activity of Ascl1. Although analysis in chick has shown the importance of Prdm13 in this process, this factor is expressed in other tissues of the CNS, such as cerebellum and retina, and its function in these organs remains unknown. Development of a Prdm13 knockout mouse model using zinc-finger nuclease technology shows that mice null for Prdm13 lose the dI4 inhibitory interneuron population, have excess dI5 excitatory interneurons, and die early neonatally. Interestingly, this model also revealed previously uncharacterized regulatory networks where Prdm13 feeds back to regulate expression of Ptf1a and Ascl1, as well as itself. Mechanistically, Prdm13 can complex with Ascl1 through the Ascl1 bHLH domain, and can bind to a subset of sites genome wide. Ongoing experiments are determining if Prdm13 functions to regulate the balance of inhibitory/excitatory neurons in other tissues such as cerebellum and retina. Together, these studies are providing insight into intrinsic programs for generating a balanced network of inhibitory/excitatory neurons.

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Characterization of Mouse Otic Sensory Lineage Specific Genes and Investigation of Mechanisms of Otic Gene Regulation

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The otic sensory lineage harbors unique potential to produce inner ear sensory cell populations including mechanosensory hair cells and supporting cells. The goals of this project are 1) to identify genes that are expressed strongly and specifically in the otic sensory lineage from early otic progenitors to differentiated sensory cells, and 2) improve our understanding of how transcriptional states and related cellular identities specific to the otic sensory lineage are initiated and maintained.

We used a comparative microarray approach to identify and rank genes that distinguish otic sensory lineage cells from the other populations of the mouse embryo. Embryonic day 10.5 microdissected otic vesicles and flow-sorted postnatal day 3 cochlear hair cell and supporting cell populations were compared to non-sensory tissues including whole embryos with the otic regions removed. Two types of otic sensory lineage consensus genes were identified based on ranked fold change over non-otic: 1) genes with high ranking in otic vesicle and supporting cells, but not hair cells, and 2) genes with high rankings in all three otic populations. The former category included

Oclin, Oc90, and Col9a2, which placed in the top 25 genes for both the otic vesicle and supporting cell populations.

Genes with inclusive consensus across all three otic populations were more rare and only one gene ranked in the top 25 for each. The ubiquitin ligase gene Fbxo2, placed in first for the otic vesicle and within the top six for both hair cells and supporting cells.

Additional methods for characterization of expression patterns of several top otic consensus genes produced results consistent with microarray predictions. Expression of Fbxo2 was found to be particularly robust and specific to the otic sensory lineage. Current investigations of development and gene regulation in the otic sensory lineage include Fbxo2 reporter mouse lines, enhancer studies, and a stem cell model of otic differentiation.

Cdx4 regulates onset of spinal cord neurogenesis.
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 cell 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 spinal cord neural primordial cells. In gain and loss of function experiments in chicks, we show that Cdx4 functions as a differentiation switch by activating neurogenic factor Pax6 while repressing the pluripotency factor Cash4. Bioinformatics analysis supports a direct regulation of Pax6 by Cdx4, as Pax6 has 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 Hes5 expression, indicating that Cdx4 also regulates other differentiation genes. Together our findings suggest that, in addition to its previous known role in patterning, Cdx have a key function in regulating onset of spinal cord neurogenesis.

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Program Abstract #463
Environmental regulation of spinal cord development
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The interplay between environmental factors and developmental processes is of interest for understanding how organisms adapt to their environment. _Xenopus laevis_ are ectotherms and the environmental temperature influences their developmental rate. However, it is unknown whether temperature influences other aspects of nervous system development, such as neuronal differentiation. Previous studies have demonstrated that spontaneous Ca^{2+} spike activity during a critical period influences neuronal phenotype specialization in _Xenopus_ spinal cord. The following study investigates how temperature influences spontaneous Ca^{2+} dynamics during this critical period in spinal cord development. _In vivo_ Ca^{2+} imaging of dorsal and ventral spinal cord of stage 22-24 embryos was performed at three different temperatures (14.5, 22.5, 26.5°C). A paired comparison of the data collected indicates that patterns of spontaneous Ca^{2+} activity are modified in response to acute changes in temperature. In dorsal neurons, decay times of both Ca^{2+} spikes and waves are longer at 14.5°C compared to 26.5°C. In ventral neurons the frequency of Ca^{2+} spikes is inversely related to the temperature, with a Ca^{2+} spike frequency about 3-fold higher at 14.5°C than at 26.5°C. To determine the potential effect that changes in Ca^{2+} activity may have on motor neuron differentiation, 3-day old tadpoles overexpressing the voltage-gated sodium channel Na_{2a}, which enhances Ca^{2+} spike activity, were processed for immunostaining with the motor neuron transcription factor Hb9. Results show that the number of motor neurons increases in Na_{2a}-overexpressing embryos. Current efforts are focused on investigating the differentiation of dorsal and ventral spinal neurons in embryos grown at different temperatures. This study will contribute to our understanding of how environmental cues interact with spontaneous Ca^{2+} activity during development to influence formation of the nervous system.

Program Abstract #464
Rorschach: The brain is never right
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Left-right structural and functional asymmetries in the central nervous system are found throughout the animal kingdom. Despite this prevalence, our understanding of the molecular mechanisms that underlie the development of asymmetry and the functional consequences of that asymmetry is far from complete. In order to identify novel genes affecting left-right patterning, we performed a forward genetic screen for mutations that cause a disruption in the normal asymmetric development of the zebrafish habenula. We isolated a mutant, _rorschach_ (rch), in which the habenula is almost fully symmetric, yet gut, heart and parapineal asymmetries are completely unaffected. The _rch_ mutation is homozygous viable, fully penetrant and has no maternal effect. _rch_ mutants have a _V1122D_ substitution in the transmembrane domain of the _cache domain containing 1_ (cachd1) gene that disrupts protein localization. Although _cachd1_ is highly conserved across vertebrates, enticingly little is known about the gene in any organism. In zebrafish, _cachd1_ is strongly expressed in the presumptive epithalamus and other neural tissue beginning at somitogenesis. Epistasis experiments and additional supporting data suggest that _Cachd1_ functions upstream of both the Notch pathway and the canonical Wnt pathway in the development of habenular asymmetry. The results of transplant studies indicate that _Cachd1_ functions non-cell autonomously. We are currently testing a model in which _Cachd1_ sequesters an elusive ‘parapineal signal’ on the left side, thus preventing the right habenula from receiving that signal and causing it to default to the ‘right’ state.

Program Abstract #465
An _atoh1_ proneural domain at the mid-hindbrain boundary is subfunctionalized for ventral isthmic neuron specification in

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152
The vertebrate atonal gene atoh1 plays essential roles in the specification of granule neurons in the cerebellum and ventral isthmic nuclei. Zebrafish have three atoh1 genes, atoh1a, 1b and 1c, all of which are expressed in granule neuron progenitors in the upper rhombic lip (URL). We have found that in addition, atoh1c is uniquely expressed in a small population of cells located at the presumptive mid-hindbrain boundary (MHB) well before its expression in the URL. After the MHB constriction has formed, atoh1c expression becomes restricted to progenitor cells in the posterior-most region of the hindbrain known as the posterior tectal membrane. This early MHB expression is under distinct regulation: through mutant and chimeric analysis we have found that MHB atoh1c expression requires the cell non-autonomous activity of Gbx and is subject to lateral inhibition by Notch. In order to understand more about the regulation, function and developmental fate of the atoh1c MHB population we used bacterial artificial chromosome recombin engineering to target a gal4FF cassette to the atoh1c locus and established a stable transgenic line that drives UAS-dependent reporter expression in the atoh1c MHB cell population. Using this transgenic line, we have found that the MHB atoh1c-expressing cells are the progenitors of an as-yet unidentified population of neurons that reside ventrally in hindbrain rhombomere 1 (r1). We hypothesize that atoh1c has been sub-functionalized in fish for the specification of these ventral isthmic neurons. Using TAL effector nucleases, we have generated several atoh1c mutant alleles. Future analysis will focus on understanding the regulation of atoh1c, the identification of the ventral r1 neuronal population and the role of atoh1c in the specification of that population. This work is supported in part by the Univ. of Washington NIH Developmental Biology Predoctoral Training Grant and the Fred Hutchinson Cancer Research Center.

**Program Abstract #466**

**Elucidating the Lineage Relationships between Neurons and Oligodendrocytes in the Forebrain**

Santos Franco, Caitlin Winkler, Brett Dwyer

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The developmental origins of neural progenitor cells that give rise to oligodendrocytes in the mammalian forebrain have recently begun to be elucidated. It is now clear that oligodendrocytes arise from progenitor cells in several distinct areas of the developing forebrain, including the dorsal neocortex. Interestingly, progenitors in the dorsal ventricular zone also give rise to various types of excitatory projection neurons. However, the lineage relationships between projections neurons and oligodendrocytes are not yet known. We have recently used genetic fate-mapping approaches to identify a subset of progenitor cells in the dorsal neocortex that are pre-specified and fate-restricted to specifically produce callosal projection neurons, but not corticofugal projection subtypes. We now have new evidence that suggests this same lineage of fate-restricted progenitors also gives rise to neocortical oligodendrocytes. We are currently undertaking studies to determine whether a single progenitor can first make callosal projection neurons and then oligodendrocytes, or whether the lineage is further subdivided. This potential lineage relationship between callosal projection neurons and oligodendrocytes raises the interesting possibility that the two cell types may have co-expanded during evolution of the corpus callosum.

**Program Abstract #467**

**Making a brain in a dish: how vascular cells influence cortical neural stem cell fate decisions**

Stephanie Snyder, Jake Cain, Natalie Crawford, Diane Darland

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Brain development requires tight cell and molecular regulation for proper neural stem cell (NSC) proliferation and differentiation. Increasing interest in NSCs has arisen due to the flexibility inherent within their proliferative capacity and their ability to generate multiple neuronal cell types. Our main focus is on the cortex, the primary integrator for higher order brain function. Cortical NSC proliferation and differentiation occur in concert with blood vessel ingress into the primitive cortex to vascularize the neuroepithelium. One potential influence derived from vascular cells is Vascular endothelial growth factor A (Vegf). Vegf has the ability to regulate both neurogenesis and angiogenesis making it an attractive candidate for a role in regulating NSC fate choice. Our hypothesis is that NSCs require vascular cells to regulate their transition from NSC to differentiated neurons and that the Vegf isoforms are key contributors in this process. To address this hypothesis we are utilizing a triculture assay system to recapitulate the microenvironment of the early cortical neuroepithelium. We have immortalized neuroepithelium and explored their potential cell fate based on the influence of microvascular cells. Our results indicate that NSCs in contact with microvasculature express neural stem cell (Pax6 & SSEA-1), neural progenitor (Id1 & Id4), and neuron (Tbr1 & Otx2) genes. Also the presence of microvascular cells lead to a significant increase in NSC proliferation and cells exhibiting a mitotic profile. Furthermore transgenic mice expressing modified Vegf isoform profiles will be used to test the hypothesis that Vegf isoforms have distinct regulatory potential in the early cortical microenvironment. These investigations are important not only for understanding normal neural developmental, but also for clarifying the potential role of NSC in neural degenerative disorders. These investigations are supported by NIH/NINDS (AREA/R15 NS057807-01/-02).

**Program Abstract #468**

**Zic1 controls cranial placodes formation by regulating retinoic acid production**

Maria Belen Jaurena, Arun Devotta, Jean-Pierre Saint-Jeannet
Cranial placodes are thickening of the embryonic head ectoderm contributing to the paired sense organs and cranial ganglia. We have previously shown that the transcription factor Zic1 is necessary and sufficient to promote placode fate in Xenopus embryos. In a microarray screen to identify targets of Zic1 we isolated lipocalin-type prostaglandin D2 synthase (LPGDS) that shows restricted expression in the anterior neural plate at neurula stage. LPGDS knockdown using morpholinos caused a reduction of expression of two pan-placodal genes, Six1 and Foxi1c, as well as several markers for individual placodal domains, Dmrt4, Pax8 and Tbx2, indicating that LPGDS is required for placode formation. One of the major functions of LPGDS is the synthesis of prostaglandin D2 (PGD2) from its precursor PGH2. This enzymatic activity depends on the presence of a cysteine residue at position 65 (Cys65). We found that wild-type mouse LPDS or a mutated version lacking Cys65, were both equally efficient at rescuing Foxi1c expression in LPGDS morphant embryos, suggesting that LPGDS regulates placode formation independently of its enzymatic activity. Another function of LPGDS is as a carrier for retinoic acid (RA). Interestingly, genes strongly activated by Zic1 in the microarray included Raldh2, Cyp26a, and Crabp2, factors also involved in RA metabolism and function. Consistent with a role of LPGDS in RA signaling, Foxi1c expression domain was rescued in LPGDS morphant embryos by treatment with RA or the RA receptor agonist TTNPB. Moreover, using a RA-response element reporter construct (RARE-GFP) we showed that RA activity in the embryo is significantly reduced in the absence of LPDGS. Finally, using animal cap explants expressing Zic1 we demonstrated that Zic1 promotes placode fate non-cell autonomously, an activity that requires active RA signaling. We propose that Zic1 controls placode progenitor formation by regulating RA production at the anterior neural plate. (NIH-R01DE014212)

Program Abstract #469
Differential Neural Ectoderm Plasticity During *X. laevis* Embryogenesis
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During early embryogenesis, cells are plastic and capable of differentiating into many different cell types. As development progresses, cells become committed and eventually give rise to a specific cell type. Our lab is interested in understanding how in-vivo dynamics influence cell differentiation and plasticity. Previous work from our lab showed that neural ectoderm cells (brain and spinal cord precursors) remain plastic and responsive to muscle-inducing signals during gastrulation. However, the duration and parameters of this plasticity remain unclear. We hypothesize that neural fate specification occurs in an anterior to posterior fashion and involves the differential loss of cell plasticity. To examine this hypothesis, we grafted fluorescently labeled cells from the prospective anterior and posterior neural ectoderm to the prospective muscle region at various stages development and ask whether the grafted cells can adopt a muscle fate. We show that cells from the prospective anterior neural region lose their ability to change their fate and form muscle fibers by the end of gastrulation (stage 12.5) whereas cells from the posterior neural ectoderm cells retain their ability to change their fate and form muscle fibers until mid neurulation (stage 18). Thus, anterior neural ectoderm cells lose their plasticity prior to posterior neural ectoderm cells. Through additional transplantation experiments, we show that prospective neural cells are differentially responsive to regionally- and temporally-specific muscle-inducing signals. Together, these results offer new insights into the ability of the embryo to regulate cell plasticity during development.

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Program Abstract #470
Sox5 is essential for BMP-mediated plasticity and patterning in the early embryonic ectoderm
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Following initial germ layer formation, the animal pole cells of early Xenopus embryos have ES cell-like properties, with potential to give rise to all embryonic cell types. At the onset of gastrulation, this potential is progressively lost as lineage restriction initiates to give rise to the neural plate, and the prospective epidermis. Interestingly, cells at the neural plate border, the neural crest (NC), retain the capacity to contribute to non-ectodermal cell types. The molecular mechanisms that control the stem cell attributes of animal pole cells and NC cells have remained unclear, however. Here, we provide evidence that the SoxD family protein, Sox5 plays a central role in controlling developmental plasticity of the early ectoderm, and formation of NC progenitor cells. Sox5 is expressed maternally and restricted to the animal hemisphere of early cleavage and blastula stage embryos. In the ectoderm, as lineage restriction progresses, Sox5 expression is lost from most ectodermal cells and is enhanced at the neural plate border/in the NC. Sox5 deficient embryos fail to form NC progenitors and have gross patterning defects, including an expansion of the neural plate at the expense of epidermis and placodes. These patterning defects are reminiscent of perturbations of BMP signaling, and we show that Sox5 is essential for BMP function and target gene activation in the early ectoderm. Sox5 physically binds to Smad1/4, both in solution and on chromatin, to direct target specificity in response to BMP signaling. Our findings identify Sox5 as the long sought BMP R-Smad DNA-binding co-factor responsible for plasticity and patterning of the early ectoderm, and likely for other essential developmental roles mediated by BMPs.

Program Abstract #471
Paladin is an antiphosphatase that reveals phosphoregulation of neural crest development
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The neural crest is a multipotent, migratory, embryonic cell type that forms a variety of derivatives including the craniofacial skeleton and peripheral nervous system. Much work has gone into assembling the network of transcription factors that regulate neural crest cell fate, but what controls the activity of these proteins is less clear. To reveal the importance and function of phosphorylation in modulating protein function during neural crest development, we are studying the antiphosphatase Paladin. Paladin is expressed throughout early neural crest development and influences the expression of a subset of neural crest transcription factors. Both gain and loss of Paladin function disrupt early neural crest migration. Based upon mutational analyses we conclude that Paladin is an antiphosphatase that protects phosphorylated residues on factors required to achieve timely neural crest migration. We are currently using Paladin to identify these phosphorylation-modulated factors using both in vitro and in vivo assays. Yeast-two hybrid interaction studies identified a small number of Paladin interacting proteins with both phosphorylation-dependent and independent interactions. One candidate interactor, Myosin Heavy Chain 9 (MYH9), is enriched in premigratory and migratory neural crest cells, and we are currently working to confirm the Paladin/MYH9 interaction in vivo. Additionally, we are beginning to query Paladin-interacting proteins using immunoprecipitation from neural crest cells followed by mass spectrometry. Together these data will help us identify proteins that modulate neural crest development in a phosphorylation-dependent manner, thus expanding our understanding of an underappreciated level of control within the neural crest developmental program.

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Program Abstract #472
Nf2-Yap signaling controls the expansion of DRG progenitors and glia during DRG Development
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The dorsal root ganglia (DRG) are clusters of sensory neurons and satellite glia found at the dorsal root of the spinal nerves. They transmit sensory information from the body to the central nervous system. Neurons and glia in the DRG are derived from neural crest (NC) cells. Although factors regulating neurogenesis and gliogenesis in DRG have been studied extensively, molecular mechanisms governing the maintenance and proliferation of DRG progenitors (multipotent NC cells within the DRG) are largely unknown. Here we investigated the function of the Hippo pathway during DRG development. The key effectors of this pathway, transcriptional coactivators Yap and Taz, are expressed in DRG progenitors and glia during DRG development but are at least partially inhibited from activating transcription. Aberrant YAP activation leads to overexpansion of DRG progenitor and glial populations. We further show that the Neurofibromatosis 2 (Nf2) tumor suppressor, an upstream regulator of the Hippo pathway, inhibits Yap during DRG development. Nf2 is coexpressed with Yap/Taz in DRG progenitors and the glial lineage. Loss of Nf2 partially inhibited from activating transcription. Our study demonstrates that Nf2-Yap signaling plays important roles in controlling the expansion of DRG progenitors and glia during DRG development.

Program Abstract #474
Deficits in neural crest lineage segregation and GI motility in the Sox10Dom mouse model of Hirschsprung disease.
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The enteric nervous system (ENS) forms by the migration of neural crest (NC) cells to the foregut and down the length of the intestine. In Hirschsprung disease (HSCR), NC-derived cells (NCDCs) fail to fully populate the intestine, leading to aganglionosis in a variable length of intestine. Many HSCR patients suffer chronic constipation despite excision of the aganglionic bowel, suggesting that HSCR mutations not only disrupt migration but also NCDC development in ganglionated regions. We hypothesized that aberrant NCDC lineage segregation contributes to post-surgical GI dysfunction in HSCR patients. To test this hypothesis, we fate-mapped NCDCs in the Sox10Doom/+ HSCR mouse model with a Sox10Cre transgene that drives ROSA26R expression. We detected no changes in Sox10Doom/+ small intestine ENS patterning or total NCDCs. Interestingly, colonic neuron and glia proportions correlated highly with the length of aganglionosis in Sox10Doom/+ mice. We noted a significant increase in excitatory motor neurons in the duodenum and ileum, but a decrease in the colon that inversely correlated with length of aganglionosis. In contrast, inhibitory motor neuron proportions were unchanged in the duodenum and ileum, but increased in the colon with increasing lengths of aganglionosis. Because motor neuron types were perturbed differentially, we assessed GI motility. As expected, all 4-week old Sox10Doom/+ exhibited significantly reduced small intestine motility rates. Interestingly, 6-week old Sox10Doom/+ males exhibited increased gastric emptying rates, while Sox10Doom/+ females showed no deficits. Our GI motility findings are congruent with other neural developmental disorders; males tend to be more affected than females and the emergence of circulating sex hormones in females provides a compensatory effect. Overall, our findings suggest a mechanism for GI dysfunction in post-operative HSCR patients and implicate a novel role for Sox10 in neuronal subtype lineage choice.

Program Abstract #475
cAMP-protein kinase A dependent iridophore development requires mitfa in zebrafish
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Danio rerio zebrafish iridophores are specified from neural crest cells and represent an intriguing, tractable system for enforcing developmental biology concepts in an undergraduate research setting. Using this system, we have investigated the role of cAMP protein kinase A (PKA) signaling in the development of iridophores during larval stages. Activation of PKA, using adenylyl cyclase activator forskolin, reduces the number of differentiated iridophores in wildtype larvae. PKA inhibitor, H89, increases iridophore number, supporting a specific role for PKA during iridophore development. To determine the developmental stage requirement for PKA signaling, we examined iridophore marker pnp4a expression in forskolin treated wildtype larvae. In situ hybridization results indicate that pnp4a expression is reduced in wildtype forskolin treated individuals, suggesting that PKA signaling regulates pnp4a expression. As mitfa/nacre mutants have extra iridophores as compared to wildtype larvae, we examined its requirement during PKA dependent iridophore development. Similar levels of pnp4a expression in forskolin treated and control mitfa/nacre mutants indicates a requirement for mitfa during PKA iridophore dependent development. Last, forskolin treatment increases neural crest marker and mitfa repressor foxd3 expression, as detected by BAC foxd3::GFP transgenic and in situ hybridization analysis. Our work suggests a model where PKA works upstream of foxd3 to regulate mitfa and pnp4a interactions during iridophore specification from zebrafish neural crest. This work was partially supported by the Melanoma Research Foundation and Washington State Univ. Vancouver Faculty Mini Grant Awards to CDC.

Program Abstract #476
Precise restriction of BMP and Wnt signaling is required for articular cartilage differentiation
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Histological and ultrastructural studies over the last few decades have generated a clear picture of the cellular changes associated with articular cartilage development. However, a well defined molecular mechanism for articular cartilage differentiation is still not completely elucidated. There are two critical questions in the field which remains controversial. First, what is the location and identity of progenitor cells that contribute to the growth of articular cartilage? And second, how does a single cartilage element acquire two distinct fates juxtaposed to each other e.g. the transient cartilage which undergoes hypertrophy and is replaced by bone while articular cartilage remains as permanent forever?
In this report, using pulse-chase of actively proliferating chondrocytes, we demonstrate that similar to longitudinal growth of transient cartilage, interstitial growth of articular cartilage take place by acquiring new cells from the proliferating chondrocytes situated near the distal ends of skeletal anlagen. Our gain-of-function experiments in chick and mice show that nascent proliferative chondrocytes are bi-potent and adopts transient or the articular cartilage fate depending on their exposure to pro-transient cartilage or pro-articular cartilage signals, for example, BMP and Wnt, respectively. By conditional knock-out of Noggin (Col2a1-Cre;Nogginfl/fl) we observed ectopic expression of transient cartilage markers at the cost of joint formation. Our results suggest that a domain of Noggin expression insulates proliferating chondrocytes at the ends of developing skeletal elements from pro-transient cartilage signals thus allowing them to adopt the articular cartilage fate under the influence of Wnt signaling. Based on our observations we have proposed a model for concomitant differentiation of transient and articular cartilage development.
NIH grant (GM 49346) R.H.

Program Abstract #477
The SR-like protein Tra2b is required for somitogenesis and regulates a novel inhibitory wnt11b isoform.
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In a previous expression-cloning screen in Xenopus embryos, we isolated cDNAs encoding numerous RNA-binding proteins. One of those, transformer-2b, encodes a serine-arginine rich SR-like protein that regulates splicing, but whose biological function in vertebrates is unknown. Here we combine the power of frog embryology with RNA-seq to identify splice changes and determine their contribution to embryogenesis. Morpholino-oligonucleotide mediated knockdown of Tra2b in X. laevis or X. tropicalis results in embryos with multiple defects, including severely shortened A-P axes and failure to form somites. Using a custom RNA-seq analysis pipeline for detecting novel isoforms, we identify 142 significant splice changes in 133 genes, of which 85% have not been previously documented. A large majority (81%) is whole intron retention or exon skipping in tra2b morphants.
One of these novel changes is retention of the last intron of wnt11b, resulting in a truncated Wnt11b ligand (Wnt11b-short) that is similar to, and at least as potent as, a previously engineered dominant-negative ligand (Tada and Smith (2000). Development 127, 2227–2238). Using animal cap experiments, we show that Wnt11b-short inhibits cardiac gene induction by Wnt+Activin. Also, like the engineered dominant-negative, Wnt11b-short inhibits pronephric tubule formation. To determine the contribution of the Wnt11b-short to the overall phenotype of tra2b morphants, we designed a splice blocking MO that specifically causes retention of the last intron in wnt11b. These morphants specifically recapitulate the somitogenesis defects, but not other defects in tra2b morphants.
In summary, we have identified a novel inhibitory Wnt ligand which is controlled by Tra2b. To our knowledge, this is the first example of regulated alternative splicing of a Wnt ligand and adds additional intricacy to an already complex signaling pathway. Future research will focus on the biology of other novel splice changes.
**Program Abstract #478**

**Canonical Wnt, FGF, and BMP signaling interact to pattern axial stem cell derived mesoderm**

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Vertebrate embryos form their body through a process called posterior growth, where the head forms first and the rest of the body develops progressively towards the posterior end. After gastrulation, a population of axial stem cells in a structure at the posterior-most end of the embryo called the tailbud, fuels the process of posterior growth by contributing cells to newly forming tissues. Axial stem cells make a basic germ layer decision between neural ectoderm and mesoderm, but it is unclear how and to what extent the newly formed mesoderm is patterned. We used zebrafish heat-shock inducible transgenic lines to temporarily inhibit or activate the canonical Wnt, FGF, and BMP pathways during tailbud stages and examined mesodermal cell fate. We found that Wnt and FGF act at different steps to promote dorsal (paraxial) mesoderm formation and inhibit ventral (vascular endothelial) mesoderm, while BMP has a reciprocal effect, promoting ventral and inhibiting dorsal mesoderm formation. Additionally, we find that within the paraxial mesoderm, somite fate (which gives rise primarily to skeletal muscle and bone) is not determined until immediately prior to physical somite formation. Our results indicate that axial stem cells generate basal mesoderm that remains highly plastic, and is patterned through a complex interaction between Wnt, FGF, and BMP signaling.

**Program Abstract #479**

**Nr2fla Act Downstream of RA Signaling to Promote Pharyngeal Muscle at the Expense of Ventricular Cardiomyocytes in Zebrafish**

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Many developmental syndromes have both cardiac and pharyngeal defects suggesting similar molecular mechanisms may control the development of these progenitor populations. Furthermore, recent studies have indicated that cardiac and pharyngeal muscle (PM) share common progenitors. However, upstream pathways that control the cardiac and PM progenitor fate decisions are not understood. Retinoic acid (RA) signaling is required for proper cardiac and PM development indicating it is a candidate to direct fate decisions between these progenitors. We identified *nr2fla* as a target of RA signaling in the anterior lateral plate mesoderm (ALPM). Interestingly, *nr2fla* depletion leads to increased ventricular cardiomyocyte (VC) number, while RA signaling depletion leads to an increase in both atrial and VC number. To determine the origin of the excess VC's in *nr2fla* deficient embryos, we performed in situ hybridization and fate-mapping experiments. We found a posterior expansion of VC progenitors (VPs) within the ALPM suggesting a possible fate transformation with adjacent mesodermal cell types. To determine if other ALPM populations were affected in *nr2fla* deficient embryos, we examined other mesodermal outcomes in our fate maps and with immunohistochemistry. We found a loss of PMs comparable to that found in RA signaling deficient embryos, while pharyngeal endothelial cells were not affected. Blastula transplantation analysis indicates *nr2fla* acts cell autonomously to direct VP and PM specification, supporting that it regulates a fate decision between VC and PM progenitors (PMPs). Interestingly, when we examined the PMP markers *tbx1*, *tcf21*, and *six1b*, they were only slightly decreased, while *Tg(tcf21:GFP)* zebrafish still show *tcf21*+ cells contributing to the remaining PMs in *nr2fla* deficient embryos. Together, our data suggest that in the ALPM, *nr2fla* acts downstream of RA signaling to promote PMP specification at the expense of VPs through a novel *tbx1/tcf21*-independent pathway.

**Program Abstract #480**

**TCF acts as a transcriptional switch to regulate fate choice in cardio-pharyngeal progenitors in the ascidian species, Ciona intestinalis**

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In invertebrates, canonical Wnt signaling plays opposing roles during heart development: it first activates cardiogenic mesoderm and then inhibits the formation of cardiomyocytes. However, due to the complexity of vertebrate embryos, these biphasic mechanisms are not still well understood. Here, I take advantage of the simplicity of cardio-pharyngeal mesoderm of the chordate model *Ciona intestinalis* to study the role of TCF, a transcription factor of the canonical Wnt pathway, in cell fate specification. *Ciona* possesses only two pairs of bilateral bipotent cardio-pharyngeal precursor cells called trunk ventral cells (TVCs). They divide asymmetrically forming median first heart precursors (FHPs) and lateral secondary TVCs, the latter giving rise to the second heart precursors (SHPs) and the pharyngeal muscle precursors, known as atrial siphon muscle (ASM) precursors. TVCs up-regulate TCF and overexpression of a dominant-negative form of TCF in the TVC progeny blocks the expression of the secondary TVC markers *Hand-like* and *Tbx1/10*, and the ASM specific transcription factor *COE*. Furthermore, using *Dickkopf* expression as a read-out of Wnt signaling, we observe that Wnt activity is likely restricted to secondary TVCs. Finally, we show that mis-expression of a TCF DNA-binding mutant induces ectopic expression of *Hand-like* in the FHPs, revealing a potential repressor function of TCF in the heart. We propose a model of a transcriptional switch, whereby asymmetric Wnt activity converts TCF into a transcriptional activator in the secondary TVCs, while TCF represses *Hand-like* in the FHPs. Thus, the dual role of TCF sharpens the fate decision between ASM and heart.
In vitro Titration of Wnt/β-catenin Signaling Enables Efficient Fate Specification of Lateral Plate Mesoderm Derivatives
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During development, dosage of morphogens like Wnt/β-catenin, Activin/nodal and BMPs are critical for defining the patterning of lateral plate mesoderm and specifying down-stream derivatives including cardiomyocyte and endothelial fates. Based on these embryological data, we used Activin A/BMP4 titration to direct hESC-derived primitive streak patterning. During the gastrulation phase of differentiation (day 2), we found that anterior (100 ng/mL Activin A and 5 ng/mL BMP4 (A100/B5)) and posterior (A50/B40) primitive streak were appropriately specified on the basis of Wnt/β-catenin signaling and gene expression analysis. As expected, cardiomyocytes developed robustly from cardiac progenitor cells (CPCs) differentiated under conditions of A100/B3 (90% cTnT+) compared to A50/B40 (14% cTnT+). Using the same Activin A/BMP4 titration in combination with conditions permissive to endothelial development, we found that endothelial cells (ECs) can be generated with equal efficiency (90% KDR+/CD34+) from A50/B40 as well as A100/B3 conditions. Human ESC-derived ECs generated greater than 90% CD31+ cells that expressed mature endothelial markers such as von Willebrand Factor and had significant capacity for sprouting angiogenesis. When placed under appropriate conditions, hESC-derived ECs had hematopoietic potential. We tested the hypothesis that endothelial cells could be redirected into the cardiac lineage by manipulating Wnt/β-catenin signaling. Inhibiting Wnt/β-catenin signaling in A100/B5–ECs but not A50/B40–ECs activated the cardiac gene program resulting in a fate switch to cardiomyocyte development with greater than 90% efficiency. We have concluded that subtle titrations of Activin A/BMP4 are sufficient to pattern an anterior to posterior polarization of hESCs that recapitulates normal events during primitive streak formation and gastrulation. Furthermore, we have revealed a novel mechanism by which to manipulate endothelial cell subtypes into the cardiac lineage.

Evidence of a lineage shift between natural (NK) killer cells and T lymphocytes in the spleen and blood of neonatally thymectomized, young adult C3H mice.
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The present study was designed to assess the influence of neonatal thymectomy on the proportions of natural killer (NK) lymphocytes and other (non-NK) lymphocytes in the spleen and blood of young adult mice. The progenitors (precursors) of both the T lymphocyte and NK cell lineages are located in the bone marrow, the organ of new cell production for both these lineages and where they both derive from a bipotential stem cell. Newborn C3H mice were thymectomized when they were 8–12 hr old by a process in standard use in our laboratory. Control infant mice, identical in every way, were sham thymectomized. When thymectomized and control mice reached 8 wk of age, their spleens and blood were prepared by our well-established methods. Smears of both organs were analyzed for their proportions of NK cells and other (non-NK) lymphocytes. The results showed that in thymectomized mice, spleen and blood had significantly increased proportions of NK cells, vs sham-thymectomized controls, while the other (non-NK) lymphocytes in both the spleen and blood were significantly decreased in thymectomized mice vs control. Moreover, the actual percentage decrease in this group of other (non-NK) lymphocytes in the thymectomized animals was almost precisely the same as the percentage gain in NK cells in both organs. We interpret these findings as evidence suggesting that in the absence of a thymus which would normally convert progenitor T cells into mature functional T lymphocytes, these progenitors which are products of the bi-potential T/NK stem cell, come under the influence of NK cell-stimulating factors, thus causing the shift toward the NK cell lineage in the 2 peripheral organs, i.e., the spleen and the blood.

Investigating the Role of Transmembrane Voltage Potentials During Embryogenesis
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Embryogenesis requires strictly regulated spatiotemporal control of cells in order to successfully develop functioning tissues and organs. In addition to genetic networks and biochemical cues, bioelectric cell signaling controls proliferation, migration, and differentiation during development. Bioelectric signaling arises from ion channels and pumps which generate voltage gradients across cell membranes, producing localized electrical fields both in individual cells and across cell populations. Brief changes in cell physiology triggered by bioelectrical signals lead to permanent changes in anatomical organization through interactions with transcriptional networks and biochemical signals. However how these physiological changes interact with the genetic components of organisms is just beginning to be investigated. Previous research has shown that disruption of endogenous voltages early in embryogenesis can lead to left-right asymmetries as well as induction of ectopic organs such as eyes. Our current work investigates the effects of manipulating bioelectric currents on the development of a larval organ, the cement gland, using the model organism Xenopus laevis. Using molecular tools designed to alter membrane voltage (e.g., the hyperpolarization or depolarization of cells via overexpression of ion channels) we examined the development and patterning of tissues during early stages of development. Interestingly these manipulations resulted in the expression of ectopic cement glands and serves to further demonstrate the importance of bioelectric cues during pattern formation and organogenesis.

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The Hippo pathway controls border cell migration through distinct mechanisms in border and polar cells of the Drosophila ovary

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The Hippo pathway is a key signaling cascade in controlling organ size. Core components are two kinases, Hippo (Hpo) and Warts (Wts), and a transcriptional coactivator Yorkie (Yki). YAP (Yki homolog in mammals) promotes epithelial-mesenchymal transition and cell migration in vitro. However, whether the Hippo pathway controls cell migration in vivo remains unclear. We use border cell migration in the Drosophila ovary as a model to study Hippo pathway functions. In the ovary, polar cells secret Unpaired (Upd), which activates JAK/STAT signaling of the neighboring cells and specifies them into border cells. The border cells form a cluster with polar cells and undergo migration. Here, we find that hpo and wts are required for border cell migration. In border cells, over-expression of hpo disrupts polarized distribution of actin cytoskeleton and migration. In polar cells, knockdown of hpo, wts, or over-expression of yki impairs border cell formation and migration. These manipulations in polar cells reduce JAK/STAT signaling activity in border cells. In upd-lacZ background, b-galactosidase is increased in yki mutant and decreased in hpo mutant polar cells. Furthermore, forced-expression of upd in polar cells rescues defects of border cell induction and migration caused by wts knockdown. These results suggest that Yki negatively regulate border cell induction by inhibiting JAK/STAT signaling. Together, our data demonstrate two distinct mechanisms for the Hippo pathway controlling border cell migration: 1) in border cells, it regulates polarized distribution of actin cytoskeleton; 2) in polar cells, it regulates upd expression to control border cell formation and migration.

Transcriptional regulation of Nodal during endoderm differentiation

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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. We then tried to understand the mechanisms involved in transcriptional regulation of Nodal during endoderm differentiation. At early somite stages, downstream genes of Nodal, Lefty2 and Pitx2 were also ectopically expressed in the foregut endoderm similar to Nodal, suggesting that the transcriptional feedback loop of Nodal observed in the left lateral plate mesoderm is active in endoderm cells of Sox17-/- embryos. To examine this possibility, a reporter transgene for the Nodal feedback regulation, LR asymmetric enhancer (ASE)-LacZ was introduced into Sox17-/- mutants. At early somite stages, the ASE-lacZ transgene recapitulated the ectopic expressions of Nodal, Lefty2 and Pitx2 in the Sox17-/- embryos; however at early bud stages, the ASE transgene was not active in endoderm of Sox17-/- embryos. We found that early expression of Nodal in endoderm is regulated by another enhancer located in the distal portion of the Nodal genomic region. We will discuss how Nodal expression is regulated during endoderm differentiation.

Hypoblast/posterior visceral endoderm drives formation of the mammalian fetal-umbilical connection

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While extraembryonic and embryonic tissues are classically thought to segregate into distinct lineages early in mammalian development, recent results have suggested that visceral endoderm (VE), thought to be an extraembryonic lineage that plays trophic and inductive roles in mammalian development, contributes to the definitive gut (Kwon et al., 2007). Given unique Hedgehog expression in posterior visceral endoderm (PVE) at the fetal-umbilical interface (Daane and Downs, 2011), and the intimate anatomical and disease association between the umbilical cord and gut (Stevenson and Hall, 2006), we tested the hypothesis that the extraembryonic hypoblast/VE of the mouse gastrula drives formation of the fetal-umbilical connection. Lipophilic fluorescent labeling revealed that PVE is compartmentalized into distinct functional regions which, unexpectedly and contrary to accepted dogmas, is systematically transformed into the full panoply of posterior extraembryonic and embryonic mesendodermal tissues. First, VE of the yolk sac contributes to both the endothelium and blood cells of the blood islands. Second, distal allantois-associated PVE is the source of the umbilical wall and hemogenic Vessel of Confluence (VOC), which together create a fixed point around which a circulatory continuum is established and remodeled between the fetus and its mother. Third, proximal allantois-associated PVE becomes part of the hindgut lip (HGL), a self-renewing growth center for the gut, umbilical cord, hemogenic arterial vasculature,
and putative primordial germ cells. Microsurgical deletion of the HGL led to a significantly reduced posterior fetal-umbilical connection. A model is presented which delineates the steps by which, in both rodents and humans, extraembryonic visceral endoderm undergoes an epithelial-to-mesenchymal transition to establish, stabilize, and reinforce the fetal-umbilical connection to ensure successful exchange between the fetus and its mother during gestation.

Program Abstract #487
Neurogenin3-directed differentiation of endoderm to insulin-expressing cells
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Neurogenin3 (NGN3) is a bHLH transcription factor sufficient for development of the pancreatic beta cell. Efforts to utilize NGN3 activity for directed differentiation of multipotent cells to endocrine lineages have primarily favored formation of glucagon-producing alpha cells. However, careful developmental timing of NGN3 activity can shift the resultant cell types toward insulin-producing beta cells. Using a drug-inducible version of NGN3 in the vertebrate model *Xenopus laevis*, we show that brief (<4hrs) and early (immediately after gastrulation, stage 12) activation of NGN3 induces ectopic and precocious formation of insulin-producing cells detectable approximately 14 hours earlier compared to control embryos. Therefore, we have established a narrow window of development (~13hrs) where we can interrogate in detail the gene regulatory network leading up to insulin expression. Using a combination of time-series and single time-point gain-of- and loss-of-function studies within the 13 hour window, we have identified many direct NGN3 targets potentially involved in beta cell differentiation, as well as two transcription factors which appear necessary for normal insulin expression. We continue to build on these data sets with additional time-series replicates, computation, and functional analyses. Our goal is to assemble a gene regulatory network useful for predicting causal relationships in the path from undifferentiated endoderm to mature beta cells. (National Institutes of Health HD073104; OD010997)

Program Abstract #488
The Developmental Origin of Two Populations of Ureteric Pacemaker Cells
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Introduction: Congenital hydronephrosis, characterized by dilatation of the urinary tract, occurs in 0.5-1% of pregnancies. While persistent postnatal dilatation requires clinical intervention, specific treatments are lacking since underlying pathogenic mechanisms are not defined. Our lab demonstrated that decreased Hedgehog (HH) signaling in kidney mesenchyme causes non-obstructive hydronephrosis, uncoordinated ureteric peristalsis and absent expression of cell markers, c-kit and Hcn3, which mark two populations of ureteric pacemaker cells (PMCs) in mice. These discoveries provide a basis to identify the lineage of origin and molecular signature of ureteric PMCs. Hypothesis: Ureteric PMCs derive from one of the cell lineages that contribute to the mature kidney and ureter. Results: Five distinct lineages of the developing urogenital system were labeled via Cre-mediated expression of ROSA-ttdTomato fluorescent protein and analyzed for co-localization with antibodies against c-kit and Hcn3 in E18.5 mice. Positive co-localization of Wnt1-Cre mediated expression of ttdTomato with c-kit and Hcn3, and negative co-localization in alternate lineages investigated (mesenchyme [Tbx18, Rarb2], stroma [FoxD1], ureteric bud [HoxB7]), indicated that both populations of PMCs originate from the neural crest. To individually analyze the molecular signature of the developing PMCs, E18.5 pelvis-kidney junctions and ureters were micro-dissected and digested into single-cell suspensions. Antibodies against c-kit and Hcn3 were applied and both populations were successfully isolated using fluorescence-activated cell sorting with >94% purity. Conclusion: Ureteric PMCs originate from the neural crest and migrate in to the developing kidney. These cells have been isolated from whole tissue, providing a basis for further investigation into their differentiation. Significance: These studies provide a platform to investigate diagnosis and treatment strategies involving PMCs in hydronephrosis.

Program Abstract #489
PI3K signaling coordinates tubulogenesis and cell fate specification
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Proper establishment of apical-basal polarity in epithelial cells is crucial for normal organ development. Little is known about how apical polarity is regulated and initiated in an organ and if this affects cell fate specification. Using the developing mouse pancreas as a model system, we show that EGFR signaling via PI3K and Rac1 coordinates cell polarity and beta-cell differentiation during pancreas development. This conclusion is based the observations that reduced expression/activity of any of these proteins leads to premature induction of apical polarity, enhanced microlumen formation, as well as reduced beta-cell differentiation. Further analysis of the beta-cell phenotype revealed a cell-autonomous requirement of Rac1 in upregulation of Ngn3 expression to reach levels that are necessary for beta-cell commitment. These findings suggests that Rac1/PI3K/EGFR signaling controls both apical polarity in an epithelial organ and differentiation of specialized cells, by inhibiting the ability of progenitors to commit to a specific fate.

Program Abstract #490
Structure-function analysis of lin-42, the *C. elegans period* homolog

160
Understanding how patterns are generated during metazoan development is a fundamental challenge in biology. A critical and largely unsolved aspect of this problem is how temporal cues are interpreted such that developmental events occur at the correct time and in the proper sequence. The nematode *C. elegans* provides a premier system for investigating how temporal information is conveyed during development, in part, because the essentially invariant cell lineage allows events as specific as single cell divisions to be monitored with respect to developmental time. This feature has allowed mutants to be isolated in which the timing of postembryonic programs have been altered. In precocious mutants, early stage-specific lineage patterns are skipped allowing later events to occur too early. In contrast, retarded mutants reiterate early events, causing subsequent events to occur abnormally late. *lin-42* hypomorphic mutants have a precocious phenotype, in which the hypodermal seam cells terminally differentiate one stage too early. In addition to the timing defect, a subset of alleles also cause molting defects. *LIN-42* is an essential component of the circadian rhythm protein PERIOD, found in flies and mammals. LIN-42 shares multiple domains with PERIOD proteins, including the protein interaction PAS domain and the less understood SYQ and LT domains. *lin-42* has three transcription units, two of which are non-overlapping and produced from two distinct promoters. We generated a null mutant with which to better dissect the contributions of each transcription unit and to test the functions of conserved protein domains. The developmental timing and molting defects in the *lin-42* null are more severe than those observed in previously described hypomorphic alleles. Progress on *lin-42* structure-function analysis will be reported.

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**Program Abstract #491**

**BMP signaling regulates cell cleavage in preimplantation mouse embryos**

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The first lineage specification of mammalian development begins with establishing the precursors of extraembryonic and embryonic lineages—the trophectoderm and the Inner Cell Mass. Regulatory interactions of transcription factors that mediate the process, such as Pou5f1, Nanog, Tead4, and Cdx2, are essential and have been extensively studied. Likewise, cell-cell signaling between blastomeres is also crucial for this lineage specification. Therefore, we investigated Bone Morphogenetic Protein (BMP) in preimplantation development. To date, BMP’s functional importance has been demonstrated as early as E6.5 in gastrulating mouse embryos. Research so far failed to disclose a role for BMP signaling at preimplantation stages, possibly due to redundancies in the signaling pathway. We directly measured BMP signaling activity using two different approaches. By examining the transcriptional response of BMP-indicator mice and by measuring the phosphorylation of Smad1/5/8 within preimplantation stages. We mapped and quantitated BMP signaling activity through high-resolution immunofluorescence imaging combined with 3D segmentation quantification. We report that Smads1/5/8 are phosphorylated as early as the 4-cell stage and become increasingly spatially restricted in the blastocyst. To uncover the function of BMP signaling, preimplantation embryos were treated with a chemical inhibitor of BMP receptor1a, LDN193189 and subjected to time-lapse imaging. We noted a significant retardation of the cell cleavage rate, which resulted in embryos with reduced cell numbers. Overexpression of dominant negative BMP receptor1a and Noggin, a known BMP antagonist, similarly slowed down cell cleavage. Molecular marker analysis of these developmentally retarded embryos suggests that regulation of cell proliferation via BMP signaling and cell lineage programming is tightly linked. Perhaps, BMP signaling functions as a clock to ensure lineage commitment occurs at the appropriate time in development.

**Program Abstract #492**

**Dynamic sorting and morphogenesis of primitive endoderm in mouse embryos and embryoid bodies**

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One of the earliest differentiation events in mouse embryonic development is the primitive endoderm (PrE) formation in blastocysts. The PrE cells first rise within the inner cell mass (ICM) in a checker box pattern and subsequently segregate from the epiblast (EPI) to form an epithelium. Transcription factor GATA6 is essential for PrE differentiation. Cell autonomous polarization within the PrE precursors are thought to be instrumental for PrE cell sorting, and the expression of Disabled-2 (Dab2), an endocytic protein involved in establishing apical polarity, is critical for cell sorting and formation of a mature primitive endoderm. We created lines of GATA6-H2BGFP and Dab2-H2BGFP knock-in ES cells and mice to track the differentiation and sorting of PrE cells in embryoid bodies and blastocysts. The heterozygous GFP knock-in models closely followed GATA6 or Dab2 expression and PrE differentiation as detected by immunofluorescence microscopy of monolayer embryonic stem cells and embryonic bodies. We conclude that the GATA6-H2BGFP and Dab2-H2BGFP knock-in models produced are reporters for us to monitor the temporal and spatial formation of PrE and to confirm the roles of GATA6 and Dab2 in PrE differentiation, cell sorting, and epithelial formation in early development. Supported by NIH RO1 CA095071, CA79716 and CA75389.

**Program Abstract #493**

**Dorsalventral patterning mechanisms of the Pacific oyster early embryo**

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The Pacific oyster Crassostrea gigas belongs to one of the most species-rich phyla, the mollusca. The molluscan embryos develop via spiral cleavage, a unique form of development that differs from those of flies, worms and vertebrates. The embryonic axes are not clearly defined and it is not known whether similar axial patterning mechanisms control oyster embryogenesis. In this study, we wanted to determine whether the conserved BMP pathway regulates dorsal-ventral (D-V) patterning of oyster embryonic development and whether the molluscan D-V axis is defined in a similar way as other vertebrate species. Using the published draft genome sequence of Crassostrea gigas, we annotated genes in the transforming growth factor b (TGFb) pathway that are involved in D-V patterning in other species, such as, bone morphogenetic proteins (BMPs) and their antagonists. We showed that the oyster genome contains most of the genes in the pathway. Examples of the name of ligand, receptor, antagonist, we are using in situ hybridization to determine the expression patterns of these genes during oyster embryogenesis and hope to resolve the roles of them regarding D-V axis formation. We also examined the role of MAPK pathway in oyster D-V patterning and showed that MAPK pathway is active in the 3D macromere, which functions as an organizer for future axial development. We will investigate the relationship between TGFb signaling pathway and MAPK pathway in 3D macromere fate specification, D-V patterning and other aspects of embryogenesis. This will provide the first glimpse of how embryonic axes are set up in a molluscan model system.

**Program Abstract #494**

**Split top: A Maternal Regulator of Dorsal-Ventral Patterning and Cell Migration in Zebrafish**

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Little is known about the maternal factors that function in body axis formation during vertebrate embryonic development. To identify these factors, our lab has performed a recessive maternal-effect mutagenesis screen in the zebrafish and found a number of mutants with defects in early developmental processes, including early morphogenesis and body axis formation. One such mutant, *split top*, exhibits a dorsalization of the embryonic axis. Clutches of embryos from *split top* mutant mothers show a characteristic dorsalized phenotype. The mutant embryos show an expansion of dorsal markers and a corresponding reduction in ventral markers during gastrulation indicating dorsalization. The dorsalization defects can be rescued by misexpression of either BMP2 or BMP7 ligands, or by derepression of BMP signaling by knockdown of BMP antagonists. The additional defects appear to be the result of altered morphogenesis, including defects in cell migration, the process by which the blastoderm cells migrate over and surround the yolk. Mutant embryos display altered microtubule and actin cytoskeletal networks in the yolk cell, which can account for the epiboly defects observed. *Split top* mutant embryos also appear to be defective in the cell movement process of convergence and extension. We mapped the *split top* mutation to chromosome 17, and have identified a candidate gene through RNA-Seq and traditional positional cloning methods.

This work was supported by NIH grant R01-GM56326, NIH training grant T32HD007516, and the PENN-PORT training program.

**Program Abstract #495**

**DNA topoisomerase III alpha interacts with the BMP-Smad pathway to specify dorsoventral patterning during early embryogenesis**

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DNA topoisomerase III (TOP3) is a Type IA topoisomerase for DNA replication and genomic stability. TOP3 has two isoforms, TOP3A and TOP3B. *top3b*-null mice can grow to maturity, but *top3a*-null mice die prematurely due to possible gastrulation defects. Surprisingly, two *top3a* zebrafish zygotic mutants show no embryonic defect, but die later due to the lack of thymocytes. To further investigate the role of Top3A during early embryonic development, we perturbed *top3a* by antisense morpholino oligonucleotides (MO) and overexpression and found dorsalized and ventralized phenotypes, respectively. *top3a* morphants also exhibited low expressions in ventral marker genes eve1 and gata1. Furthermore, the dorsalized phenotypes could be specifically rescued by overexpression of *top3a*. To understand how *top3a* regulates dorsoventral patterning, we used co-immunoprecipitation assay and discovered that TOP3A interacts with Smad1, a core component of BMP signaling. Chromatin immunoprecipitation assay further showed that knockdown or overexpression of *top3a* decreases or enhances the binding of smad1 to the eve1 and gata1 promoter regions, respectively. We also observed the enhanced accessibility of nuclear chromatin to nucleases in top3aMO-treated ZF4 cells that could be rescued by overexpression of wild-type *top3a* but not the truncated *top3a* with only the C-terminal region. Thus, TOP3A may mediate dorsoventral patterning via creating a Smad favored chromatin structure for transcription. The ventralized phenotype of *top3a* overexpression fish was rescued by BMP-specific inhibitor dorsomorphin. Most importantly, dorsalized phenotype of *top3a* morphants could be complemented by overexpression of *top3a*, human TOP3A, Smad1 or Smad5, but not TOP3b<sub>cyt</sub> active-site mutant. Together, these results suggest that zTOP3A participate in BMP-mediated ventral patterning via enhancing Smads-directed transcription in zebrafish.

**Program Abstract #496**

**Role of chemokine ligand Ccl19.1 and calcium signaling in zebrafish axis formation**

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During vertebrate embryonic axis specification activation of the Wnt/b-catenin signaling pathway promotes the formation of the dorsal organizer, and modulation of intracellular Ca\(^{2+}\) signaling has been implicated in axis specification by inhibiting b-catenin activities. Previous studies from our laboratory demonstrated that Ccl19.1/Ccr7 chemokine G protein-coupled receptor (GPCR) functions to limit b-catenin-mediated axis formation in zebrafish likely via Ca\(^{2+}\) signaling. However, the specific molecular mechanisms remain unclear. Here we generated mutations in the zebrafish ccl19.1 genes using TALENs to further characterize its role during this process. Depletion of maternal and zygotic (MZ) ccl19.1 function results in slightly oval shape morphology at tail bud stage in zebrafish, suggesting a mild dorsalization phenotype of the MZccl19.1 mutants. Consistently, qRT-PCR and in situ hybridization analyses revealed an upregulation of b-catenin-dependent dorsal markers and the corresponding downregulation of ventral markers at blastula and gastrula stages. Moreover, MZccl19.1 mutants display more severe dorsIALIZED phenotypes, in comparison with WT embryos, when injected with low doses of b-catenin RNA, indicating MZccl19.1 mutants are sensitive to b-catenin levels. Indeed, we observed a suppression of the ventralized Michabod/b-catenin2 mutant phenotype in Mcc19.1; Michabod/b-catenin2 double mutants. Ongoing studies aim to fully understand the mechanisms trough which Ccl19.1 regulates embryo patterning. We are also generating tools to test how the intracellular Ca\(^{2+}\) signaling is modulated in a spatiotemporal manner to inhibit b-catenin activities during zebrafish axis formation using the GCaMP6s and Opto1AR transgenic lines.

Program Abstract #497
Wnt8a post-transcriptional regulation and vertebrate axis development
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Wnt/beta-catenin signaling in early vertebrate embryos patterns the D/V and A/P axes, and wnt8a is one of the earliest acting genes that interconnects both D/V and A/P patterning. In the zebrafish, wnt8a, the earliest zygotically expressed Wnt gene, acts over broad domains to regulate D/V and A/P patterning of the mesoderm and ectoderm. Wnt8a acts in two phases to regulate different developmental choices. In early gastrula stages, Wnt8a signaling prevents expansion of the Dorsal Organizer and induces posterior neural plate fates. During mid to late gastrulation, Wnt signaling promotes growth of the posterior embryo. Because of its critical role during axis patterning, wnt8a regulation is likely to occur at several levels, including post-transcriptionally. We have used a combination of transgenic and transient sensor assays and target protector morpholinos in the zebrafish to test the hypothesis that post-transcriptional regulation of wnt8a occurs through the action of microRNAs. Our results show that wnt8a is a target of several microRNAs during early development, a major regulator being miR430. We identified two miR430 binding sites in wnt8a UTR elements, and these sites are necessary and sufficient for wnt8a post-transcriptional regulation. Protecting wnt8a transcripts from miR430 regulation results in elevated wnt8a expression and embryo patterning defects. Interestingly, our results also indicate that the two Wnt8a proteins differentially regulate neural and mesodermal patterning. Thus, microRNA-dependent regulation of wnt8a is crucial to normal embryonic axis patterning.

Program Abstract #498
Loss of ripply1 restores mesp-ba expression in the absence of foxc1a during somitogenesis
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Expression of mesp-ba in somitogenesis is tightly regulated by the activator tbx6 and the repressor ripply1 via a feedback regulatory network. Loss of foxc1a function in zebrafish prevents anterior somite formation and reduces mesp-ba expression. To determine how foxc1a functions to regulate mesp-ba expression in somitogenesis, we investigated how foxc1a interacts with the tbx6-ripply regulatory network in zebrafish somitogenesis. We found that in foxc1a morphants, somite formation was not observable at 12.5 hours post fertilization (hpf). Irregularly formed posterior somites were eventually seen, whereas anterior somites were still absent, by 22 hpf. In ripply1 morphants no somites were observed by 22 hpf. mesp-ba expression, usually detected in one or two stripes in the anterior presomitic mesoderm (PSM), was greatly reduced in the foxc1a morphants and expanded anteriorly in ripply1 morphants. tbx6 expression, which spans the entire posterior PSM, was unaffected in the foxc1a morphants, but was expanded anteriorly in the ripply1 morphants. Double knockdown of foxc1a and ripply1 resulted in a lack of anterior somite formation while posterior somites did form, suggesting a rescue of the ripply1 phenotype. However, unlike the single foxc1a morphant, expression of mesp-ba was detected as 1 to 3 stripes in the anterior PSM. tbx6 expression was expanded anteriorly in the double morphants. In conclusion, both foxc1a and ripply1 morphants displayed defects in somitogenesis, but their individual loss of function had opposing effects on mesp-ba expression. Loss of ripply1 appears to have rescued the mesp-ba expression in the foxc1a morphant, suggesting that intersection of these two parallel regulatory mechanisms is required for normal mesp-ba expression and somite formation. This work was funded by a CIHR grant awarded to FBB.

Program Abstract #499
Conserved transcriptional regulatory modules in mouse, chicken and zebrafish somitogenesis networks.
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The metanercic segmentation of the vertebrate body is established during somitogenesis, when a cyclic spatial pattern of gene expression is created within the mesoderm of the developing embryo. Although several studies have been devoted to the subject, the mechanism controlling vertebrate somitogenesis is still not fully understood. The process strongly relies on transcriptional
regulation. We characterized the spatiotemporal regulation patterns using a model-based timing of approach to precisely identify the moments in time, when the particular genes are active. This method, based on maximum entropy deconvolution, allows to select potential causal dependencies within the underlying genetic, signaling and transcriptional networks. We have shown that the regulation is very precisely timed, and that the genes involved are transcribed just before their products are used in their respective pathways. Nonetheless, the generally accepted "clock and wavefront" model involving Notch, Fgf and Wnt signaling pathways appears to be partially redundant and some of its components may be unessential. To identify the essential genes and interactions in the somitogenesis network, we used a maximum-likelihood model to compare results of timing analysis from three different vertebrate species: mouse, chick and zebrafish. As a result, we obtained the list of causal interactions in the somitogenesis network that are evolutionarily conserved and are thus the primary candidates for the most essential, core modules of the regulatory network. We expect that our results will lead to identifying the regulatory interaction between somitogenesis and other conserved developmental processes, including morphogenesis and regulation of genes in Hox clusters.

Program Abstract #501
Apolipoprotein C-I mediates Wnt/Ctnnb1 signaling during neural border formation and is required for neural crest development
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In vertebrates, the neural crest and placodes originate in the neural border, which is located between the neural plate and epidermal ectoderm. The neural crest and placodes give rise to a vast array of cell types. Formation of neural crest is a multistep process, in which Wnt signals are used reiteratively, but if a Wnt signal is required for neural border formation is currently not well understood. Here, we have identified apolipoprotein C-I (apoc1) in a screen for genes regulated by Wnt/Ctnnb1 signaling in late blastula stage Xenopus tropicalis embryos. We show that Xenopus laevis apoc1 encodes a small, secreted protein, and is induced by Wnt/Ctnnb1 signaling. Depletion of Apoc1 protein results in a neural border formation defect and loss of border fates, including neural crest cells. However, unlike another Wnt/Ctnnb1 target, gbx2.2, apoc1 is not required for patterning of the neural border. We further show that gbx2.2 and apoc1 are independently regulated by Wnt signaling. Our results thus suggest that Wnt regulates border formation and patterning by distinct genetic mechanisms.

Program Abstract #502
Polarity proteins regulate the localization of a spindle-positioning mediator, LET-99
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Spindle positioning is essential for asymmetric divisions, where the spindle must align with the axis of cell polarity. In many systems, conserved PAR proteins establish polarization of the cell and regulate spindle movements via a complex including Ga, GPR and LIN-5. LET-99 is a key regulator of GPR asymmetry in C. elegans embryos. LET-99 is asymmetrically localized at the cortex in a posterior-lateral band pattern, where it inhibits GPR localization. Analysis of LET-99 mutant backgrounds showed that PAR-3 and the kinase PAR-1 are required to inhibit cortical LET-99 localization at the anterior and posterior-most cortex respectively. In other systems, phosphorylation of targets by PAR-1 and the PAR-3 associated kinase PKC-3 generates binding sites for 14-3-3 proteins, which alters the targets’ localization. We found that the 14-3-3 protein PAR-5 bound to His::LET-99 in wild-type embryo extracts, but PAR-5 binding was greatly diminished after PAR-1 depletion. Computer predictions and yeast-two hybrid data identified two LET-99 serine residues essential for PAR-5 binding. We then introduced S-to-A mutations into an otherwise full-length rescuing LET-99 transgene (LET-99-AA). In a let-99 deletion background, the LET-99-AA protein mislocalized to the entire posterior cortex of the one-cell embryo, similar to LET-99 in par-1 mutant embryos. These and other results support a model that PAR-1 phosphorylates LET-99 to create binding sites for PAR-5, which prevents LET-99 association with the posterior-most cortex. To begin to determine how LET-99 localization is restricted from the anterior, we analyzed LET-99 after depletion of anterior PAR components. We found that PAR-3 associations proteins, PAR-6 and PKC-3, restrict LET-99 localization from the anterior. We are now testing if PAR-1 and PKC-3 directly phosphorylate LET-99 in vitro and how phosphorylation may regulate LET-99 affinity for the cell cortex.
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Program Abstract #503
Investigating the role of the G-protein regulator LET-99 in cytokinesis
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Asymmetric cell division is required to generate cellular diversity during normal development. Asymmetric division relies on communication between the PAR polarity proteins that determine cytoplasmic and cortical asymmetries, and an asymmetrically positioned mitotic spindle to properly position components of the contractile ring to specify the plane of division. We have previously shown that LET-99, a negative regulator of G-alpha proteins, is required for asymmetric cell division in the C. elegans one-cell embryo. During spindle positioning, LET-99 localizes in a posterior lateral band where it regulates the force generation machinery so that the spindle first orients onto the AP axis then displaces towards the posterior. An additional role for LET-99 in the
Although some pairwise protein-protein interactions between members of the PAR system have been demonstrated, an unanswered question is how these proteins are organized biochemically into a signaling network that can mediate cell polarization. Although the localization and genetic requirements of PAR proteins have been studied in detail, a fundamental in vivo unknown which of these interactions occur in vivo is whether these interactions are temporally regulated during the establishment and maintenance of cell polarity.

Program Abstract #504
Determining the mechanism of EMS spindle positioning in response to Wnt and Src polarity cues
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Asymmetric divisions generate cellular diversity. In the four-cell Caenorhabditis elegans embryo, the EMS cell undergoes an asymmetric division to generate mesoderm (MS) and endoderm (E) founder cells. The neighboring P2 cell polarizes EMS via redundant Wnt and Src cues, which induce the EMS nuclear-centrosome complex to rotate onto the anterior/posterior (A/P) axis. Single mutations in either Wnt or Src pathway genes often result in late EMS spindle orientation, while double mutants show a complete failure in spindle positioning. Wnt and Src signaling lead to the cortical enrichment of the dynein regulatory complex, Nodin, at the EMS-P2 contact. However, how this enrichment is regulated and what other components act downstream of signaling to generate asymmetric cortical pulling forces to position the EMS spindle are yet to be determined. At the one-cell stage, conserved PAR polarity proteins regulate a non-canonical G protein pathway including Gα1/2 and LIN-5, which interact with regulators of dynein to generate cortical microtubule pulling forces. LET-99, a DEP containing protein, negatively regulates the cortical localization of Gα/GPR/LIN-5 complex and leads to asymmetric force generation. Gα and LET-99 mutants also have defects in spindle positioning in the EMS cell. We hypothesize that Src and Wnt act through the LET-99/G protein pathway to recruit dynactin/dynein and position the EMS spindle. Using let-99(ts) embryos shifted to the restrictive temperature at the four-cell stage, we found that LET-99 is required for EMS spindle positioning in the absence of earlier division abnormalities. We are currently examining lin-5(ts) and Ga(ts) mutants. Next, we will determine if a given G protein component is a part of the Wnt or/and Src pathway/s using genetic analysis. This work was funded by R01GM068744 to LR.

Program Abstract #505
Developing an approach for single molecule analysis of PAR protein complexes during cell polarization
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The conserved PAR protein system mediates polarization in a wide variety of animal cell types including epithelia, neurons and stem cells. The first division of the C. elegans zygote is a valuable model system for studying how PAR proteins mediate cell polarization. This cell division is asymmetric, with the anterior and posterior daughter cells adopting different fates. PAR-3, PAR-6, atypical Protein Kinase C (aPKC) and CDC-42 localize to the anterior of the zygote prior to division, while PAR-1, PAR-2 and Lethal Giant Larvae (LGL-1) localize to the posterior. All of these proteins are required (some redundantly) for a normal polarized division. Although the localization and genetic requirements of PAR proteins have been studied in detail, a fundamental unanswered question is how these proteins are organized biochemically into a signaling network that can mediate cell polarization. Although some pairwise protein-protein interactions between members of the PAR system have been demonstrated in vitro, it is unknown which of these interactions occur in vivo, how these interactions might be regulated, and how this regulation could contribute to cell polarity. To address this, we have developed a method that allows us to interrogate protein-protein interactions in individual, precisely staged C. elegans embryos. Single embryos are crushed in microfluidic chambers, generating lysates in a small volume with minimal dilution. Protein complexes are captured inside this device by antibodies, and can then be counted using single-molecule TIRF microscopy. We are using this assay to test which of the reported interactions between members of the PAR system occur in vivo, and whether these interactions are temporally regulated during the establishment and maintenance of cell polarity.

Program Abstract #506
Localization of the C. elegans polarity regulator PAC-1 by components of the cadherin-catenin complex
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The PAR proteins play an essential role in asymmetrically partitioning cellular molecules and in determining the different fates of future daughter cells. Beginning at the four-cell stage, PAR proteins in the C. elegans embryo localize symmetrically to the cell cortex. Specifically, PAR-3, PAR-6 and PKC-3 occupy the contact-free surfaces of cells. The asymmetric localization of PAR proteins is induced by the RhoGAP protein PAC-1. PAC-1 itself is found at sites of cell-cell contact, and is needed to exclude PAR proteins from these sites. How PAC-1 recognizes cell contacts and localizes there to exclude PAR proteins is unknown. In a two-
hybrid screen, we identified a PAC-1-interacting protein, named PICC-1, that also interacts with the cadherin-catenin complex by binding to JAC-1/p120-cat. Moreover, the E-cadherin homologue HMR-1 was shown to help recruit PAC-1 to cell contacts. To test the hypothesis that PICC-1 acts as a physical linker between the cadherin-catenin complex and PAC-1, we performed misexpression experiments. Mislocalizing the intracellular domain of HMR-1/E-cad around the entire cortex of the cell is sufficient to recruit PAC-1. However, mislocalizing PICC-1 was not sufficient to recruit PAC-1, suggesting that other components of the cadherin-catenin complex work together with PICC-1 to recruit PAC-1—a hypothesis that we are now testing. By understanding the processes of early cellular polarity in worms, we hope to learn more about how cell contacts polarize cells in the human embryo—an event that is important for segregation of embryonic and extra-embryonic fates. This research was supported in part by Choose Development!, an NSF (grant IOS-1239422) sponsored program through the SDB.

Program Abstract #507
Instructive polarization of early embryonic cells by the cadherin-catenin complex and the RhoGAP PAC-1
Diana Klompstra, Dorian Anderson, Jeremy Nance
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Early embryonic cells in many species polarize radially by distinguishing their contacted and contact-free surfaces. In C. elegans, radial polarity begins at the four-cell stage, when cell contacts restrict the PAR polarity proteins to contact-free surfaces. We previously identified the RhoGAP PAC-1 as an upstream regulator that is required to exclude PAR proteins from contacted surfaces of early embryonic cells. PAC-1 is recruited specifically to sites of cell contact and directs PAR protein asymmetries by inhibiting the Rho GTPase CDC-42. How PAC-1 is able to sense where contacts are located and localize to these sites is unknown. We identified an N-terminal fragment of PAC-1 that is sufficient for localization to cell contacts and showed that its localization depends on HMR-1/E-cadherin. HMP-1/a-catenin and JAC-1/p120-catenin, which interact with the HMR-1 cytoplasmic tail, function redundantly to recruit the PAC-1 N-terminus. We identified a conserved adaptor protein that physically links the PAC-1 N-terminus to the cadherin-catenin complex. E-cadherin has a conserved role in promoting contact-induced cell polarization, but there has been debate as to whether it functions instructively (defining contact sites) or permissively (promoting sufficient adhesion for other polarity regulators to operate). We show that ectopically localizing the intracellular domain of HMR-1 to contact-free surfaces of cells recruits full-length PAC-1 and depolarizes cells, demonstrating that HMR-1 plays an instructive role in polarization by recruiting a symmetry-breaking polarity regulator to cell contacts.

Program Abstract #508
E2a diversifies the functions of Nodal signaling in the embryo
Andrea Wills, Se-Jin Yoon, Rakhi Gupta, Julie Baker
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Transcription factor complexes can have varied effects on cell fate and behavior depending on their position or conformation. But what variables direct the choice of where a transcription factor will bind, and whether the outcome of binding has an activating, repressive, or neutral effect on the expression of the target? The Nodal signaling pathway has many biological functions, including mesoderm and endoderm specification, left/right patterning, stem cell pluripotency and cancer progression. All these roles converge on the transcription factors Smad2/3, which can associate with many potential cofactors. Alternative usage of these cofactors may therefore provide a mechanism for modulating the transcriptional targets and outcome of Smad2/3 binding. To test the hypothesis that the association of Smad2/3 with specific cofactors helps diversify its functions, we used a ChIP-SEQ approach in human embryonic stem cells, endoderm, and Xenopus tropicalis embryos to identify cofactors associated with Smad2/3. We find that the bHLH protein E2a associates with the Smad2/3 complex, and is required for mesendoderm specification and gastrulation movements in the early embryo. We then investigated how E2a diversifies the functions of Nodal signaling. We perturbed E2a in X. tropicalis embryos, and examined global patterns of Smad2/3 binding and gene expression. Unexpectedly, we discovered that Smad2/3 binding is not sufficient for target gene transcription, but requires E2a for transcriptional activation at many critical regulators of dorsal fate and morphogenesis. By a separate mechanism, E2a also directs the position of Smad2/3 binding near the Nodal inhibitor lefty. We show that E2a directly represses lefty transcription, and that this regulation is critical for mesendoderm specification. Overall, we find that E2a acts as both an activator and repressor to direct and partition the effects of Nodal signaling in the embryo.

Program Abstract #509
Cyp26 Enzymes are Required within the Anterior Lateral Plate Mesoderm to Balance Cardiac and Vascular Lineages
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Normal heart development requires appropriate levels of retinoic acid (RA) signaling as too much or too little can be teratogenic. One way that RA signaling levels are moderated is by Cyp26 enzymes, which metabolize RA into easily degraded derivatives. Previous studies using mouse knockouts have shown that loss of Cyp26a1 or both Cyp26a1 and Cyp26c1 (referred to here as Cyp26 deficient) leads to vascular and cardiac defects. However, the mechanisms underlying these defects in Cyp26 deficient embryos have not been addressed. We have found that in zebrafish, Cyp26a1 and Cyp26c1 are expressed in the anterior lateral plate mesoderm (ALPM) predominantly overlapping with vascular progenitors and not cardiac progenitors. Although singular
knockdown of Cyp26a1 or Cyp26c1 does not overtly affect cardiovascular development, Cyp26 deficient embryos have increased atrial cells and reduced cranial vasculature cells. Within the ALPM, Cyp26 deficient embryos exhibit an anterior shift in the expression of cardiac progenitor markers and a corresponding truncation of the adjacent anterior vascular progenitor markers. We further examined the ALPM lineages by performing fate-mapping in Cyp26 deficient embryos and found that, in addition to an anterior shift, the atrial progenitor frequency was significantly increased while the vascular progenitor frequency was significantly decreased, suggesting that there is an expansion of the atrial progenitors at the expense of the ventricular progenitors. Surprisingly, in a series of complementary blastula transplantation experiments, we found that both gain and loss of Cyp26 enzyme expression can have cell non-autonomous consequences on the local environment. Therefore, our results suggest that Cyp26 enzymes are required within the ALPM to limit local RA signaling levels, which is necessary for proper placement of progenitor boundaries to balance the cardiac and vascular lineages.

Program Abstract #510
Live Dynamic Imaging and Analysis of Developmental Cardiac Defects in Mouse Models with Optical Coherence Tomography
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Understanding mouse embryonic development is an invaluable resource for our interpretation of human embryology. Our research is focused on developing methods for live imaging and dynamic characterization of early embryonic cardiovascular development in mouse models of human diseases and using these methods to study congenital defects in humans through multidisciplinary approaches, which include optical coherence tomography (OCT), live mouse embryo manipulations and static embryo culture, molecular biology, advanced image processing and computational modeling. We have developed an OCT based approach, which can be used for imaging live early mouse embryos (E7.5 - E10) cultured on an imaging stage and visualizing developmental events with spatial resolution of a few micrometers (less than the size of individual cells) and a frame rate of up to hundreds of frames per second. We have successfully used this approach to visualize the structure of whole embryos, analyze blood flow profiles and reconstruct cardiodynamics in 4D (3D+time). We are now using these methods to study how specific embryonic lethal mutations affect cardiac morphology and function during early development.

Program Abstract #511
Rapid inhibitor expression in response to excess Nodal signaling
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Secrepted activator/inhibitor pairs drive much of the pattern formation that occurs during development. The Nodal/Lefty activator/inhibitor pair patterns mesendoderm during vertebrate embryogenesis. lefty is a target of Nodal signaling, and expression of both nodal and lefty normally commences during the late blastula stage. Previous studies with morpholinos suggested that Lefty loss results in dramatic upregulation of Nodal target genes as early as the late blastula stage. However, we show that most Nodal targets are not dramatically upregulated until early gastrulation in embryos mutant for both zebrafish leftys. The exception was lefty itself: both leftys were strongly upregulated in lefty double mutants at the late blastula stage, concurrent with the onset of Nodal expression. Modeling and NanoString data suggest that leftys respond to Nodal signaling with unusually rapid transcription kinetics, exhibiting transcription rates >20-fold higher than ~90% of Nodal target genes. Our results suggest that highly responsive inhibitor expression may allow rapid dampening of excess activator signaling before excess signaling is converted into misexpression of mesendodermal genes.

Program Abstract #512
Studies of Cyclopia: Cloning and characterization of the Daphnia magna hedgehog gene
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During Daphnia magna embryonic development, a unique phenomenon occurs: two distinct eye spots within an eye field, which are present at a very early embryonic stage, gradually fuse into one cyclopean eye. Though these freshwater microcrustaceans are attractive model organisms to study, no details regarding the molecular genetic basis of this eye fusion event are known. This study focused on cloning a Daphnia magna candidate gene, hedgehog, and characterizing the developmental time-course of its expression using PCR and in situ hybridization. The homologous human gene, sonic hedgehog, when mutated, is associated with holoprosencephaly (HPE), a disorder that can be manifested in a variety of midline defects including cyclopia. The present study reveals that Hedgehog amino acid sequence identity between the D. magna and human Sonic hedgehog sequences is 79.6% in the amino-terminal domain with only 35.1% identity in the C-terminal region. A similar pattern of sequence identity was also observed between the D. magna Hedgehog and the M. musculus Sonic hedgehog (80.3% in the N-terminus and 35.3% in the C-terminus), while a moderate sequence identity was found between the D. magna Hedgehog and the D. melanogaster Hedgehog in both regions (68.8% in the N-terminus and 46.9% in the C-terminus). Preliminary expression studies in D. magna reveal hedgehog mRNA transcripts are present throughout early embryogenesis (RT-PCR) and in the anterior midline of the developing animal (in situ hybridization). Further studies of the Hedgehog signaling pathway using genetic and pharmacological methods are being conducted to determine what role Hedgehog plays in Daphnia magna cyclopean eye development.
Program Abstract #513
Temporal expression of trunk Hox genes in embryos of the freshwater prawn Macrobrachium olfersi (Decapoda, Palaemonidae)
Dib Ammar, Michael Jaramillo, Christian Paese, Evelise Nazari, Yara Müller
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Hox genes have been discovered in many species and playing role in specifying regional identity along the anterior-posterior axis of animals during embryonic development. Crustacean that presenting a variety of body plans, unlike encountered in other class or phylum of the Metazoa, has been shown to possess a single set of Hox genes like insect. In body plan of crustaceans, different Hox genes act to control the development of different segments and tagmosis. Therefore, the aim of this study was to evaluate the temporal expression of the trunk Hox genes Antennapedia (Antp), Ultrabithorax (Ubx) and abdominal A (abd-A) in embryos of the malacostracan crustacean Macrobrachium olfersi. Samples of embryos at E3 to E9 stages were processed for (i) RNA extraction, cDNA synthesis and PCR, and (ii) whole mount preparations for immunohistochemistry analysis. RT-PCR analysis showed that the expression of Antp and Ubx genes were detected at E4 to E9 embryonic stages, which coincides with the development of nauplius and early post-nauplius stages. However, the expression of abd-A gene was detected only at E7 to E9 embryonic stages. By immunohistochemistry using antibodies anti-Antp, anti-Ubx, anti-abd-A, anti-engrailed and anti-even-skipped, we found that these product genes are observed in well-defined and discrete domains, which coincide with the boundaries of body segments of the embryos. Our results contribute to the understanding of the mechanisms involved in the embryogenesis of M. olfersi. In addition, our results confirm that products of the trunk Hox genes are evolutionarily conserved transcription factors that control elaborated developmental processes in animals. Studies in our laboratory are in progress to sequencing of the amplicons and subsequent bioinformatic analysis by comparison with other arthropods Hox gene sequences deposited in the GenBank database.

Program Abstract #514
Trends in Gene Expression Dynamics within the Drosophila Early Embryo Identified using NanoString
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The syncytial Drosophila blastoderm embryo is a rapidly changing system, with 13 nuclear divisions occurring in the three hours between egg deposition and gastrulation. During this time, maternally deposited transcripts are used and degraded by the embryo concurrent with the onset of zygotic transcription. The new zygotic transcripts and gene products begin to take control of the embryo as the syncytial nuclei cellularize. This maternal to zygotic transition (MZT) is known to occur between syncytial nuclear cycles 12-14, and the degradation of maternal transcripts is well understood, but the early sequence of zygotic gene activation, however, is not as well defined. There are several key transcription factors present in the embryo, including Dorsal and Zelda, that are active early in the syncytial embryo. Dorsal is essential in the dorsal-ventral gene network, and nuclear concentrations of Dorsal protein increase during each syncytial nuclear cycle until gastrulation. This dynamic and consistent increase in nuclear concentrations of Dorsal provides a unique system to use for characterizing the sequence of zygotic gene activation during the MZT. Using the NanoString nCounter instrument, we have observed the activation of the dorsal-ventral gene network in a precisely staged time course at 10 time points spanning syncytial nuclear cycles 10-14 and into gastrulation. We observed that the dorsal-ventral gene network is activated in discrete stages, with different functional classes of genes and signaling pathways being acted upon in a sequential pattern. Our results provide several gene targets and testable predictions for further study in the activation of the dorsal-ventral gene network.

Program Abstract #515
Role of Syntabulin in Xenopus Primordial Germ Cell Formation
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In anuran amphibian and teleost embryos, cytoplasmic localizations in the egg are essential for establishing initial differences in cell fates. Microtubule mediated movements in the first cell cycle are critical for determining the dorsal axis. Additionally, cytoplasmic inheritance of germ plasm, a mixture of mitochondria, germ line granules, and various localized RNAs, is required for specifying the primordial germ cells (PGCs). The germ plasm originates within the mitochondrial cloud of early oocytes, which is thought to serve as an initial hub for localization of germ plasm RNAs. The roles of several of these localized RNAs in PGC formation have been well characterized, including nos1, vasa, dazl and dnd1. Recently however, it has become apparent that a subset of germ plasm RNAs in the vegetal cortex may also have roles in dorsal axis formation. These include trim36 and dnd1 in Xenopus, which are thought to facilitate microtubule assembly during cortical rotation, and syntabulin (sybu) in zebrafish, which encodes a kinesin motor protein linker. Sybu is defective in ventralized tokkeabi mutants and is thought to function in distribution of dorsal determinants in the fish egg. Here we examine the function of Sybu in early Xenopus development. Sybu is expressed in the mitochondrial cloud and vegetal cortex of oocytes, similar to the case in zebrafish. In contrast however, Xenopus sybu mRNA remains localized to the germ plasm of early embryos through the gastrula stage. Maternal antisense inhibition of sybu results in embryos with abnormal PGC migration patterns and eventual loss of PGCs. We further present experiments to identify more
precisely the role of Sybu in germline and axis development in *Xenopus* and zebrafish. This research is supported in part by the NIGMS/NIH under award number R01GM083999 to DWH.

Program Abstract #516

**Vasa and Nanos Protein Localization within the Germ Line in Penaeid Shrimp**  
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To improve penaeid shrimp aquaculture, genetically improved strains of faster growing and disease resistant shrimp have been developed. Producers now seek methods to sterilize them as a way to protect their financial investment. Efforts to produce sterilized shrimp by ionizing radiation and polyploidization have proven unsuccessful. An alternative approach to induce sterility would be to inhibit specific genes required for germ line development and gamete formation. Despite the economic importance of penaeid shrimp, little is known about the development of the germ line. Based on *Peneaus japonicus* sequences, custom monoclonal antibodies were made against 10 peptide sequences of Vasa and Nanos. Immunoblotting of ovary tissue was used to identify four Vasa antibodies and three Nanos antibodies recognizing proteins of the expected size of 75 kDa and 45 kDa, respectively. The Vasa and Nanos monoclonal antibodies were used to immunostain sectioned shrimp ovary tissue and whole embryos, nauplius larvae, and protozoea larvae. Vasa was found in the cytoplasm but not the nucleus of mature oocytes and embryos. However, Nanos was evenly distributed within the nucleus of mature oocytes and embryos. Vasa and Nanos both localized to a RNA-rich granular structure (ICB) which is hypothesized to be a germ granule. Vasa and Nanos showed a similar localization pattern in the nauplii and protozoa. In nauplius 1, Vasa and Nanos staining was found in cells of mesendoderm origin on the ventral side. In protozoa 1, Vasa and Nanos expression was observed in the dorsal abdomen where the adult gonad later forms. The Vasa and Nanos antibodies will eventually be used to trace the germ line throughout development to provide a better understanding of shrimp germ line development and analyze the results of loss-of-function studies. Supported by CSIRO Food Futures Flagship Cluster on “Sex ratio and sterility for commercial animal production.”

Program Abstract #517

**Gap junction-mediated signalling regulates the proliferation and differentiation of somatic cyst cells in the *Drosophila* testis**

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Gametogenesis is a conserved process in animals that requires intricate signalling between germ cells, which will give rise to sperm or eggs, and somatic cells, which support germline development. A key feature of gametogenesis is the involvement of specialized stem cells that give rise to both the soma and the germline. Previous work in *Drosophila* has illustrated that soma-germline interactions control stem cell behaviour. Failure to achieve proper regulation of germline-soma communication within the stem cell niche or during spermatogenesis can result in infertility or the formation of tumours. Flies containing mutations in the gene zero population growth/innexin4 (*zpg*) are sterile and possess tiny gonads. *zpg* has been shown to code for an innexin, a gap junction protein. Previous studies indicate that Zpg functions in the germ line to regulate germ cell function but the precise role of Zpg has not yet been elucidated. Our preliminary data support the idea that Zpg mediates communication from the germline to the soma. We have uncovered previously uncharacterized defects in the soma of *zpg* mutants, including overproliferation and delayed differentiation. In addition, we have analysed the function of Innexin2 in the testis, showing that Zpg in the germline interacts with Innexin2 in the soma to regulate spermatogenesis. Our observations support the assertion that Zpg helps form gap junctions between the soma and germline. To further analyse the function of Zpg, we are carrying out a structure/function analysis of the Zpg protein. Altogether, our studies are beginning to provide mechanistic insight into germline-soma communication and the role of gap junctions in regulating stem cell proliferation and differentiation.  

This work has been funded by grants from NSERC and CIHR.

Program Abstract #518

**Profilin and septate junctions are required in the soma for the formation a permeability barrier around the germline in *Drosophila* testes**

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Gametogenesis requires cooperation between the germline, which develop into sperm or eggs, and the soma, which surrounds and supports the developing germline. In the *Drosophila* testis, differentiating germ cells are encapsulated by two somatic cells, which surround the germline throughout spermatogenesis. To identify genes that are required for the formation and maintenance of proper soma-germline interactions we carried out a candidate screen. Our screen identified a role for Chickadee, the fly ortholog of profilin, in the soma for mediating germline encapsulation. We find that EGF signaling is disrupted upon depletion of Chickadee and asked whether defective encapsulation disrupts the milieu in which such signaling events take place. To this end, we developed a permeability assay to assess the accessibility of the germline to signaling molecules during various stages of spermatogenesis. Using this permeability assay, we show that for germline stem cells, gonialblast, and early spermatogonial stages the permeability barrier is not established. This is surprising because it shows that encapsulation of the germline, by itself, does not insulate the germline. Furthermore we find that septate junction markers become upregulated and localize as a belt connecting the two somatic cells that surround the germline during these stages. Importantly, knockdown of either Chickadee or septate junction components disrupts the
formation and/or maintenance of the permeability barrier around the germline.
Our work identifies the formation of permeability barrier between the somatic cells that surround the germline that could play a major role in shaping the signalling events that occur between the soma and the germline in the testes.

This work has been funded by grants from NSERC and the CIHR.

Program Abstract #519
Regulated cell cycle dependent transcriptional silencing promotes germline stem cell differentiation.
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Loss of stem cell differentiation has been shown to have implications in ageing and in diseases such as cancer. Therefore it is critical to identify mechanisms that regulate stem cell self-renewal and differentiation. The germ line is the ultimate stem cell as it is both totipotent and immortal. Therefore, paradigms established in the germ line can easily be extended to other stem cell systems.

Drosophila germ line stem cells (GSCs) are a great model system to understand differentiation as its germline is well characterized. During Drosophila melanogaster embryogenesis, polar granule component (pgc), a global transcriptional silencer, represses transcription of somatic genes in primordial germ cells promoting germ line fate. Surprisingly, we have discovered that Pgc is expressed in a very different developmental stage, during oogenesis. We found that Pgc is expressed in the stem cell daughter or the pre-cystoblast. However, we observed that Pgc is expressed in the pre-cystoblasts for a part of its life cycle suggesting that it may be expressed in a cell cycle dependent manner. To determine in which stage of the cell cycle Pgc is being expressed, we used different cell cycle markers simultaneously with a reporter for Pgc and found that majority of the pre-cystoblast cells expressed pge in the early G2 phase. Additionally, we have shown that in loss of pge, cell cycle is disrupted and majority of the pre-cystoblasts are accumulated in the G1/S-phase indicating the role of Pgc in regulating cell cycle during differentiation. Therefore, our results show that cell cycle dependent expression of a transcriptional silencer, Pgc, drives stem cell differentiation.

Program Abstract #520
The Drosophila Deadbeat protein reveals a new link between a sperm nuclear basic protein and paternal chromosome maintenance in early embryogenesis
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Paternal effect mutations reveal the contributions that fathers make to ensure the successful development of their offspring. Compared to maternal effect or zygotic mutations, paternal effect mutations are rare and only a few paternally provided products have been molecularly identified. Here we describe the analysis of ddbt, a paternal effect mutation of Drosophila melanogaster.

We show that the Ddbt protein is expressed during spermatogenesis, localized post-meiotically to spermad tid telomeres and retained on mature sperm. Males that fail to express Ddbt produce spermatids that lose the telomere-capping complex during the dramatic chromatin remodeling process that transforms the spherical spermatid nucleus into the condensed head of the mature sperm. Although sperm produced by ddbt mutant fathers are fertilization-competent, progeny die due to telomeric fusions and fragmentation of paternal chromosomes. The Ddbt protein is an SNBP, a sperm nuclear basic protein. Many SNBPs have been biochemically identified from a wide variety of animals and these proteins are generally implicated in spermad tid nuclear condensation. Our functional analysis of Ddbt reveals an essential role for one SNBP that is independent of nuclear condensation but required for the maintenance of the telomere-capping complex. We suggest that other members of the SNBP family may also have crucial post-fertilization roles with potential consequences on paternal effects and chromosomal imprinting.

Program Abstract #521
Novel domains of expression for orphan receptor tyrosine kinase Ror2 in the human and mouse reproductive system
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The orphan receptor tyrosine kinase Ror2 has been associated with Recessive Robinow syndrome (RRS) and dominant Brachydactyly type B. The phenotypes of mouse mutants implicate Ror2 in the development of the heart, lungs, bone and craniofacial structures, which are affected in RRS. Following a recently identified role of Ror2 in the migration of mouse primordial germ cells, which are a functionally pluripotent cell lineage in the embryo, we extensively characterized its expression throughout the fetal and postnatal internal reproductive system. We show that transcript and protein are present in the germ cells and somatic cells of the testis and the ovary of both the mouse and human fetus. Complex interactions between the developing germ cell, and the surrounding somatic cells in the gonad are essential for optimal development of the sperm or oocyte. Expression of Ror2 in the developing germ cell and its supporting microenvironment raises the intriguing possibility of Ror2 involvement in both intrinsic development programs and niche interactions of germ cells. In reproductive tract structures, we find that Ror2 is expressed in the mesonephros, developing Wolffian and Müllerian ducts and later in their derivatives, the epididymal epithelium and uterine epithelium. Currently we are using conditional mice, along with the drug-inducible and tissue specific Cre alleles to analyze requirement of Ror2 in oocytes, gonad support cells, postnatal gametogenesis and reproductive tract development. This study sets the stage to explore function for this tyrosine kinase receptor in these novel regions of expression in the developing reproductive system in both mouse and human.

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**Program Abstract #522**

**Mouse antral NSN oocytes developmental arrest is due to lack of MATER and cytoplasmic lattices**

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In most mammals, the antral compartment of the ovary contains two different kinds of oocytes, SN and NSN (Surrounded Nucleolus and Not Surrounded Nucleolus), whose main differentiating characteristic lies in their acquisition of developmental competence: still inexplicably only the SN (70% of the antral oocytes population) are able to develop to the blastocyst stage while the NSN arrest at the two-cell stage. Using proteomics and microscopy techniques, we analyzed both SN and NSN oocytes, showing for the first time that NSN-derived embryo arrest at the two-cell stage is due to the under-regulation of some maternal proteins together with a lack of cytoplasmic lattices (CPLs). The SN and NSN proteomic profile showed very few differences: in particular, the down-regulation of the proteins MATER and FILIA in NSN. MATER is responsible for the acquisition of meiotic competence, in fact Mater<sup>tm</sup> oocytes do not have CPLs and arrest at the two-cell stage. Based on our results, we speculated that the Mater<sup>tm</sup> antral population should be composed mostly of NSN. To confirm this hypothesis we isolated oocytes from the Mater<sup>tm</sup> mice, showing that 84% of them are of the NSN type. Transmission Electron Microscopy analysis also revealed that NSN contain no or very few CPLs as compared to SN. Through the morphometric analysis of lipid droplets (LD) content, we demonstrated that NSN oocytes contain a significantly higher amount of LD than SN. This is further evidence of the role played by CPLs in the resumption of meiosis and of LD being good candidate markers for oocyte developmental competence.

Our results provide the first molecular evidence that accounts for NSN-derived embryo’s inability to progress beyond the two-cell stage and, thus, for some of the naturally occurring pre-implantation losses in mammals. These data will also give basic knowledge about the function of the miniature, wonderfully organized laboratory of molecular biology that is the oocyte.

**Program Abstract #523**

**Role of the inhibitory kinase WEE-1.3 in regulating the meiotic cell cycle and fertility in C. elegans**

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Meiosis is a specialized cell cycle by which the haploid gametes, oocytes and sperm, are produced. It is of crucial importance for successful sexual reproduction and human health, as defects during meiosis have serious deleterious outcomes such as infertility, birth defects, and tumorigenesis. Meiosis is controlled via dualing regulatory phosphorylation events on the cyclin-dependent kinase (Cdk1) component of maturation promoting factor (MPF). The Wee1/Myt1 kinases provide inhibitory phosphorylations that keep MPF inactive, and it was previously shown that depletion of the Myt1 ortholog in C. elegans, WEE-1.3, results in precocious oocyte maturation and infertility. To further investigate the function of WEE-1.3 during oocyte maturation, we performed an extensive study of the precocious oocyte maturation phenotype observed upon WEE-1.3 depletion. This demonstrated that WEE-1.3-depleted proximal germlines containing precocious oocytes have begun to transcribe embryonic genes and inappropriately express proteins in patterns normally found in embryos. We performed an RNA interference (RNAi) suppressor screen of the infertility phenotype exhibited upon WEE-1.3 depletion and identified 57 genes, that when co-depleted along with WEE-1.3, restore fertility to the animals. The 57 suppressors were placed in to three different classes based on their degree of suppression. We are currently studying the mechanism of how one suppressor, ETR-1 (an RNA-binding protein), is acting. ETR-1 is characterized as being muscle-specific and Not Surrounded Nucleolus, whose main differentiating characteristic lies in their acquisition of developmental competence: still inexplicably only the SN (70% of the antral oocytes population) are able to develop to the blastocyst stage while the NSN arrest at the two-cell stage. Using proteomics and microscopy techniques, we analyzed both SN and NSN oocytes, showing for the first time that NSN-derived embryo arrest at the two-cell stage is due to the under-regulation of some maternal proteins together with a lack of cytoplasmic lattices (CPLs). The SN and NSN proteomic profile showed very few differences: in particular, the down-regulation of the proteins MATER and FILIA in NSN. MATER is responsible for the acquisition of meiotic competence, in fact Mater<sup>tm</sup> oocytes do not have CPLs and arrest at the two-cell stage. Based on our results, we speculated that the Mater<sup>tm</sup> antral population should be composed mostly of NSN. To confirm this hypothesis we isolated oocytes from the Mater<sup>tm</sup> mice, showing that 84% of them are of the NSN type. Transmission Electron Microscopy analysis also revealed that NSN contain no or very few CPLs as compared to SN. Through the morphometric analysis of lipid droplets (LD) content, we demonstrated that NSN oocytes contain a significantly higher amount of LD than SN. This is further evidence of the role played by CPLs in the resumption of meiosis and of LD being good candidate markers for oocyte developmental competence.

Our results provide the first molecular evidence that accounts for NSN-derived embryo’s inability to progress beyond the two-cell stage and, thus, for some of the naturally occurring pre-implantation losses in mammals. These data will also give basic knowledge about the function of the miniature, wonderfully organized laboratory of molecular biology that is the oocyte.

**Program Abstract #524**

**A Novel Function for the C. elegans Torsin OOC-5 in Nuclear Pore Function**

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OOC-5 is one of three Torsin family AAA+ ATPases in C. elegans. Torsins localize to the lumen of the endoplasmic reticulum and contiguous nuclear envelope (NE), and mutation in human TorsinA leads to a neuromuscular disease. TorsinA interacts with integral NE proteins, and loss of torsins in mice and flies leads to NE membrane abnormalities. However, the precise cell biological roles of torsins are still being elucidated. In C. elegans, ooc-5 mutants are maternal effect lethal and have disrupted polarity and germ-line defects that give rise to small oocytes. We investigated the role of OOC-5 at the NE in the C. elegans germ line and report a novel role of a Torsin in nuclear pore biology. In ooc-5 mutants, nucleoporins (Nups) were mislocalized in large plaques beginning at the transition zone, where germ cells enter meiosis, and persisted throughout meiosis. Moreover, SUN/KASH proteins were mislocalized in ooc-5 gonads. Abnormal Nup plaques also formed in adult intestinal nuclei, and to a lesser extent in embryos from mutant mothers. Examination of L1 and L4 stage larvae showed that the Nup phenotype did not correlate with either differentiation
or absolute age of the worm. EM analysis showed ultrastructural defects in the NE in both intestinal and germ cell nuclei. Large vesicle-like structures were seen in the perinuclear space, similar to defects reported in other Torsin mutants. Despite NE and Nup defects, the size exclusion barrier of nuclear pores was maintained and pores were capable of import in ooc-5 nuclei. However, using a kinetic assay in embryos, we found that the nuclear import of PIE-1 was delayed. Together our data show that OOC-5 is important in the biology of the NE and nuclear pores, a role that is likely conserved. We are currently examining cause and effect relationships of germline phenotypes, and how these correlate with embryonic polarity defects.

Funding: Dystonia Medical Research Foundation and NIGMS grant 1F32GM103041-01A1

Program Abstract #525
ERK dependent Dicer Phosphorylation Coordinates Oocyte-to-Embryo Transition in Caenorhabditis elegans
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Cellular and molecular mechanisms that suppress small RNAs in oocytes while maintaining them in zygotes remain unknown. We show that ERK directly phosphorylates Dicer on two conserved residues in its RNAse IIIb and dsRNA-binding domains, respectively; and phosphorylation of these residues is necessary and sufficient to trigger Dicer’s nuclear translocation in worms, flies, mice, and human cells. Phosphorylation of the dsRNA-binding domain confers on Dicer the ability to negatively regulate ERK activity and that phosphorylation of the RNAse IIIb domain inhibits Dicer function. Moreover, we show that ERK must phosphorylate and inactivate Dicer during meiosis I for oogenesis to proceed normally in C. elegans and that this inhibitory grip must be released before fertilization for embryogenesis to proceed normally. Thus, the ERK-Dicer nexus is a key regulator of the oocyte-to-embryo transition and a fundamental mechanism that couples extracellular cues to small RNA production.

Program Abstract #526
Characterization of xnd-1 function during Caenorhabditis elegans germline development
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As the only cells with the ability to produce haploid gametes, germ cells are key to passing genetic material from generation to generation. Proper development of germ cells is therefore critical to prevent infertility, birth defects, and other germline abnormalities. The nematode, Caenorhabditis elegans is a model system for studying germ cell development, because from birth to adulthood we can observe germ cells in situ 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. Because many mutant phenotypes of xnd-1 animals share striking resemblance with that of mitogen activated protein kinase (MAPK) mutants, 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(lov) mutations gave opposite results, with the former decreasing fecundity and the latter increasing in the xnd-1 background. Further progress on understanding the role of xnd-1 and MAPK signaling in germ cell specification will be presented. This research was supported in part by Choose Development!, an NSF (grant IOS-1239422) sponsored program through the SDB.

Program Abstract #527
Specification and differentiation of the nematode germ line are regulated by the chromatin associated factor XND-1
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Despite the central importance of germ cells for the transmission of genetic material between generations, very little is known about the molecular programs that regulate their development. Defects during germ cell formation and differentiation can lead to infertility, birth defects, and formation of germ cell cancers. Work in our lab has identified xnd-1 (X nondisjunction factor-1) as a key regulator of germ cell development in the nematode, Caenorhabditis elegans. Our analysis has revealed that XND-1 is one of the first proteins turned on in the primordial germ cell nuclei (PGC). Consistent with a role in early PGC differentiation, co-depletion of xnd-1 and nos-2, one of C. elegans Nanoshomologs, leads to a synthetic sterile phenotype. We show that xnd-1 regulates the division of the PGCs and that mutant animals have reduced germline size and resultant broods. We also show that xnd-1 is required for various aspects of germline differentiation. Our prior work revealed a role for xnd-1 in meiotic recombination and in establishing germline chromatin architecture (Wagner et al., 2010). Current studies reveal that xnd-1 is required for maintaining germline immortality, for genome stability, for oocyte growth, and for proper germline organization. Our progress on elucidating the genetic basis of these phenotypes will be discussed.
**Program Abstract #528**

**Sequencing and Visualizing Germline miRNAs in *C. elegans***

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More than 175 microRNAs have been identified in the *C. elegans* genome. To determine whether any are specific for the germline, we compared the small RNA transcriptome of worms with and without a germline using RNAseq. Our results indicate that \(\sim30\%\) of the annotated *C. elegans* miRNAs are likely to be enriched in the germline. Remarkably, we found that the levels of five of the eight *mir-35* family members were more than 200-300-fold reduced in worms lacking a germline, raising the possibility that in adult worms this family might be germline-specific. To test this idea further, we developed an in situ hybridization protocol to visualize the distribution of miRNAs in fixed worms. We found that the *mir-35* cluster miRNAs are strongly expressed in the germline, starting in the pachytene region and perduring through oogenesis and early embryogenesis. Consistent with these findings, mothers homozygous for a deletion that removes the *mir-35* cluster lay 100% dead embryos (Miska et al., 2007). We conclude that the *mir-35* cluster encodes microRNAs that are produced during oogenesis and are critical for maternal inheritance by embryos. This research was funded by the NSF and the NIH.

**Program Abstract #529**

**The role of TGFß signaling in *C. elegans* germ line stem cells 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 developing *C. elegans* germ line as a model for understanding how this decision is influenced by external environmental conditions. In *C. elegans* germ line, progenitors accumulate during larval development to form an adult pool from which gametes are produced. We found that members of TGFß/*daf-7* 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/hibernation-like decision and in lifespan regulation. *daf-7* encodes a TGFß-like ligand. The level of *daf-7* expression in ASI chemosensory neurons provides a link between environmental changes perceived by the animal and the appropriate proliferation of germ cells. The TGFß-receptor signaling complex and the downstream transcriptional regulatory complex act in the distal tip cell (DTC), the germline stem cell niche (Dalfo D. et al., 2012). We are curious to understand how TGFß-receptor signaling in the DTC leads to a response in the germ line. Our previous results indicate that TGFß-receptor signaling modulates germline differentiation in parallel with the GLP-1/Notch pathway. We are investigating this further. The Notch signaling pathway is essential for germline development and maintenance; the ligands are produced in the DTC and the receptor is present in the germ line. Based on more recent results, we hypothesize that TGFß/*daf-7*-mediated accumulation of undifferentiated germ cells in favorable environmental conditions may also modulate the level of Notch pathway signaling.

The project is funded by NIH R01 GM102254.

**Program Abstract #530**

**H3K27 methylation and PRC2 epigenetically transmit a memory of repression across generations and during development***

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To retain cell identity during development, cells must remember patterns of gene expression and repression through cell division. Although repression via methylation of histone H3 on Lys 27 (H3K27me) by Polycomb Repressive Complex 2 (PRC2) is conserved and well studied, the diverse developmental roles of repression and its transmission are not well understood. Our studies reveal that the sole essential role of PRC2 in *Caenorhabditis elegans* is repression of the X chromosomes in germ cells. This repression is transmitted to embryos by both sperm and oocytes. By generating embryos containing some chromosomes with and some without H3K27me, we show that in embryos lacking PRC2, H3K27me is transmitted to daughter chromatids through several rounds of cell division. In embryos with PRC2, the mosaic pattern of H3K27me is perpetuated through embryogenesis. These results demonstrate that H3K27me and PRC2 each contribute to transmitting the memory of repression during development and across generations. This work was supported by NIH T32GM008646, UCSC Dissertation Year Fellowship, and ARCS Foundation Award to LG, and NIH R01GM034059 to SS.

**Program Abstract #531**

**Chemical biology in the embryo: Imaging sulfur in cartilage matrix of proteoglycan mutants***

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Osteoarthritis is caused in part by the loss of proteoglycans (PGs, which are sugar-coated proteins) in cartilage matrix. Sulfation, or the addition of sulfate groups as esters, of PGs is thought to be important for at least two biological roles of cartilage matrix that are
relevent to defects in osteoarthritis patients: tissue integrity and growth factor signaling. Despite the proposed role for PG sulfation in the function of cartilage matrix, however, sulfation in cartilage matrix has never been demonstrated directly.

Here, we use synchrotron-based X-ray fluorescence imaging (XFI) to detail the distribution of total sulfur and sulfate esters in developing cartilage of zebrafish. Our quantitative analyses of these images demonstrate that total sulfur and sulfate esters decrease in cartilage matrix as it differentiates, suggesting a functional link between sulfur content of cartilage matrix and the differentiation of resident cells (chondrocytes). Supporting this hypothesis, we find that sulfate esters are decreased in cartilage of a proteoglycan synthesis mutant, which has precocious chondrocyte differentiation. Surprisingly, total sulfur in this mutant was not affected, suggesting that sulfate is distributed in an alternative chemical form when cartilage proteoglycan production is below normal. The relevance of these findings to human osteoarthritis is currently under investigation.

Program Abstract #532
Mechanism of maxillary suture fusion and midfacial hypoplasia in Apert syndrome
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Midfacial hypoplasia is a frequent comorbidity of craniosynostosis and pathognomonic of disorders such as Apert and Crouzon syndromes that both result from gain-of-function mutations in fibroblast growth factor receptor 2 (Fgfr2). Using mouse models of these syndromes, we have shown that midface hypoplasia is caused by premature fusion of facial sutures that precedes ossification of the cranial base synchondroses and cranial sutures. Here, we used the Wnt1-Cre driver to conditionally activate expression of the Apert syndrome mutant Fgfr2 in the neural crest cell lineage (Fgfr2NC-S252W+) that gives rise to all the bones of the midface as well as frontal skull bones. Fgfr2NC-S252W+ mice developed severe midfacial hypoplasia with fusion of premaxillary-maxillary sutures. Coronal sutures, which are derived from a combination of neural crest and paraxial mesoderm lineages, were not prematurely fused in these mice. To determine how mutant FGFR affects fusion of premaxillary-maxillary sutures, we used an osterix-Cre (Osx-Cre) driver to conditionally activate expression of mutant Fgfr2 in osteoprogenitor cell lineages (Fgfr2OP-S252W+) at the osteogenic front of the sutures. Unlike the neural crest-specific condition mutant that targets the entire facial sutural mesenchyme, Osx-Cre activity was found in a subset of sutural mesenchyme. Furthermore, Fgfr2OP-S252W+ mice only developed mild craniofacial defects without midfacial hypoplasia. We found that the premaxillary-maxillary sutures as well as coronal sutures remain patent even at weaning age in Fgfr2OP-S252W+ mice, suggesting that premature suture fusion may not result from increased osteoblast differentiation at the osteogenic front as previously concluded but could result from early osteogenic commitment of sutural mesenchyme due to enhanced FGFR signaling during development.

This work was supported by NIDCR grant R01DE022561.

Program Abstract #533
Modeling aberrant progenitor cell segregation in the X-linked neurocristopathy craniofrontonasal syndrome
Audrey O'Neill, Andrew Larson, Seungil Kim, Jeffrey Bush
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Craniofrontonasal syndrome (CFNS) is an X-linked neurocristopathy caused by mutations in the EPHRIN-B1 gene that results in dramatic craniofacial, neurological and skeletal abnormalities in heterozygous females, whereas hemizygous males are mildly affected. Ephrin-B1 is a transmembrane member of the Eph/ephrin family of signaling proteins and has both ligand and receptor signaling capabilities. Bidirectional signaling has been proposed to be critical for Eph/ephrin-mediated cell sorting, a phenomenon wherein Eph-expressing cells segregate from ephrin-expressing cells. Ephrin-B1 mosaicism and subsequent cell sorting has been proposed to underlie the unusual heterozygous severity observed in CFNS, but the cellular and developmental mechanisms have not been elucidated, and the timing of and relevant contexts for this action are not clear. By analyzing a series of targeted inducible and signaling mutations in mice, we demonstrate that ephrin-B1 drives cell segregation in the neuroepithelium prior to neural crest emigration. Forward signaling by ephrin-B1 is necessary and sufficient for cell sorting and requires signaling through Rho-family small GTPases to mediate its effects. Finally, we have generated human iPS cell lines as a developmentally relevant model for understanding CFNS etiology. Together, our data contribute in vivo understanding of the mechanistic basis of Eph/ephrin-mediated cell sorting and further demonstrate that this cellular process is fundamental to the pathogenesis of this unusual disease.

Program Abstract #534
Effect of maternal glucocorticoid exposure on sex-specific changes in mouse embryonic development
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Prenatal stress is known to cause intrauterine growth retardation of fetus and is also associated with various metabolic and neurodevelopmental diseases in adult offspring as long-term effects. In addition, many of the diseases associated with prenatal stress exhibit a sex bias. Perturbations and vulnerability to prenatal stress are often more profound for the male, however, the mechanisms responsible for this relationship are not clear. We have previously shown that administration of dexamethasone at gestational day 7.5, 8.5 and 9.5, a critical time point on early placenta development, induces placental defects as well as embryonic growth restriction. In this study, we examined the effect of maternal dexamethasone on male testis development as well as on the sex-
specific changes in embryonic and placental growth. The prenatal administration of dexamethasone at the same time point led to growth restriction in both females and males at E11.5. Afterward, females, but not males, showed restoration to near normal levels at E18.5. During the testis development, sex-determining genes (Sry, Sox9, and other downstream genes) were dysregulated by prenatal dexamethasone. Moreover, prenatal exposure to dexamethasone disrupted testosterone level in males. Taken together, these data provide a valuable resource for finding possible mechanisms that cause sex-specific effects in response to prenatal stress.

Program Abstract #535
**α-melanocyte-stimulating hormone protects developing chicken retina from glutamate-induced excitotoxicity via MCR/miR194 pathway**
Qian Han1,2,3, Qiyu Bo1,2,3, Guangwei Yu1,2,3, Lijie Dong1,2,3, Xun Liu1,2,3, Mian Liu1,2,3, Xiaorong Li1,2,3, Yan Zhang1,2,3
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**Aims:** Study protective effects of α-melanocyte-stimulating hormone (α-MSH) on glutamate-induced excitotoxicity on developing chicken retinas, identify the signaling pathway mediating the protective effects.

**Methods and Results:** Retinas isolated from chick embryos at embryonic day 9 (E9) were cultured as explants in media containing 10% and 15% fetal bovine serum (FBS). Based on morphology comparison revealed by H&E staining, culture media containing 15% FBS were used thereafter. Real-time PCR showed that MC1R and MC5R were predominant melanocortin receptor subtypes expressed in the explants, and their expression patterns were similar to those in retinas in ovo. The excitotoxicity was induced in 4-day-explants by incubation with glutamate for 24 and 48 h. α-MSH was applied 30 min before and during glutamate treatment. TUNEL staining showed that significant amount of apoptotic cells were detected in explants at both time points, which were significantly reduced by α-MSH. LDH activity in culture media was significantly increased at 24 h after glutamate stimulation, and α-MSH normalized the elevated activity. Real-time PCR showed that eNOS and GFAP expression were up-regulated after 24 h glutamate treatment, whereas α-MSH dramatically down-regulated eNOS, GFAP, and iNOS expression in the explants. Intravitreal injection of glutamate into post-hatch chicks induced retinal apoptosis and dampened amplitudes of a wave and flicker in ERG, both of which were significantly alleviated by α-MSH. The miR array indicated miR194 as the most up-regulated miR induced by glutamate, this up-regulation was confirmed by real-time PCR and normalized by α-MSH.

**Conclusions:** Application of α-MSH greatly ameliorated cell apoptosis and tissue destruction, and dramatically reduced eNOS, iNOS, and GFAP expression in the retinal explants stimulated by glutamate. α-MSH administration in glutamate-treated post-hatch chick retinas mitigated apoptosis and improved electrophysiological functions.

Program Abstract #536
**Genetic Influences on Zebrafish Enteric Nervous System Development**
Julia Ganz, Ellie Melancon, Angel Amores, Peter Batzel, Marie Strader, Ingo Braasch, John Postlethwait, Judith Eisen
Institute of Neuroscience, 1254 Univ. of Oregon, Eugene OR 97403, USA

The enteric nervous system (ENS) regulates essential gut functions including motility, secretion, and homeostasis and is composed of diverse neuronal subtypes and glia. Abnormal ENS development causes human diseases, for example Hirschsprung disease (HSCR), in which the distal gut is uninnervated and nonmotile. HSCR is a multifactorial disease and although causative mutations of diverse neuronal subtypes and glia. Abnormal ENS development causes human diseases, for example Hirschsprung disease (HSCR), in which the distal gut is uninnervated and nonmotile. HSCR is a multifactorial disease and although causative mutations

Program Abstract #537
**Coiled-coil domain containing protein 103 regulates myeloid development**
Sarah Beckman, Joshua Waxman
Cincinnati Children’s Hospital Medical Center, USA

Myeloid cells have many important roles including immune surveillance and tissue remodeling. Abnormal myeloid function can result in myeloproliferative disorders such as leukemias. Therefore, understanding regulators of myelopoiesis and myeloid function will lead to novel therapies for myeloid-related disorders. The coiled-coil domain containing protein 103 (Ccdc103) was identified as being mutated in *smh*, a zebrafish model of primary ciliary dyskinesia (PCD), as well as being mutated in humans with PCD. Ccdc103 has a role in dynein arm assembly and cilia motility. We found that Ccdc103 is expressed in the anterior lateral plate...
Program Abstract #538
A novel model of oxidative heart disease in *Xenopus laevis*
Kyle Jewhurst, Kelly McLaughlin
*Tufts Univ., USA*

Although oxidative stress is a major component of morbidity and mortality in human heart disease, due to technical limitations it has been difficult to directly examine the effect of oxidative stress in isolation on the heart. In recent years, however, optogenetic tools have been developed for directly controlling intracellular reactive oxygen species (ROS) levels *in vivo*. Fluorescent proteins such as KillerRed and miniSOG can be used to generate ROS upon excitation with specific wavelengths of light. Our research takes advantage of these new optogenetic tools to create and study a novel model of cardiac disease in the externally developing vertebrate model organism *Xenopus laevis*. We have now begun to characterize the histological, morphological, and functional response of the *Xenopus* heart to our model of oxidative stress, to better determine the role that apoptotic cell death plays in cardiac pathology. Interestingly, our results suggest that some tissues in the embryonic *Xenopus* heart are less sensitive to ROS-induced apoptosis than others. We are currently investigating the underlying factors that allow these cell populations to avoid an apoptotic fate when subjected to high levels of oxidative stress, with a focus on early cardiac developmental genes.

Funding: NSF, AHA (K. McLaughlin)

Program Abstract #539
Expression of FGF9/16/20 and Foxp1 in Larval, Juvenile and Adult *Ciona intestinalis*
Brittany Prioleau
*Winthrop Univ., USA*

*Ciona intestinalis*, commonly known as sea squirt, is a useful model system for studying developmental processes due to its close evolutionary relationship to vertebrate cardiac gene programs and conserved chordate features during heart formation. Two conserved genes that have a great impact on heart development in vertebrates are Foxp1 and FGF9/16/20. FGF9/16/20 is an important gene in heart development and Foxp1 is a transcriptional repressor that regulates myocardial growth which is significant in maintaining proper function of the heart. FGF9/16/20 was shown to be a necessary factor in heart formation and knocking out this gene resulted in the disruption of the expression of heart lineage markers which facilitates in heart formation. Other studies demonstrated that knocking out Foxp1 during heart development decreased the rate of cardiomyocyte proliferation. Based on the results from previous studies, our hypothesis is that Foxp1 and FGF9/16/20 also play an important role in heart development and regeneration in *Ciona*. Here, the expressions of these genes were examined via RT-PCR and quantitatively analyzed in the *Ciona* model. Preliminary results demonstrate FGF9/16/20 expression in the juvenile and in the adult heart; however, expression of Foxp1 was not found. In addition, real time RT-PCR is being used to quantitatively determine gene expression levels of FGF9/16/20 and Foxp1 in the larval, juvenile, and adult *Ciona*. Additionally, the technique *in situ* hybridization is being used to examine tissue specific expression in juvenile and adult hearts using Digoxigenin-labeled RNA probes for FGF9/16/20 and Foxp1. Understanding the expression patterns during development of these genes will help elucidate conserved mechanisms of cardiac myocyte proliferation and heart development in chordates.

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Program Abstract #540
Chemical screening in *Drosophila* reveals a side effect of chemotherapy that induces stem cell hyper proliferation
Michele Markstein1, Samantha Dettorre1, Julio Cho2, Ralph Neumuller2, Soren Craig-Müller2, Norbert Perrimon2
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Here we report the development of an *in vivo* system to study the interaction of stem cells with drugs using a tumor model in the adult *Drosophila* intestine. Strikingly, we find that some FDA-approved chemotherapeutics that can inhibit the growth of *Drosophila* tumor stem cells can paradoxically promote the hyper-proliferation of their wild type counterparts. These results reveal an unanticipated side effect on stem cells that may drive tumor recurrence. We show that this side effect is mediated by the stem cell microenvironment, which we found responds to a wide spectrum of chemotherapy drugs. Importantly, we find that the side effect is mediated by the evolutionarily conserved JAK-STAT inflammation pathway, raising the possibility that a similar side effect may...
Patterning plant epidermis: Cell fate and communication
Keiko U. Torii
Howard Hughes Medical Institute and Dept. of Biology, Univ. of Washington, Seattle, WA 98195 USA

Multicellular organisms produce complex tissues with specialized cell types for functionality. Our group is interested in understanding the molecular mechanism of how a cell constituting a multicellular organism communicates with each other to execute decision-making processes. Specifically, we are focusing on stomatal patterning on developing epidermis as a model for two-dimensional spatial patterning for its simplicity, accessibility, and availability of molecular-genetic and imaging tools. Stomatal patterning occurs according to positional cues and requires critical cellular decision-making steps of whether or not to become stomata. During development of photosynthetic organs, a selected population of undifferentiated protodermal cells undergoes asymmetric cell divisions that initiate the stomatal cell lineage. A stomatal precursor cell reiterates asymmetric cell division and eventually differentiates into guard cells. Recent progress by our group and others has led to the discovery of key molecules and pathways controlling stomatal patterning and differentiation: (1) Sequential and combinatorial actions of five bHLH transcription factors specifying stomatal precursor cell state transitions; (2) A ligand-receptor system enforcing frequency and orientation of asymmetric cell division; (3) Intrinsic polarity and cellular constituents that are required for creating and maintaining asymmetry. The next challenge is to understand how these regulatory components: ligands, receptors, transcription factors and polarity components are put together in the context of two-dimensional tissue patterning in the plant epidermis. Combining high-resolution live imaging approaches, biochemistry, mathematical modeling, and large-
scale genomics and epigenomics, we seek to unravel multi-scale regulatory mechanisms coordinating stomatal patterning in real time and space.

Program Abstract #544
Notch signaling controls lineage decisions during zebrafish sensory hair cell development and regeneration
Tatjana Piotrowski1, Andres Romero-Carvajal1,2, Agne Kozlowskaja-Gumbriene1
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Sensory hair cell loss in the inner ear leads to permanent hearing loss. Lateral line hair cells are homologous to mammalian inner ear hair cells and are an excellent model for the study of hair cell regeneration. The lateral line sense organs (neuromasts) consist of a central core of sensory hair cells that are surrounded by inner support cells and mantle cells. While mammals are unable to regenerate hair cells, hair cells in the zebrafish lateral line system readily regenerate via inner support cell proliferation. To identify neuromast stem cells we performed fate analyses of all dividing cells in 72 hour time lapse movies and BrdU analyses of homeostatic and regenerating sensory organs. Support cell divisions either produce two support cells to amplify the support cell pool (amplifying divisions) or they produce two hair cells (differentiating divisions). To uncover the molecular mechanisms that control this cellular behavior, we performed gene expression analyses in regenerating neuromasts. Changes in cell behavior indeed correlate with quantitative and spatial expression changes of several signaling pathways, most notably the Notch pathway. These changes lead to cell cycle re-entry of support cells. High resolution cell fate analyses demonstrate that loss of Notch signaling during regeneration does not affect proliferation itself but that it leads to a shift from amplifying to differentiating cell divisions. Our data shows that Notch signaling is at the center of cross talk between several signaling pathways that together orchestrate proliferation and cell division patterns during homeostasis and regeneration.

Program Abstract #545
Evolution of embryonic organizing activity
Elaine C. Seaver, Aldine R. Amiel
Whitney Laboratory for Marine Bioscience, USA

The highly stereotypic early development program called spiral cleavage is shared among many members of the Lophotrochozoa, a large bilaterian clade that exhibits enormous body plan diversity. Such conservation allows for comparison of homologous cells across diverse taxa such as annelids and mollusks, and for analysis of cell lineage evolution. Previous studies in mollusks identified an ‘organizing activity’, which influences development of surrounding embryonic cells. This organizing activity originates from a single cell, called 3D, a progenitor of endomesoderm. However, characterization of organizing activity is unknown for the large clade of polychaete annelids. Using available fate map information for the annelid Capitella teleta, we used single cell laser cell deletions to dissect the role of individual cells on patterning of the larval body. Morphological features and cell and tissue-specific markers were employed to analyze patterning of the body axes following deletion of thirteen uniquely identifiable individual cells in the early stage embryos. Our results demonstrate the clear presence of an organizing activity in C. teleta, although surprisingly, the cellular identity and timing of this activity are different from that of other spiralians. In C. teleta, organizing activity is restricted to the primary ectodermal somatoblast, the cell called 2d, and not the progenitor of endomesoderm. Specifically, deletion of 2d leads to larvae that lack bilateral symmetry, a dorso-ventral axis and eyes, with additional defects in the brain, foregut and mesoderm. In addition, we show that ERK/MAPK signaling does not appear to be involved in mediating organizing activity in C. teleta, which contrasts with data from mollusks and the polychaete Hydrodies elegans, and highlights variation among spiralian embryos at the molecular level. These results contribute to our understanding of the evolution of developmental programs. Funded by NSF (IOS09-23754 to ECS).

Program Abstract #546
Mitotic membrane turnover coordinates differential induction of the heart progenitor lineage
Brad Davidson, Christina Cota
Swarthmore College, USA

Anchorage dependent growth factor signaling impacts fate specification and morphogenesis. Anchored integrin receptor complexes regulate signaling through participation in transduction cascades or alterations of local plasma membrane composition. In vitro studies indicate that anchorage dependent membrane remodeling compartmentalizes growth factor signaling components. Although signaling often occurs in actively dividing cells, the in vivo impact of mitosis on adhesion dependent signaling remains poorly characterized. Here we show that mitotic membrane turnover orchestrates adhesion dependent signal compartmentalization. We investigate in vivo signaling dynamics in the invertebrate chordate, Ciona intestinalis. In Ciona, matrix adhesion polarizes Fibroblast Growth Factor (FGF)-dependent induction of the heart progenitor lineage. We found that matrix adhesion promotes localized enrichment of FGF receptors by inhibiting mitotic internalization and degradation. Through mutational analysis of beta-integrin subunits, we have experimentally defined the motif required for membrane stabilization and mitotic FGFR retention. Furthermore, our results indicate that Caveolin-rich membrane domains function downstream of adhesion to retain FGFR during mitotic rounding. Our results support a revised model of anchorage-dependent signal transduction in which mitotic membrane turnover resets historic, pre-mitotic receptor distribution according to contemporaneous adhesive cues.
Program Abstract #547
Assessing teaching and learning effectiveness
William Anderson¹, Sally Shuler¹, Virginia Anderson², Steve Klein³, Charles Sullivan³, Rebecca Landsberg⁴
¹Washington Informal Science Education (WISE) Consortium, USA; ²Towson Univ., USA; ³National Science Foundation, USA; ⁴The College of St. Rose, USA; ⁵Harvard Univ., USA
Assessment of student learning is an essential component for developing and improving teaching and research programs in the life sciences. Assessment can help institutions by promoting program successes and emphasize the broader impact of their research programs. There are many challenges for establishing an effective assessment strategy, including the lack of an institutional culture that places value on assessment as well as the need to identify tools that would enhance the development of best practices. This session will provide information on how to effectively conduct an assessment of student learning under various conditions: classroom, lab, research projects/programs, independent study, seminar/discussion session, etc. Presenters will discuss the basic requirements to conduct any informative assessment and will focus on useful tools and strategies specific to the field.

Program Abstract #548
Open explorations of the microcosmos
Manu Prakash
Stanford Univ., USA
Using principles of "frugal science" - I will discuss a few ideas from our group where we are imagining how to enable open explorers (traditional and non-traditional scientists) around the world to ask and discover curiosity driven questions in biological, ecological and health related domains. More specifically I will describe the history and making of "Foldscope", an ultra-low cost origami based print-and-fold paper microscope that brings microscopy out of the lab and into the hands of kids and adults globally. Manufactured with a roll-to-roll printed optics that applies paper folding to make optical instruments, Foldscope is a field microscope that enables a broad range of biological explorations in field conditions. Our long term vision is to bring "microscopy to every child in the world"; and enable a curiosity driven approach to exploring biological mysteries at an early age and encourage explorations of the micro-cosmos.

Program Abstract #549
Asymmetric specification of motile cells in Drosophila oogenesis
Lathiena A. Manning, Ann Marie Weideman, Bilal Moiz, Bradford Peercy, Michelle Starz-Gaiano
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Cellular responses to morphogens are impacted by parameters that govern concentration including release, diffusion, uptake, and decay rates. We propose that the domain created by the landscape of adjacent tissue influences local morphogen concentrations and thereby cell fates. To examine this idea, we are leveraging the simple tissue architecture of the Drosophila melanogaster egg chamber. We focus on the specification of a small set of somatic cells, border cells. When correctly fated, border cells exit the follicular epithelium and collectively migrate into neighboring germline cells toward the oocyte. Border cell specification depends on the secretion of Unpaired (Upd) from two central cells, the polar cells. Upd activates JAK/STAT signaling in a gradient of nearby cells. Previous studies have shown follicle cells closest to the polar cells have the highest STAT activity and therefore become motile border cells. We are using a novel technique to view egg chambers in an upright position in combination with genetic and mathematical approaches to illustrate morphogen responses in the epithelium. This atypical view of egg chambers revealed unexpected differences in STAT activity. Surprisingly, in a large percentage of egg chambers, cells are activated asymmetrically, with only a few border cells touching the polar cells. Genetic analysis revealed mutations that effectively expand STAT activation restore a radial pattern of border cell specification. To characterize morphogen activity in the irregular extracellular domain, we developed a mathematical model of Upd movement. Our model supports the idea that extracellular gaps generated by underlying tissue are sufficient to produce an asymmetric distribution of signaling molecules, and the patterns of activated cells we observe. This work illustrates a novel aspect to morphogen patterning in juxtaposed complex tissues, and sheds light on the acquisition of cell motility.
<table>
<thead>
<tr>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abassah-Oppong, Samuel</td>
<td>297</td>
</tr>
<tr>
<td>Abbruzzese, Genevieve</td>
<td>443</td>
</tr>
<tr>
<td>Abrams, Elliott</td>
<td>494</td>
</tr>
<tr>
<td>Abramyan, John</td>
<td>276</td>
</tr>
<tr>
<td>Abzhanov, Arkhat</td>
<td>274</td>
</tr>
<tr>
<td>Ackerman, Kristin M.</td>
<td>110</td>
</tr>
<tr>
<td>Adam, Mike</td>
<td>348</td>
</tr>
<tr>
<td>Anderson, K.V.</td>
<td>129</td>
</tr>
<tr>
<td>Adler, Carolyn E.</td>
<td>79</td>
</tr>
<tr>
<td>Agapito, Maria</td>
<td>224</td>
</tr>
<tr>
<td>Agarwal, Vikram</td>
<td>306</td>
</tr>
<tr>
<td>Agrawal, Puja</td>
<td>212</td>
</tr>
<tr>
<td>Aguilar, Cristian</td>
<td>98</td>
</tr>
<tr>
<td>Ahn, Jonathon</td>
<td>196</td>
</tr>
<tr>
<td>Ajima, Rieko</td>
<td>347</td>
</tr>
<tr>
<td>AJioka, Itsuki</td>
<td>320</td>
</tr>
<tr>
<td>Akerberg, Brynn</td>
<td>336</td>
</tr>
<tr>
<td>Akiyama, Ryutaro</td>
<td>183</td>
</tr>
<tr>
<td>Al-Rekabi, Zeinab</td>
<td>390, 446</td>
</tr>
<tr>
<td>Albert Hubbard, E. Jane</td>
<td>529</td>
</tr>
<tr>
<td>Alder, Olivia</td>
<td>291</td>
</tr>
<tr>
<td>Aldiri, Issam</td>
<td>320</td>
</tr>
<tr>
<td>Aleman, Abigail</td>
<td>222</td>
</tr>
<tr>
<td>Alfishandi, Dominique</td>
<td>411, 443</td>
</tr>
<tr>
<td>Allen, Anna</td>
<td>523</td>
</tr>
<tr>
<td>Allende, Miguel</td>
<td>101</td>
</tr>
<tr>
<td>Allgood, Miguel</td>
<td>429</td>
</tr>
<tr>
<td>Allison, W. Ted</td>
<td>498</td>
</tr>
<tr>
<td>Almonte, Vanessa</td>
<td>223</td>
</tr>
<tr>
<td>Altman, Eran</td>
<td>521</td>
</tr>
<tr>
<td>Alvarez, Anthony</td>
<td>157, 235</td>
</tr>
<tr>
<td>Amacher, Sharon</td>
<td>197</td>
</tr>
<tr>
<td>Amack, Jeffrey</td>
<td>424</td>
</tr>
<tr>
<td>Amarnath, Smita</td>
<td>375</td>
</tr>
<tr>
<td>Ambalavanan, Manoj</td>
<td>368</td>
</tr>
<tr>
<td>Ambrose, Barbara</td>
<td>22</td>
</tr>
<tr>
<td>Amemiya, Chris</td>
<td>261</td>
</tr>
<tr>
<td>Ameri, Jacqueline</td>
<td>489</td>
</tr>
<tr>
<td>Amiel, Aldine R.</td>
<td>545</td>
</tr>
<tr>
<td>Amin, Bansari</td>
<td>372</td>
</tr>
<tr>
<td>Ammar, Dib</td>
<td>186, 192, 513</td>
</tr>
<tr>
<td>Amores, Angel</td>
<td>536</td>
</tr>
<tr>
<td>Anani, Shihadeh</td>
<td>67</td>
</tr>
<tr>
<td>Anastassiadis, Konstantinos</td>
<td>26</td>
</tr>
<tr>
<td>Anderson, Dorian</td>
<td>45, 506, 507</td>
</tr>
<tr>
<td>Anderson, Kathryn V.</td>
<td>162</td>
</tr>
<tr>
<td>Anderson, Rebecca</td>
<td>225</td>
</tr>
<tr>
<td>Anderson, Ryan</td>
<td>107</td>
</tr>
<tr>
<td>Anderson, Virginia</td>
<td>547</td>
</tr>
<tr>
<td>Anderson, William</td>
<td>71, 547</td>
</tr>
<tr>
<td>Andraso, Greg</td>
<td>77</td>
</tr>
<tr>
<td>Andrey, Guillaume</td>
<td>297</td>
</tr>
<tr>
<td>Angelini, David</td>
<td>245</td>
</tr>
<tr>
<td>Anglin, Chris</td>
<td>365</td>
</tr>
<tr>
<td>Ankamreddy, Harinarayana</td>
<td>362, 364</td>
</tr>
<tr>
<td>Anoju, Chibuzo</td>
<td>120</td>
</tr>
<tr>
<td>Antczak, Doug</td>
<td>19</td>
</tr>
<tr>
<td>Antin, Parker</td>
<td>166, 286</td>
</tr>
<tr>
<td>Antoku, Susumu</td>
<td>452</td>
</tr>
<tr>
<td>Appel, Bruce</td>
<td>66, 457, 459</td>
</tr>
<tr>
<td>Apte, Sunee S.</td>
<td>150</td>
</tr>
<tr>
<td>Archambeault, Sophie</td>
<td>263</td>
</tr>
<tr>
<td>Arlotta, Paola</td>
<td>29</td>
</tr>
<tr>
<td>Armenti, Stephen</td>
<td>450</td>
</tr>
<tr>
<td>Armstrong, Benjamin</td>
<td>105</td>
</tr>
<tr>
<td>Aronow, Bruce</td>
<td>413</td>
</tr>
<tr>
<td>Arora, Ripla</td>
<td>521</td>
</tr>
<tr>
<td>Artavanis-Tsakonas, Spyros</td>
<td>220</td>
</tr>
<tr>
<td>Artinger, Kristin</td>
<td>440</td>
</tr>
<tr>
<td>Artinger, Kristin Bruk</td>
<td>441</td>
</tr>
<tr>
<td>Arur, Swathi</td>
<td>525</td>
</tr>
<tr>
<td>Aschbacher-Smith, Lindsey</td>
<td>377</td>
</tr>
<tr>
<td>Asimeng, Rhoda</td>
<td>138</td>
</tr>
<tr>
<td>Askary, Amjad</td>
<td>418</td>
</tr>
<tr>
<td>Aspiras, Ariel</td>
<td>20, 267</td>
</tr>
<tr>
<td>Åstrand, Carolina</td>
<td>501</td>
</tr>
<tr>
<td>Atsuta, Yuji</td>
<td>130</td>
</tr>
<tr>
<td>Attia, Aria</td>
<td>360</td>
</tr>
<tr>
<td>Atukorala, ADS</td>
<td>175</td>
</tr>
<tr>
<td>Avila, Julian</td>
<td>226</td>
</tr>
<tr>
<td>Bain, Emily</td>
<td>190, 252, 253</td>
</tr>
<tr>
<td>Bain, Virginia</td>
<td>343, 344</td>
</tr>
<tr>
<td>Baker, Clare V.</td>
<td>428</td>
</tr>
<tr>
<td>Baker, Julie</td>
<td>508</td>
</tr>
<tr>
<td>Baker, Ruth E.</td>
<td>439</td>
</tr>
<tr>
<td>Bakken, Trygve</td>
<td>290</td>
</tr>
<tr>
<td>Balashova, Olga A.</td>
<td>126</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Balasubramanian, Revathi</td>
<td>404</td>
</tr>
<tr>
<td>Balciunas, Darius</td>
<td>104</td>
</tr>
<tr>
<td>Balcumiene, Jorune</td>
<td>104</td>
</tr>
<tr>
<td>Balezerski, Bartosz</td>
<td>177</td>
</tr>
<tr>
<td>Baldwin, Emily</td>
<td>92</td>
</tr>
<tr>
<td>Bandyopadhyay, Amitabha</td>
<td>476</td>
</tr>
<tr>
<td>Barbosa-Sabanero, Karla</td>
<td>111</td>
</tr>
<tr>
<td>Barna, Maria</td>
<td>301</td>
</tr>
<tr>
<td>Barragan, Jessica</td>
<td>108</td>
</tr>
<tr>
<td>Barraud, Perrine</td>
<td>428</td>
</tr>
<tr>
<td>Barresi, Michael</td>
<td>108, 429</td>
</tr>
<tr>
<td>Barsi, Julius</td>
<td>41</td>
</tr>
<tr>
<td>Bartel, David</td>
<td>306, 308</td>
</tr>
<tr>
<td>Bashiruddin, Sarah</td>
<td>108, 429</td>
</tr>
<tr>
<td>Bates, Katie</td>
<td>400</td>
</tr>
<tr>
<td>Batzel, Peter</td>
<td>536</td>
</tr>
<tr>
<td>Baugh, Ryan</td>
<td>195</td>
</tr>
<tr>
<td>Baumholtz, Amanda</td>
<td>128</td>
</tr>
<tr>
<td>Bazarsky, Michael</td>
<td>233</td>
</tr>
<tr>
<td>Bazzini, Ariel</td>
<td>304</td>
</tr>
<tr>
<td>Beckerle, Mary</td>
<td>321</td>
</tr>
<tr>
<td>Beckman, Matthew L.</td>
<td>512</td>
</tr>
<tr>
<td>Beckman, Sarah</td>
<td>537</td>
</tr>
<tr>
<td>Behringer, Richard R.</td>
<td>265</td>
</tr>
<tr>
<td>Beier, David</td>
<td>288, 373</td>
</tr>
<tr>
<td>Beier, David R.</td>
<td>371</td>
</tr>
<tr>
<td>Beiriger, Anastasia</td>
<td>58</td>
</tr>
<tr>
<td>Belgacem, Yesser H.</td>
<td>463</td>
</tr>
<tr>
<td>Bell, Russell</td>
<td>321</td>
</tr>
<tr>
<td>Bendall, Andrew J.</td>
<td>388</td>
</tr>
<tr>
<td>Benfey, Philip</td>
<td>4</td>
</tr>
<tr>
<td>Benkovics, Anna</td>
<td>62</td>
</tr>
<tr>
<td>Bennett, Cassandra</td>
<td>10</td>
</tr>
<tr>
<td>Bennett, Karen</td>
<td>69, 528</td>
</tr>
<tr>
<td>Berg, Celeste</td>
<td>131, 144</td>
</tr>
<tr>
<td>Berkey, Samantha</td>
<td>106</td>
</tr>
<tr>
<td>Bermingham-McDonogh, Olivia</td>
<td>361</td>
</tr>
<tr>
<td>Bernadskaya, Yelena</td>
<td>224, 448</td>
</tr>
<tr>
<td>Bernard, Amy</td>
<td>290</td>
</tr>
<tr>
<td>Bernstein, Irwin</td>
<td>481</td>
</tr>
<tr>
<td>Berry, Fred B.</td>
<td>498</td>
</tr>
<tr>
<td>Betzig, Eric</td>
<td>26, 49</td>
</tr>
<tr>
<td>Bevilacqua, Ariana</td>
<td>312</td>
</tr>
<tr>
<td>Bhat, Shivani</td>
<td>67</td>
</tr>
<tr>
<td>Biechele, Steffen</td>
<td>51</td>
</tr>
<tr>
<td>Biersmith, Bridget H.</td>
<td>158</td>
</tr>
<tr>
<td>Bilenky, Misha</td>
<td>291</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisson, Joseph</td>
<td>205</td>
</tr>
<tr>
<td>Blaine, W.G.</td>
<td>241</td>
</tr>
<tr>
<td>Blecher, Ronen</td>
<td>199</td>
</tr>
<tr>
<td>Bleyl, Steven</td>
<td>333</td>
</tr>
<tr>
<td>Bloomekatz, Joshua</td>
<td>135, 162</td>
</tr>
<tr>
<td>Bloomsburg, Sam</td>
<td>196</td>
</tr>
<tr>
<td>Blum, Martin</td>
<td>24</td>
</tr>
<tr>
<td>Bo, Qiyu</td>
<td>535</td>
</tr>
<tr>
<td>Boateng, Ruby</td>
<td>523</td>
</tr>
<tr>
<td>Bobkova, Yelena</td>
<td>232</td>
</tr>
<tr>
<td>Boin, Nathan</td>
<td>326</td>
</tr>
<tr>
<td>Bok, Jinwoong</td>
<td>362, 364</td>
</tr>
<tr>
<td>Bonneau, Ashley</td>
<td>304</td>
</tr>
<tr>
<td>Borodinsky, Laura</td>
<td>127</td>
</tr>
<tr>
<td>Borodinsky, Laura N.</td>
<td>126, 463</td>
</tr>
<tr>
<td>Borowsky, Richard</td>
<td>20</td>
</tr>
<tr>
<td>Borras-Castells, Ferran</td>
<td>242</td>
</tr>
<tr>
<td>Borromeo, Mark D.</td>
<td>458</td>
</tr>
<tr>
<td>Bosada, Fernanda</td>
<td>336</td>
</tr>
<tr>
<td>Boualia, Sami</td>
<td>345</td>
</tr>
<tr>
<td>Bouaquina, Mohamed</td>
<td>152</td>
</tr>
<tr>
<td>Bouchard, Maxime</td>
<td>191, 345</td>
</tr>
<tr>
<td>Bouldin, Cortney M.</td>
<td>61</td>
</tr>
<tr>
<td>Bouldin, Cortney M.</td>
<td>375</td>
</tr>
<tr>
<td>Bourckhardt, Gilian Fernando</td>
<td>186</td>
</tr>
<tr>
<td>Bouzas, Santiago</td>
<td>295</td>
</tr>
<tr>
<td>Box Power, Olivia</td>
<td>2</td>
</tr>
<tr>
<td>Box, Andrew</td>
<td>48</td>
</tr>
<tr>
<td>Braasch, Ingo</td>
<td>264, 536</td>
</tr>
<tr>
<td>Brade, Thomas</td>
<td>208</td>
</tr>
<tr>
<td>Brady, Siobhan M.</td>
<td>12</td>
</tr>
<tr>
<td>Braendle, Christian</td>
<td>391</td>
</tr>
<tr>
<td>BrainSpan Consortium (Bruce Fischl, Mark Gerstein, Daniel Geschwind, Ian Glass, Robert Hevner, Hao Huang, Pat Levitt)</td>
<td>290</td>
</tr>
<tr>
<td>Branchfield, Kelsey</td>
<td>367</td>
</tr>
<tr>
<td>Brissette, Daniel</td>
<td>322</td>
</tr>
<tr>
<td>Broday, Limor</td>
<td>82</td>
</tr>
<tr>
<td>Broitman-Maduro, Gina</td>
<td>10</td>
</tr>
<tr>
<td>Bronner, Marianne</td>
<td>49, 295, 309</td>
</tr>
<tr>
<td>Brooks, Eric</td>
<td>171</td>
</tr>
<tr>
<td>Brosnahan, Margaret</td>
<td>19</td>
</tr>
<tr>
<td>Brown, Alexander</td>
<td>294</td>
</tr>
<tr>
<td>Brown, Alexander S.</td>
<td>189</td>
</tr>
<tr>
<td>Brown, Emilee A.</td>
<td>265</td>
</tr>
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<td>Brown, Jeff</td>
<td>400</td>
</tr>
<tr>
<td>Brown, Nadean L.</td>
<td>405</td>
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<td>Name</td>
<td>Page(s)</td>
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<td>Brunetti, Tonya</td>
<td>293</td>
</tr>
<tr>
<td>Bryk, Jenny</td>
<td>300</td>
</tr>
<tr>
<td>Buac, Kristina</td>
<td>302</td>
</tr>
<tr>
<td>Buchholz, Daniel</td>
<td>323</td>
</tr>
<tr>
<td>Buchholz, Daniel R</td>
<td>324</td>
</tr>
<tr>
<td>Buchtova, Marcela</td>
<td>180, 419</td>
</tr>
<tr>
<td>Bui, Andrew</td>
<td>404</td>
</tr>
<tr>
<td>Burdick, Joshua</td>
<td>426</td>
</tr>
<tr>
<td>Burnham, Justin L.</td>
<td>265</td>
</tr>
<tr>
<td>Burrell, Laura</td>
<td>200, 397, 398</td>
</tr>
<tr>
<td>Bush, Jeffrey</td>
<td>445, 533</td>
</tr>
<tr>
<td>Bush, Jeffrey O.</td>
<td>133, 212, 436</td>
</tr>
<tr>
<td>Busser, Brian</td>
<td>298</td>
</tr>
<tr>
<td>Buttitta, Laura</td>
<td>385</td>
</tr>
<tr>
<td>Butts, John</td>
<td>256</td>
</tr>
<tr>
<td>Buzzi, Ailin</td>
<td>295, 309</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Choi, Jae Young 362
Choi, Jinyoung 324
Choi, Yujung 336
Chou, Tsui-Fen 2
Chow, Ida 69
Christiaen, Lionel 46, 448, 480
Christian, Jan 211
Christopher, Kasey 57
Chuong, Cheng-Ming 278
Cifuentes-Rojas, Catherine 14
Cinquin, Olivier 491
Ciruna, Brian 35
Citarella, Mathew 231
Clark, James 223
Clark-Hachtel, Courtney 243
Clarke, Christine 390, 446
Clay, Matthew 450
Cleaver, Ondine 54, 341
Clements, Wilson K. 119
Coate, Thomas 431
Cobb, John 297
Cockburn, Katie 51
Cohen, Ethan David 205
Collins, Jim 25
Condeelis, John 452
Conlon, Frank 416
Constantinou, S.J. 241
Cooley, James 166
Coombs, Daniel 384
Cooper, Cynthia 475
Cooper, Kimberly 19
Cooper, W. James 230
Copenhagen, Philip 27
Cortez Rossi, Christy 440
Costantini, Frank 122, 347, 349
Cota, Christina 546
Cottingham, Naiga 526
Cousin, Helene 443
Cox, Timothy 170, 172, 275, 532
Cox, Timothy C. 371
Craig-Müller, Soren 540
Crawford, Natalie 467
Crespi, Erica 97, 185
Cretekos, Chris J. 265
Crimp, James 369
Cripps, Richard M. 300
Cristea, Ileana 416
Crow, Karen D. 263
Crump, Gage 177, 412, 418
Cruz, Joesfa 9
Cullum, Rebecca 291, 337
Cundiff, Jennifer 97, 185
Cunningham, Doreen 409
Cunningham, Michael L. 390, 446
Cunningham, Thomas J. 208, 313
Curran, Kevin 475
Cusack, Brian 372
Czerniecki, Stefan 384
Czerwinski, Michael 246
Dabizljevic, Sonja 322
Dahia, Chitra 6
Dale, Rodney 322
Dalgin, Gokhan 355
Damdimopoulou, Pauliina 305
Damerla, Rama 447
Damjanovski, Sashko 151
Danescu, Adrian 179
Dang, Chinh 290
Dao, Anh 87
Darland, Diane 467
Darnell, Diana 75, 286
Das, Raibatak 384
Das, Rhiju 301
Dasi, Lakshmi Prasad 331
Dauer, William T. 524
Davey, Crystal 434, 435
Davey, Megan 421
Davey, Sean 286
Davidson, Brad 546
Davis, Adam 142
Davis, George 54
de Bellard, Maria Elena 438
De La Rosa, Richard 157
de Miguel, Cristina 9
de Santa Barbara, Pascal 328
Degnan, Bernard 229
Deimling, Steven 35
Del Rio-Tsonis, Katia 111
del Viso, Florencia 304
Delacruz, Timothy 240
Delgado Cuenca, Paulina 330
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delgado, Irene</td>
<td>420</td>
</tr>
<tr>
<td>Demby, Celia</td>
<td>406</td>
</tr>
<tr>
<td>Deneris, Evan</td>
<td>31</td>
</tr>
<tr>
<td>Denetclaw, Wilfred</td>
<td>194</td>
</tr>
<tr>
<td>Denholm, Barry</td>
<td>350</td>
</tr>
<tr>
<td>Deol, Gurjoth</td>
<td>210</td>
</tr>
<tr>
<td>Der, Channing J.</td>
<td>391</td>
</tr>
<tr>
<td>Deshwar, Ashish</td>
<td>332</td>
</tr>
<tr>
<td>Deshwar, Ashish R.</td>
<td>334</td>
</tr>
<tr>
<td>Dettorre, Samantha</td>
<td>540</td>
</tr>
<tr>
<td>Devasthali, Vidusha</td>
<td>336</td>
</tr>
<tr>
<td>Devoto, Stephen</td>
<td>108, 415, 429</td>
</tr>
<tr>
<td>Devotta, Arun</td>
<td>468</td>
</tr>
<tr>
<td>Di Stilio, Veronica</td>
<td>227</td>
</tr>
<tr>
<td>Dichmann, Darwin</td>
<td>477</td>
</tr>
<tr>
<td>Dickinson, Daniel J.</td>
<td>1, 505</td>
</tr>
<tr>
<td>Dickson, Benjamin</td>
<td>210</td>
</tr>
<tr>
<td>Ding, Song-Lin</td>
<td>290</td>
</tr>
<tr>
<td>Dixon, Gary</td>
<td>307</td>
</tr>
<tr>
<td>Djabrayan, Nareg</td>
<td>9</td>
</tr>
<tr>
<td>Dohn, Tracy</td>
<td>479</td>
</tr>
<tr>
<td>Domingo, Carmen</td>
<td>469</td>
</tr>
<tr>
<td>Domingo, PhD, Carmen</td>
<td>437</td>
</tr>
<tr>
<td>Domyan, Eric</td>
<td>367</td>
</tr>
<tr>
<td>Dong, Lijie</td>
<td>535</td>
</tr>
<tr>
<td>Donlin, Milene</td>
<td>360</td>
</tr>
<tr>
<td>Donohue, Duncan</td>
<td>32</td>
</tr>
<tr>
<td>Donovan, Lauren</td>
<td>31</td>
</tr>
<tr>
<td>Doris, Rosemarie</td>
<td>415</td>
</tr>
<tr>
<td>Dorr, Kerry</td>
<td>416</td>
</tr>
<tr>
<td>Dosedelova, Hana</td>
<td>180</td>
</tr>
<tr>
<td>Dou, Wei</td>
<td>7</td>
</tr>
<tr>
<td>Downes, Gerald</td>
<td>407</td>
</tr>
<tr>
<td>Downs, Karen</td>
<td>486</td>
</tr>
<tr>
<td>Drain, Stephen</td>
<td>184</td>
</tr>
<tr>
<td>Drake, Melanie</td>
<td>525</td>
</tr>
<tr>
<td>Drapek, Colleen</td>
<td>425</td>
</tr>
<tr>
<td>Drerup, Catherine</td>
<td>30</td>
</tr>
<tr>
<td>Driefer, Wolfgang</td>
<td>5</td>
</tr>
<tr>
<td>Driver, Elizabeth</td>
<td>165</td>
</tr>
<tr>
<td>Drzymalski, Monika</td>
<td>138</td>
</tr>
<tr>
<td>Du, Fang</td>
<td>292</td>
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<td>Duan, Cunming</td>
<td>204</td>
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<td>Dubrulle, Julien</td>
<td>511</td>
</tr>
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<td>Duester, Gregg</td>
<td>208, 313</td>
</tr>
<tr>
<td>Dumaine, Anne Marie</td>
<td>251</td>
</tr>
<tr>
<td>Duman-Scheel, Molly</td>
<td>259</td>
</tr>
<tr>
<td>Duncan, Kent</td>
<td>196</td>
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<td>Dunn, Ariel</td>
<td>135</td>
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<td>Dunn, Ian</td>
<td>421</td>
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<td>Durruthy-Durruthy, Robert</td>
<td>461</td>
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<td>Dwyer, Brett</td>
<td>466</td>
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<td>Dyer, Michael</td>
<td>50, 320</td>
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<td>E, Tian-Fa</td>
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<td>455</td>
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<td>Easterling, Marietta</td>
<td>97, 185</td>
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<td>Easton, Ann</td>
<td>200</td>
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<td>Ebbert, Amanda</td>
<td>290</td>
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<td>Ebert, Alicia M</td>
<td>207</td>
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<td>490</td>
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<td>375</td>
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<td>Eisen, Judith</td>
<td>536</td>
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<td>Eivers, Edward</td>
<td>222</td>
</tr>
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<td>Eldon, Elizabeth</td>
<td>240</td>
</tr>
<tr>
<td>Ellis, Andrea</td>
<td>157, 235</td>
</tr>
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<td>Ellis, Stephanie J.</td>
<td>146, 152</td>
</tr>
<tr>
<td>Engel, Michael</td>
<td>321</td>
</tr>
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<td>England, Samantha</td>
<td>406</td>
</tr>
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<td>Enomoto, Hideki</td>
<td>349</td>
</tr>
<tr>
<td>Enriquez, Michael</td>
<td>398</td>
</tr>
<tr>
<td>Eom, Dae Seok</td>
<td>190, 202</td>
</tr>
<tr>
<td>Eppig, Janan</td>
<td>287</td>
</tr>
<tr>
<td>Epstein, Douglas J.</td>
<td>189</td>
</tr>
<tr>
<td>Erickson, Steve</td>
<td>475</td>
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<td>Ernfors, Patrik</td>
<td>428</td>
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<td>449</td>
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<td>Espiritu, Eugenel</td>
<td>502</td>
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<td>Essig, Jaclyn</td>
<td>99</td>
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<td>Essner, Jeff</td>
<td>262</td>
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<td>123</td>
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<td>Fadul, John</td>
<td>321</td>
</tr>
<tr>
<td>Fairchild, Michael J.</td>
<td>152, 518</td>
</tr>
<tr>
<td>Faltine-Gonzalez, Dylan</td>
<td>374</td>
</tr>
<tr>
<td>Farahabadi-Hosseini, Sara</td>
<td>179</td>
</tr>
<tr>
<td>Faro, Ana</td>
<td>464</td>
</tr>
<tr>
<td>Farr III, Gist H.</td>
<td>16</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
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<td>-----------------------</td>
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<td>Gibson, Daniel</td>
<td>367</td>
</tr>
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<td>Gilbert, Scott</td>
<td>247</td>
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<td>399</td>
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<td>472</td>
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<td>304</td>
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<td>Glavic, Alvaro</td>
<td>393</td>
</tr>
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<td>Gleghorn, Jason</td>
<td>124</td>
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<td>Glenn, Thomas D.</td>
<td>402</td>
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<td>Gline, Stephanie</td>
<td>46</td>
</tr>
<tr>
<td>Gochnauer, Heather</td>
<td>247</td>
</tr>
<tr>
<td>Gokirmak, Tufan</td>
<td>449</td>
</tr>
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<td>Golden, Andy</td>
<td>523</td>
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<td>Golding, Id</td>
<td>155</td>
</tr>
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<td>Goldstein, Bob</td>
<td>1, 505</td>
</tr>
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<td>Gomes, Anielle</td>
<td>378</td>
</tr>
<tr>
<td>Gomez, Alan</td>
<td>105</td>
</tr>
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<td>Gomez, Carlos</td>
<td>82</td>
</tr>
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<td>Gonzalez, Federico</td>
<td>114</td>
</tr>
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<td>Gonzalez, Gabriel</td>
<td>525</td>
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<td>Goodman, Miriam B.</td>
<td>34</td>
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<td>Goodwin, Katie</td>
<td>146</td>
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<tr>
<td>Gopalaiahgari, Mallika</td>
<td>187</td>
</tr>
<tr>
<td>Gordon, Julie</td>
<td>13, 344</td>
</tr>
<tr>
<td>Gorny, Anne</td>
<td>443</td>
</tr>
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<td>Götzfried, Jessica</td>
<td>18</td>
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<td>410</td>
</tr>
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<td>152</td>
</tr>
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<td>Graefelman, Michelle</td>
<td>512</td>
</tr>
<tr>
<td>Grainger, Robert</td>
<td>78</td>
</tr>
<tr>
<td>Grajevskaja, Viktorija</td>
<td>104</td>
</tr>
<tr>
<td>Grant, Kelly</td>
<td>77</td>
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<tr>
<td>Grapi-Botton, Anne</td>
<td>340</td>
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<td>541</td>
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<td>Gray, Jessica</td>
<td>39</td>
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<td>Gray, Ryan</td>
<td>273</td>
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<td>305</td>
</tr>
<tr>
<td>Greco, Todd</td>
<td>416</td>
</tr>
<tr>
<td>Grego-Bessa, Joaquim</td>
<td>162</td>
</tr>
<tr>
<td>Gregory, Clint</td>
<td>132</td>
</tr>
<tr>
<td>Greiner, Thomas U</td>
<td>489</td>
</tr>
<tr>
<td>Grewal, Savraj</td>
<td>386</td>
</tr>
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<td>Greico, Theresa</td>
<td>176</td>
</tr>
<tr>
<td>Greico, Theresa M.</td>
<td>277</td>
</tr>
<tr>
<td>Griffith, Malachi</td>
<td>291</td>
</tr>
<tr>
<td>Grim, Carl</td>
<td>397</td>
</tr>
<tr>
<td>Groner, Anna</td>
<td>267</td>
</tr>
<tr>
<td>Gronostajski, Richard</td>
<td>294</td>
</tr>
<tr>
<td>Gros, Jerome</td>
<td>267</td>
</tr>
<tr>
<td>Grout, Megan</td>
<td>202</td>
</tr>
<tr>
<td>Grover, Sumant</td>
<td>254, 255</td>
</tr>
<tr>
<td>Grubb Jones, Alice</td>
<td>245</td>
</tr>
<tr>
<td>Gullerme, Ezequiel</td>
<td>295</td>
</tr>
<tr>
<td>Gunder, Meredith</td>
<td>203</td>
</tr>
<tr>
<td>Gupta, Bhagwati P.</td>
<td>238</td>
</tr>
<tr>
<td>Gupta, Rakhi</td>
<td>508</td>
</tr>
<tr>
<td>Gupta, Tripti</td>
<td>494</td>
</tr>
<tr>
<td>Guseman, Jessica</td>
<td>55, 395</td>
</tr>
<tr>
<td>Gutierrez, Daniela</td>
<td>101</td>
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<tr>
<td>Gutierrez, Edgar</td>
<td>164</td>
</tr>
</tbody>
</table>

**H**

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ha, Dae-Gon</td>
<td>451</td>
</tr>
<tr>
<td>Ha, Seunghsin</td>
<td>371, 373</td>
</tr>
<tr>
<td>Haani, Virjee</td>
<td>383</td>
</tr>
<tr>
<td>Habib, Shukry</td>
<td>26</td>
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<td>531</td>
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<tr>
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<td>358</td>
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<td>Hadjantonakis, Anna-Katerina</td>
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<td>481</td>
</tr>
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<td>68</td>
</tr>
<tr>
<td>Haimovich, Julian</td>
<td>298</td>
</tr>
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<td>Hákornardóttir, Guðlaug Katrin</td>
<td>384</td>
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<td>524</td>
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<td>87</td>
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<td>449</td>
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<td>96</td>
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<td>458</td>
</tr>
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<td>315</td>
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<td>365</td>
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<td>535</td>
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<td>200, 398</td>
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<td>375</td>
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<td>Harfe, Brian</td>
<td>266</td>
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<td>Harland, Richard</td>
<td>268, 307, 476, 477</td>
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<td>265</td>
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<td>420</td>
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<td>461</td>
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<td>396</td>
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<td>287</td>
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<td>Hayes, Naomi</td>
<td>442</td>
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<td>307</td>
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<td>418</td>
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<td>Hedrick, Stephen</td>
<td>117</td>
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<td>34</td>
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<td>Helde, Kathryn</td>
<td>159</td>
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<td>461</td>
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<td>96</td>
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<td>432</td>
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<td>74</td>
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<td>14</td>
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<td>157</td>
</tr>
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<td>Hernandez, Lydia</td>
<td>153</td>
</tr>
<tr>
<td>Hernandez-Lagunas, Ana-Laura</td>
<td>441</td>
</tr>
<tr>
<td>Hernández-Martínez, R.</td>
<td>129</td>
</tr>
<tr>
<td>Hernández Rodríguez, Benjamin</td>
<td>427</td>
</tr>
<tr>
<td>Herrera Reyes, Alejandra</td>
<td>384</td>
</tr>
<tr>
<td>Herrera, Antonio</td>
<td>138</td>
</tr>
<tr>
<td>Herrmann, Alyssa</td>
<td>383</td>
</tr>
<tr>
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<td>3</td>
</tr>
<tr>
<td>Hertzler, Philip</td>
<td>516</td>
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<td>Hess, Katja</td>
<td>489</td>
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<td>Hesselson, Daniel</td>
<td>107</td>
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<td>Hester, S.D.</td>
<td>241</td>
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<td>274</td>
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<tr>
<td>Higuchi, Ryo</td>
<td>163</td>
</tr>
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<td>Hilinski, William</td>
<td>406</td>
</tr>
<tr>
<td>Hill, D. Ashley</td>
<td>366</td>
</tr>
<tr>
<td>Hill, Robert</td>
<td>421</td>
</tr>
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<td>455</td>
</tr>
<tr>
<td>Hindes, Anna</td>
<td>159</td>
</tr>
<tr>
<td>Hinds, Thomas</td>
<td>395</td>
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<tr>
<td>Ho, Diana</td>
<td>220</td>
</tr>
<tr>
<td>Hockman, Dorit</td>
<td>428</td>
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<tr>
<td>Hofferek, Vinzenz</td>
<td>381</td>
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<tr>
<td>Hoffman, Laura</td>
<td>321</td>
</tr>
<tr>
<td>Hoffman, Matthew P.</td>
<td>358</td>
</tr>
<tr>
<td>Hofstetter, Katrina S.</td>
<td>265</td>
</tr>
<tr>
<td>Hohmann, John</td>
<td>290</td>
</tr>
<tr>
<td>Holley, Scott</td>
<td>149</td>
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<td>Honarbhakhsh, Maryam</td>
<td>224</td>
</tr>
<tr>
<td>Hong, Ray</td>
<td>236</td>
</tr>
<tr>
<td>Hong, Ray L.</td>
<td>237</td>
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<tr>
<td>Hoodless, Pamela A.</td>
<td>337</td>
</tr>
<tr>
<td>Hoopes, Maria</td>
<td>89</td>
</tr>
<tr>
<td>Hopyan, Sevan</td>
<td>35</td>
</tr>
<tr>
<td>Horb, Marko</td>
<td>78, 487</td>
</tr>
<tr>
<td>Hou, Hanxiao</td>
<td>116</td>
</tr>
<tr>
<td>Houk, Andrew</td>
<td>330</td>
</tr>
<tr>
<td>Houston, Douglas W.</td>
<td>515</td>
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<td>Hovatta, Outi</td>
<td>305</td>
</tr>
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<td>428</td>
</tr>
<tr>
<td>Hromowyk, Kimberly</td>
<td>197</td>
</tr>
<tr>
<td>Hu, Di</td>
<td>345, 482</td>
</tr>
<tr>
<td>Hu, Jimmy</td>
<td>182, 267</td>
</tr>
<tr>
<td>Hu, Nan</td>
<td>350</td>
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<tr>
<td>Hu, Yuzhi</td>
<td>302</td>
</tr>
<tr>
<td>Huang, Di</td>
<td>298</td>
</tr>
<tr>
<td>Huang, Kao-Hua</td>
<td>90</td>
</tr>
<tr>
<td>Huang, Lei</td>
<td>289</td>
</tr>
<tr>
<td>Huang, Yongzhao</td>
<td>170</td>
</tr>
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<td>Huangfu, Danwei</td>
<td>114</td>
</tr>
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<td>Hudish, Laura</td>
<td>66, 457</td>
</tr>
<tr>
<td>Huebner, Robert J.</td>
<td>123</td>
</tr>
<tr>
<td>Hueston, Catherine</td>
<td>28</td>
</tr>
<tr>
<td>Huh, Sung-Ho</td>
<td>363</td>
</tr>
<tr>
<td>Hunt, Adam</td>
<td>428</td>
</tr>
<tr>
<td>Hunter, E. Sidney</td>
<td>89</td>
</tr>
<tr>
<td>Hurd, Thomas</td>
<td>3</td>
</tr>
<tr>
<td>Hutson, Mary</td>
<td>203</td>
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<td>210</td>
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<td>110</td>
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</table>

**I**

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
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<td>480</td>
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<td>432</td>
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<td>239</td>
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<td>451</td>
</tr>
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<td>350</td>
</tr>
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<td>Ingersoll, Eric P.</td>
<td>76</td>
</tr>
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<td>268</td>
</tr>
<tr>
<td>Irish, Vivian</td>
<td>21</td>
</tr>
<tr>
<td>Irizarry, Jihyun</td>
<td>382</td>
</tr>
<tr>
<td>Isgrig, Kevin</td>
<td>431</td>
</tr>
<tr>
<td>Islam, Fayeza</td>
<td>517</td>
</tr>
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<td>433</td>
</tr>
<tr>
<td>Ito, Mayumi</td>
<td>95</td>
</tr>
</tbody>
</table>

**J**

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackman, William</td>
<td>369</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------</td>
</tr>
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<td>Jahren, Neal R</td>
<td>84</td>
</tr>
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<td>249</td>
</tr>
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<td>James, Jennifer</td>
<td>198</td>
</tr>
<tr>
<td>Jang, Seunghee</td>
<td>396</td>
</tr>
<tr>
<td>Jaramillo, Michael</td>
<td>513</td>
</tr>
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<td>Jaszczyk, Jacob</td>
<td>87</td>
</tr>
<tr>
<td>Jaurena, Maria Belen</td>
<td>468</td>
</tr>
<tr>
<td>Jausoro, Ignacio</td>
<td>401</td>
</tr>
<tr>
<td>Javier, Anna L</td>
<td>491</td>
</tr>
<tr>
<td>Jeffay, Susan</td>
<td>89</td>
</tr>
<tr>
<td>Jeffery, William</td>
<td>262</td>
</tr>
<tr>
<td>Jensen, Amber</td>
<td>139</td>
</tr>
<tr>
<td>Jewhurst, Kyle</td>
<td>538</td>
</tr>
<tr>
<td>Ji, Hongkai</td>
<td>292</td>
</tr>
<tr>
<td>Ji, Yongchang</td>
<td>424</td>
</tr>
<tr>
<td>Jia, Shihai</td>
<td>181, 413</td>
</tr>
<tr>
<td>Jiang, Rulang</td>
<td>181, 346, 377, 413</td>
</tr>
<tr>
<td>Jiang, Ting-Xin</td>
<td>278</td>
</tr>
<tr>
<td>Jing, Yuan-Ya</td>
<td>209</td>
</tr>
<tr>
<td>Johnson, Aaron</td>
<td>326</td>
</tr>
<tr>
<td>Johnson, Andrew</td>
<td>100</td>
</tr>
<tr>
<td>Johnson, Brennan</td>
<td>331</td>
</tr>
<tr>
<td>Johnson, Dianna</td>
<td>320</td>
</tr>
<tr>
<td>Johnson, Edward</td>
<td>421</td>
</tr>
<tr>
<td>Johnson, Jane E.</td>
<td>458</td>
</tr>
<tr>
<td>Johnson, Kimberly</td>
<td>108</td>
</tr>
<tr>
<td>Johnson, Nathan M.</td>
<td>16</td>
</tr>
<tr>
<td>Johnson, Samuel</td>
<td>132</td>
</tr>
<tr>
<td>Jones, Allan</td>
<td>290</td>
</tr>
<tr>
<td>Jones, Daniel</td>
<td>481</td>
</tr>
<tr>
<td>Jones, Kimberly</td>
<td>336</td>
</tr>
<tr>
<td>Jones, Steven</td>
<td>291</td>
</tr>
<tr>
<td>Jong, Brigette</td>
<td>469</td>
</tr>
<tr>
<td>Jorgensen, Erik</td>
<td>490</td>
</tr>
<tr>
<td>Joshi, Piyush</td>
<td>462</td>
</tr>
<tr>
<td>Jowdry, Andrea</td>
<td>369</td>
</tr>
<tr>
<td>Joyner, Alexandra</td>
<td>214, 374</td>
</tr>
<tr>
<td>Juarez, Matthew</td>
<td>222</td>
</tr>
<tr>
<td>Judge, Chelsey</td>
<td>111</td>
</tr>
<tr>
<td>Juliano, Celina</td>
<td>18</td>
</tr>
<tr>
<td>Jülich, Dörthe</td>
<td>149</td>
</tr>
<tr>
<td>Junco, Marisa</td>
<td>420</td>
</tr>
<tr>
<td>Jung, Han-Sung</td>
<td>370, 380</td>
</tr>
<tr>
<td>Kundmanizade, Taimaa</td>
<td>108</td>
</tr>
<tr>
<td>Kundmanizade, Taimaa</td>
<td></td>
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<tr>
<td>K</td>
<td></td>
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<tr>
<td>Kadin, James</td>
<td>287</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Kirby, Margaret</td>
<td>203</td>
</tr>
<tr>
<td>Kirmizitas, Arif</td>
<td>399</td>
</tr>
<tr>
<td>Kirschner, Marc</td>
<td>39</td>
</tr>
<tr>
<td>Kivelhan, Emily</td>
<td>383</td>
</tr>
<tr>
<td>Klarmann, Kimberly</td>
<td>118</td>
</tr>
<tr>
<td>Klavins, Eric</td>
<td>396</td>
</tr>
<tr>
<td>Klein, Ophir</td>
<td>182</td>
</tr>
<tr>
<td>Klein, Steve</td>
<td>547</td>
</tr>
<tr>
<td>Klimke, Alexander</td>
<td>307</td>
</tr>
<tr>
<td>Klimov, Eugene</td>
<td>210</td>
</tr>
<tr>
<td>Klompstra, Diana</td>
<td>45, 506, 507</td>
</tr>
<tr>
<td>Kniss, Sarah</td>
<td>451</td>
</tr>
<tr>
<td>Knosp, Wendy M.</td>
<td>358</td>
</tr>
<tr>
<td>Knowles, James</td>
<td>290</td>
</tr>
<tr>
<td>Knox, Sarah M.</td>
<td>213, 358</td>
</tr>
<tr>
<td>Kocot, Kevin</td>
<td>231, 232</td>
</tr>
<tr>
<td>Kohn, Andrea</td>
<td>231, 232</td>
</tr>
<tr>
<td>Kong, Yong</td>
<td>57</td>
</tr>
<tr>
<td>Konys, Claire</td>
<td>254</td>
</tr>
<tr>
<td>Koo, Yeon</td>
<td>54</td>
</tr>
<tr>
<td>Koto, Catherine</td>
<td>170</td>
</tr>
<tr>
<td>Kovenock, Marlies</td>
<td>395</td>
</tr>
<tr>
<td>Kowalko, Johanna</td>
<td>262</td>
</tr>
<tr>
<td>Kozlowskaja-Gumbriene, Agne</td>
<td>544</td>
</tr>
<tr>
<td>Kramer, Joseph</td>
<td>139</td>
</tr>
<tr>
<td>Kramer, Sunita</td>
<td>139</td>
</tr>
<tr>
<td>Kravarik, Kellie M.</td>
<td>11</td>
</tr>
<tr>
<td>Krawchuk, Dayana</td>
<td>67</td>
</tr>
<tr>
<td>Krejci, Pavel</td>
<td>419</td>
</tr>
<tr>
<td>Krieg, Michael</td>
<td>34</td>
</tr>
<tr>
<td>Krieg, Paul</td>
<td>75</td>
</tr>
<tr>
<td>Kristen, Clements</td>
<td>383</td>
</tr>
<tr>
<td>Kudlicki, Andrzej</td>
<td>499</td>
</tr>
<tr>
<td>Kulesa, Paul</td>
<td>48</td>
</tr>
<tr>
<td>Kulesa, Paul M.</td>
<td>439</td>
</tr>
<tr>
<td>Kulkarni, Saurabh</td>
<td>323</td>
</tr>
<tr>
<td>Kumar, Sandeep</td>
<td>208, 313</td>
</tr>
<tr>
<td>Kump, Kevin</td>
<td>442</td>
</tr>
<tr>
<td>Kuntaas-Tatli, Ezgi</td>
<td>221</td>
</tr>
<tr>
<td>Kuo, Bryan</td>
<td>92</td>
</tr>
<tr>
<td>Kuo, Calvin</td>
<td>336</td>
</tr>
<tr>
<td>Kuratani, Shigeru</td>
<td>268</td>
</tr>
<tr>
<td>Kusakabe, Rie</td>
<td>268</td>
</tr>
<tr>
<td>Kweon, Junghun</td>
<td>416</td>
</tr>
<tr>
<td>Kwon, Hye-Joo</td>
<td>410</td>
</tr>
<tr>
<td>Kwon, Hyeon Kyu</td>
<td>502</td>
</tr>
<tr>
<td>Kwon, Hyuk-Jae</td>
<td>181</td>
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<td>Labonne, Carole</td>
<td>470</td>
</tr>
<tr>
<td>Ladbury, John</td>
<td>525</td>
</tr>
<tr>
<td>Lai, Wan-Jung</td>
<td>495</td>
</tr>
<tr>
<td>Lai, Yun-Ju</td>
<td>90</td>
</tr>
<tr>
<td>Lai, Yung-Chih</td>
<td>278</td>
</tr>
<tr>
<td>Laird, Diana</td>
<td>521</td>
</tr>
<tr>
<td>Lakomy, David</td>
<td>339</td>
</tr>
<tr>
<td>Lampe, Paul</td>
<td>217</td>
</tr>
<tr>
<td>Lan, Yu</td>
<td>181, 346, 413</td>
</tr>
<tr>
<td>Lancotot, Amy</td>
<td>55, 395</td>
</tr>
<tr>
<td>Lander, Arthur</td>
<td>63</td>
</tr>
<tr>
<td>Landsberg, Rebecca</td>
<td>547</td>
</tr>
<tr>
<td>Langdon, Yvette</td>
<td>494</td>
</tr>
<tr>
<td>Lapan, Sylvain W.</td>
<td>11</td>
</tr>
<tr>
<td>LaPlante, Matt</td>
<td>400</td>
</tr>
<tr>
<td>Larin, Kirill</td>
<td>510</td>
</tr>
<tr>
<td>Larina, Irina</td>
<td>510</td>
</tr>
<tr>
<td>Larsen, Hjalte List</td>
<td>340</td>
</tr>
<tr>
<td>Larson, Andrew</td>
<td>533</td>
</tr>
<tr>
<td>Laske, Roman</td>
<td>461</td>
</tr>
<tr>
<td>Lau, Kimberly</td>
<td>35</td>
</tr>
<tr>
<td>Lavy, Rotem</td>
<td>498</td>
</tr>
<tr>
<td>Lazic, Savo</td>
<td>35, 332</td>
</tr>
<tr>
<td>LeBlanc, Michelle</td>
<td>456</td>
</tr>
<tr>
<td>Ledon-Rettig, Cris</td>
<td>250</td>
</tr>
<tr>
<td>Lee, Chih-Yung</td>
<td>528</td>
</tr>
<tr>
<td>Lee, Daniel</td>
<td>222</td>
</tr>
<tr>
<td>Lee, Eunice</td>
<td>294</td>
</tr>
<tr>
<td>Lee, Jeannie</td>
<td>14</td>
</tr>
<tr>
<td>Lee, Ji-Yeon</td>
<td>534</td>
</tr>
<tr>
<td>Lee, Jiae</td>
<td>140</td>
</tr>
<tr>
<td>Lee, Jong-Min</td>
<td>370, 380</td>
</tr>
<tr>
<td>Lee, Joon Sub</td>
<td>307</td>
</tr>
<tr>
<td>Lee, Keun</td>
<td>414</td>
</tr>
<tr>
<td>Lee, Miler</td>
<td>304</td>
</tr>
<tr>
<td>Lee, Sam</td>
<td>291</td>
</tr>
<tr>
<td>Lee, Shyh-Jye</td>
<td>495</td>
</tr>
<tr>
<td>Legent, Kevin</td>
<td>392</td>
</tr>
<tr>
<td>Lehmann, Ordan</td>
<td>187</td>
</tr>
<tr>
<td>Lehmann, Ruth</td>
<td>3, 456</td>
</tr>
<tr>
<td>Lehner, Ben</td>
<td>43</td>
</tr>
<tr>
<td>Lei, Kai</td>
<td>81</td>
</tr>
<tr>
<td>Leichsenring, Manuel</td>
<td>5</td>
</tr>
<tr>
<td>Lein, Ed</td>
<td>290</td>
</tr>
</tbody>
</table>
Leinonen, Kalle 325  Linz, David 243, 244
Lekven, Arne 497  Lipinski, Robert 373
Lemaire, Laurence Anne 340  Liro, Malgorzata 504
Leming, Matthew T. 259  Lishko, Polina 307
Lemke, Kristi 447  List, Mary 299
Leonard, Andrea 390, 446  Liszewski, Walter 451
Lesperance, Megan 138, 383  Little, Melissa 122
Lessnick, Stephen 321  Littleford, Hana 369
Lettice, Laura 421  Liu, Aimin 389
Levine, Mike 408  Liu, Haijiao 35
Levy, Karine 233  Liu, Han 181, 346, 377, 413
Lewandoski, Mark 118, 208, 266, 347, 367  Liu, Jinling 389
Lewandowski, Jordan 292  Liu, Jonathan 2
Lewandowski, Jordan P. 375  Liu, Karen 169
Lewis, Ace E. 436  Liu, Mian 535
Lewis, Katharine 406  Liu, Na 18
Lewis, Victor M. 237  Liu, Xiaqin 447
Lhamo, Tashi 433  Liu, Xun 535
Li, Ang 278  Liu, Yunzhang 204
Li, Bing 413  Liu, Zhaowei 215
Li, Chunying 182  Lize, Muriel 307
Li, Danyi 35  Lo, Cecilia 372
Li, Dong 289  Lo, Cecilia W. 447
Li, Hongwen 289  Lo-Castro, Adriana 187
Li, Li-Wen 370  Lodato, Simona 29
Li, Lydia 200  Loer, Curtis 460
Li, Ming-Yang 90  Löf-Öhlin, Zarah M 489
Li, Nanbing 159  Logan, Malcolm 422
Li, Qiang 375  Loh, Sarah 6
Li, Qinggang 121, 387  Lohmer, Lauren 450
Li, Qingyin 28  Lombaert, Isabelle M. A. 358
Li, Tiansen 260  Longabaugh, William 325
Li, Tsai-Kun 495  Longaker, Michael 94
Li, Wenjie 275  Longmore, Greg 159
Li, Xiaorong 535  Looger, Loren 246
Li, Xuyan 387  López Ceballos, Pablo 384
Li, You 447  López Falcón Piza, Brenda Araceli 427
Li, Yun 204  Lopez, Andrew 510
Lichtarge, Olivier 155  Lopez, Davys 506
Liebl, Eric 430  Lopez, Suhujey 269, 270
Lin, Haifan 18  Lopez-Ceballos, Pablo 152
Lin, Tzu-Huai 484  Lostchuck, Emily 146, 152
Lin, Wen Yang 140  Louie, Kristin 177
LIN, WEN-YANG 196  Love, Crystal 58
Linbo, Tor 134  Loza, Andrew 159
Linden, Lara 47  Lozano, Elliott 194
Ling, Ka Yi 486  Lu, Ling 204
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lua, Rhonald C.</td>
<td>155</td>
</tr>
<tr>
<td>Luedke, Kory</td>
<td>196</td>
</tr>
<tr>
<td>Lujan, Sean</td>
<td>167</td>
</tr>
<tr>
<td>Lumpe, Andrew</td>
<td>74</td>
</tr>
<tr>
<td>Luong, Mui</td>
<td>491</td>
</tr>
<tr>
<td>Luttrell, Shawn</td>
<td>88</td>
</tr>
<tr>
<td>Luu, Elizabeth</td>
<td>491</td>
</tr>
<tr>
<td>Luz-Madrigal, Agustin</td>
<td>111</td>
</tr>
<tr>
<td>Lyons, Deirdre</td>
<td>37</td>
</tr>
<tr>
<td>Lyson, Peter</td>
<td>322</td>
</tr>
<tr>
<td>Lyvers, Ben</td>
<td>251</td>
</tr>
<tr>
<td>Ma, Ji Hyun</td>
<td>362</td>
</tr>
<tr>
<td>Ma, Le</td>
<td>367</td>
</tr>
<tr>
<td>Ma, Li</td>
<td>262</td>
</tr>
<tr>
<td>Ma, Taylor</td>
<td>159</td>
</tr>
<tr>
<td>Ma, Wenxiu</td>
<td>294</td>
</tr>
<tr>
<td>Ma, Yiqin</td>
<td>385</td>
</tr>
<tr>
<td>Macabenta, Frank</td>
<td>139</td>
</tr>
<tr>
<td>MacGregor, Grant</td>
<td>491</td>
</tr>
<tr>
<td>Machanda, Zarin</td>
<td>71</td>
</tr>
<tr>
<td>Machikas, Alexa</td>
<td>406</td>
</tr>
<tr>
<td>Mackem, Susan</td>
<td>347</td>
</tr>
<tr>
<td>MacKenzie, R. Katie</td>
<td>388</td>
</tr>
<tr>
<td>Madaan, Uday</td>
<td>318</td>
</tr>
<tr>
<td>Madissoon, Elo</td>
<td>305</td>
</tr>
<tr>
<td>Maduro, Morris</td>
<td>10</td>
</tr>
<tr>
<td>Magella, Bliss</td>
<td>348</td>
</tr>
<tr>
<td>Mager, Jesse</td>
<td>312, 352</td>
</tr>
<tr>
<td>Mah, In Kyoung</td>
<td>117</td>
</tr>
<tr>
<td>Mahmud, Abdullah Al</td>
<td>316</td>
</tr>
<tr>
<td>Mahoney, Eric</td>
<td>6</td>
</tr>
<tr>
<td>Maier, Jennifer</td>
<td>19</td>
</tr>
<tr>
<td>Maini, Philip K.</td>
<td>439</td>
</tr>
<tr>
<td>Mamidi, Anant</td>
<td>342</td>
</tr>
<tr>
<td>Man, Kin Suen</td>
<td>525</td>
</tr>
<tr>
<td>Mandal, Amrita</td>
<td>329</td>
</tr>
<tr>
<td>Mandalapu, Sailaya</td>
<td>224</td>
</tr>
<tr>
<td>Mandt, Rebecca</td>
<td>451</td>
</tr>
<tr>
<td>Manley, Nancy</td>
<td>344</td>
</tr>
<tr>
<td>Mann, Zoë</td>
<td>165</td>
</tr>
<tr>
<td>Manning, Alyssa</td>
<td>168</td>
</tr>
<tr>
<td>Manning, Lathiena A.</td>
<td>549</td>
</tr>
<tr>
<td>Mansfield, Jennifer</td>
<td>306</td>
</tr>
<tr>
<td>Mansour, Suzanne</td>
<td>164, 333</td>
</tr>
<tr>
<td>Mansour, Ahmed</td>
<td>420</td>
</tr>
<tr>
<td>Mao, Haibin</td>
<td>395</td>
</tr>
<tr>
<td>Marchant, Jonathan S</td>
<td>84</td>
</tr>
<tr>
<td>Marcho, Chelsea</td>
<td>312</td>
</tr>
<tr>
<td>Marcotte, Michael</td>
<td>345</td>
</tr>
<tr>
<td>Mariani, Francesca</td>
<td>56, 117</td>
</tr>
<tr>
<td>Marin, Ignacio</td>
<td>80</td>
</tr>
<tr>
<td>Marini, Melisa</td>
<td>295</td>
</tr>
<tr>
<td>Markstein, Michele</td>
<td>540</td>
</tr>
<tr>
<td>Marlow, Florence</td>
<td>494</td>
</tr>
<tr>
<td>Marlow, Florence L.</td>
<td>159, 402</td>
</tr>
<tr>
<td>Marra, Marco</td>
<td>291</td>
</tr>
<tr>
<td>Martik, Megan</td>
<td>37, 141</td>
</tr>
<tr>
<td>Martin, Benjamin</td>
<td>478</td>
</tr>
<tr>
<td>Martindale, Mark Q.</td>
<td>234</td>
</tr>
<tr>
<td>Martinez, Darwin</td>
<td>438</td>
</tr>
<tr>
<td>Martinez-Gomez, Jesus</td>
<td>227, 228</td>
</tr>
<tr>
<td>Martins, Rodrigo</td>
<td>378</td>
</tr>
<tr>
<td>Marty-Santos, Leilani</td>
<td>341</td>
</tr>
<tr>
<td>Marzolo, Maria Paz</td>
<td>401</td>
</tr>
<tr>
<td>Mason, D. Adam</td>
<td>138, 383</td>
</tr>
<tr>
<td>Matei, M.T.</td>
<td>241</td>
</tr>
<tr>
<td>Mathewson, Andrew</td>
<td>434</td>
</tr>
<tr>
<td>Mathewson, Andrew W.</td>
<td>435</td>
</tr>
<tr>
<td>Matus, David Q.</td>
<td>147</td>
</tr>
<tr>
<td>Maurer, Kate A.</td>
<td>405</td>
</tr>
<tr>
<td>Mauri, Pierluigi</td>
<td>522</td>
</tr>
<tr>
<td>May-Simera, Helen</td>
<td>260</td>
</tr>
<tr>
<td>Mazza-Curll, Kathleen</td>
<td>17</td>
</tr>
<tr>
<td>Mc Ardle, Adrian</td>
<td>94</td>
</tr>
<tr>
<td>McCann, Anna</td>
<td>190</td>
</tr>
<tr>
<td>McCartney, Brooke M.</td>
<td>221</td>
</tr>
<tr>
<td>McClay, David</td>
<td>37, 141</td>
</tr>
<tr>
<td>McCright, Ingeborg</td>
<td>287</td>
</tr>
<tr>
<td>McCulloch, Katherine</td>
<td>490</td>
</tr>
<tr>
<td>McDowell, William</td>
<td>48</td>
</tr>
<tr>
<td>McEwen, Tamara</td>
<td>528</td>
</tr>
<tr>
<td>McGlinn, Edwina</td>
<td>306</td>
</tr>
<tr>
<td>McGraw, Hillary</td>
<td>134</td>
</tr>
<tr>
<td>McIntyre, Daniel</td>
<td>85</td>
</tr>
<tr>
<td>McKay, Andrew</td>
<td>336</td>
</tr>
<tr>
<td>McKey, Jennifer</td>
<td>328</td>
</tr>
<tr>
<td>McKinney, Sean A.</td>
<td>79</td>
</tr>
<tr>
<td>McKnite, Autumn</td>
<td>211</td>
</tr>
<tr>
<td>McLaughlin, Kelly</td>
<td>483, 538</td>
</tr>
<tr>
<td>McLennan, Rebecca</td>
<td>48, 439</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
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<tr>
<td>-------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>McMahon, Andrew</td>
<td>418</td>
</tr>
<tr>
<td>McMenamin, Sarah</td>
<td>190</td>
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<tr>
<td>McMillen, Patrick</td>
<td>149</td>
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<td>231</td>
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<td>421</td>
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<td>Melancon, Ellie</td>
<td>536</td>
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<td>Mellor, Josh</td>
<td>138</td>
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<td>492</td>
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<td>Merzdorf, Christa</td>
<td>167</td>
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<td>Messenberg, Anat</td>
<td>517</td>
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<td>Meulemans Medeiros, Dan</td>
<td>412</td>
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<td>427</td>
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<td>26</td>
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<td>Meyer-Miner, Anne</td>
<td>157, 235</td>
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<td>Michaud, Jacques L.</td>
<td>316</td>
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<tr>
<td>Michelson, Alan</td>
<td>298</td>
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<td>Michiue, Tatsuo</td>
<td>161</td>
</tr>
<tr>
<td>Michos, Odysse</td>
<td>122</td>
</tr>
<tr>
<td>Miller, Adam</td>
<td>282, 283, 285</td>
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<td>Miller, Jeremy</td>
<td>290</td>
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<td>451</td>
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<td>482</td>
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<td>398</td>
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<td>Mitchell-Dick, Aaron</td>
<td>296</td>
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<td>Miura, Grant</td>
<td>330</td>
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<td>Miura, Takashi</td>
<td>163</td>
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<tr>
<td>Miyata, Takaki</td>
<td>163</td>
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<tr>
<td>Moens, Cecilia</td>
<td>159, 282, 283, 285, 434, 452</td>
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<td>Moens, Cecilia B.</td>
<td>435, 465</td>
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<td>Moiz, Bilal</td>
<td>549</td>
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<td>Monahan, Kimberly B.</td>
<td>391</td>
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<td>Montero Barrera, Daniel</td>
<td>427</td>
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<tr>
<td>Montgomery, Mary K.</td>
<td>72, 319</td>
</tr>
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<td>Monti, Manuela</td>
<td>522</td>
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<td>Moody, Sally A.</td>
<td>411</td>
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<td>333</td>
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<td>65</td>
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<td>Moore, Robert</td>
<td>379, 492</td>
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<td>Moravec, Trevor</td>
<td>475</td>
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<td>Moriarty, Chelsea</td>
<td>108</td>
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<td>Mork, Lindsey</td>
<td>246, 412, 418</td>
</tr>
<tr>
<td>Moroz, Leonid</td>
<td>231, 232</td>
</tr>
<tr>
<td>Morris, Ann</td>
<td>187, 188</td>
</tr>
<tr>
<td>Morrison, Jason</td>
<td>48, 439</td>
</tr>
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<td>Morrison, Nicole</td>
<td>196</td>
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<tr>
<td>Morrissey, Meghan</td>
<td>148</td>
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<tr>
<td>Morrissy, A.Sorrana</td>
<td>291</td>
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<tr>
<td>Moskowitz, Ivan</td>
<td>416</td>
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<tr>
<td>Moss, Britney</td>
<td>55, 395</td>
</tr>
<tr>
<td>Mukherjee, Somdutta</td>
<td>15</td>
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<tr>
<td>Mukoyama, Yosuke</td>
<td>198, 354</td>
</tr>
<tr>
<td>Muller, Yara</td>
<td>186, 192, 513</td>
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<td>200</td>
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<td>350</td>
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<td>Murdoch, Barbara</td>
<td>113</td>
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<td>426</td>
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<tr>
<td>Murray, John Isaac</td>
<td>15</td>
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<td>Murry, Charles</td>
<td>115, 481</td>
</tr>
<tr>
<td>Musser, Melissa</td>
<td>474</td>
</tr>
<tr>
<td>Musumeci, Sebastiano</td>
<td>187</td>
</tr>
<tr>
<td>Musunuru, Kiran</td>
<td>71</td>
</tr>
<tr>
<td>Muthukrishnan, Sree Deepthi</td>
<td>216</td>
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<tr>
<td>Myhre, J. Layne</td>
<td>327</td>
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<tr>
<td>Myhre, Layne</td>
<td>178</td>
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<td>Mysore, Keshava</td>
<td>259</td>
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<td>Naegeli, Kaleb</td>
<td>47</td>
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<tr>
<td>Nagandla, Harika</td>
<td>269, 270</td>
</tr>
<tr>
<td>Nagasaka, Arata</td>
<td>163</td>
</tr>
<tr>
<td>Nagayama, Masaharu</td>
<td>163</td>
</tr>
<tr>
<td>Nagy, L.M.</td>
<td>241</td>
</tr>
<tr>
<td>Nagy, Lisa M.</td>
<td>239</td>
</tr>
<tr>
<td>Nahmad, Marcos</td>
<td>63</td>
</tr>
<tr>
<td>Naiche, L. A.</td>
<td>118</td>
</tr>
<tr>
<td>Naiche, L.A.</td>
<td>367</td>
</tr>
<tr>
<td>Nakahara, Hiroko</td>
<td>455</td>
</tr>
<tr>
<td>Nakamoto, Ayaki</td>
<td>241</td>
</tr>
<tr>
<td>Nakamura, Tetsuya</td>
<td>264</td>
</tr>
<tr>
<td>Nakanishi, Nagayasu</td>
<td>229</td>
</tr>
<tr>
<td>Nam, Sang-Chul</td>
<td>156</td>
</tr>
<tr>
<td>Nance, Jeremy</td>
<td>45, 450, 506, 507</td>
</tr>
<tr>
<td>Nandadasa, Sumeda</td>
<td>150</td>
</tr>
<tr>
<td>Nascone-Yoder, Nanette</td>
<td>142, 143, 250, 351, 353</td>
</tr>
<tr>
<td>Natarajan, Anirudh</td>
<td>246</td>
</tr>
<tr>
<td>Nazari, Evelise</td>
<td>186, 192, 513</td>
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<td>Page(s)</td>
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<tr>
<td>Nechiporuk, Alex</td>
<td>30, 134</td>
</tr>
<tr>
<td>Neelathi, Uma</td>
<td>403</td>
</tr>
<tr>
<td>Neely, David</td>
<td>421</td>
</tr>
<tr>
<td>Neilson, Karen M.</td>
<td>411</td>
</tr>
<tr>
<td>Nelson, Celeste</td>
<td>120, 124</td>
</tr>
<tr>
<td>Nelson, Courtney</td>
<td>150</td>
</tr>
<tr>
<td>Nelson, Paul</td>
<td>258</td>
</tr>
<tr>
<td>Nemhauser, Jennifer</td>
<td>55, 395, 396</td>
</tr>
<tr>
<td>Nerurkar, Nandan</td>
<td>36</td>
</tr>
<tr>
<td>Nesmith, Jessica</td>
<td>523</td>
</tr>
<tr>
<td>Neugebauer, Judith</td>
<td>211</td>
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<td>540</td>
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<tr>
<td>Newmark, Phillip</td>
<td>25</td>
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<tr>
<td>Ng, Joanna</td>
<td>188</td>
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<tr>
<td>Ng, Lydia</td>
<td>290</td>
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<tr>
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<td>2</td>
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<td>Nichols, Harriette</td>
<td>89</td>
</tr>
<tr>
<td>Nichols, Megan</td>
<td>360</td>
</tr>
<tr>
<td>Nie, Qing</td>
<td>278</td>
</tr>
<tr>
<td>Nimmagadda, Suresh</td>
<td>184</td>
</tr>
<tr>
<td>Nishinakamura, Ryuichi</td>
<td>183</td>
</tr>
<tr>
<td>Nisperos, Sean</td>
<td>80</td>
</tr>
<tr>
<td>Niswander, Lee</td>
<td>125</td>
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<td>Noorassa, Anisa</td>
<td>395</td>
</tr>
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<td>Nordin, Kara</td>
<td>470</td>
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<tr>
<td>Norrie, Jacqueline L.</td>
<td>375</td>
</tr>
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<td>Norris, Megan L.</td>
<td>454</td>
</tr>
<tr>
<td>Norris, Rachael</td>
<td>217</td>
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<tr>
<td>Novotny, Karel</td>
<td>180</td>
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<tr>
<td>Nusse, Roel</td>
<td>26, 91</td>
</tr>
<tr>
<td>Neichchouk, Daria</td>
<td>5</td>
</tr>
<tr>
<td>Ornitz, David</td>
<td>363</td>
</tr>
<tr>
<td>Ornitz, David M.</td>
<td>366</td>
</tr>
<tr>
<td>Ostrander, Elaine A.</td>
<td>42</td>
</tr>
<tr>
<td>Otani, Tomoki</td>
<td>428</td>
</tr>
<tr>
<td>Ovcharenko, Ivan</td>
<td>298</td>
</tr>
<tr>
<td>Oviedo, Nestor J.</td>
<td>82</td>
</tr>
<tr>
<td>O'Brien, Lucy Erin</td>
<td>542</td>
</tr>
<tr>
<td>O'Connell, Joyce</td>
<td>13</td>
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<td>541</td>
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<td>533</td>
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<td>110</td>
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<td>259</td>
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<td>415</td>
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<td>420</td>
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<td>515</td>
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<td>Oh, Sunghee</td>
<td>413</td>
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<tr>
<td>Okamoto, Mayumi</td>
<td>163</td>
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<tr>
<td>Okuthe, Grace</td>
<td>279</td>
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<tr>
<td>Olsen, Doug</td>
<td>28</td>
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<tr>
<td>Ongoro, Kerilyn C.</td>
<td>83</td>
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<td>Onguka, Ouma</td>
<td>200</td>
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<tr>
<td>O'Brien, Larissa</td>
<td>253</td>
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<td>Patterson, James G.</td>
<td>174</td>
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<tr>
<td>Pauli, Andrea</td>
<td>454</td>
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<tr>
<td>Pay, Gina</td>
<td>397</td>
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<td>78</td>
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<td>Peery, Bradford</td>
<td>549</td>
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<td>Pegram, Kelly</td>
<td>203</td>
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<td>529</td>
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<td>28</td>
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<td>347</td>
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<td>Perrimon, Norbert</td>
<td>540</td>
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<td>Peshkin, Leonid</td>
<td>487</td>
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<td>Peters, Janet</td>
<td>288</td>
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<td>144</td>
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<td>Philipson, Elliot</td>
<td>150</td>
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<td>290</td>
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<td>531</td>
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<td>Pickett, Melissa</td>
<td>353</td>
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<td>Pierre-Jerome, Edith</td>
<td>396</td>
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<tr>
<td>Pilgrim, Dave</td>
<td>327</td>
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<tr>
<td>Pillai-Kastoori, Lakshmi</td>
<td>187, 188</td>
</tr>
<tr>
<td>Pineault, Kyriel</td>
<td>417</td>
</tr>
<tr>
<td>Pineda, Christopher</td>
<td>397</td>
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<td>Piotrowski, Tatjana</td>
<td>544</td>
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<tr>
<td>Piper, Michael</td>
<td>294</td>
</tr>
<tr>
<td>Pitcairn, Emily</td>
<td>483</td>
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<tr>
<td>Pitstick, Rose</td>
<td>288</td>
</tr>
<tr>
<td>Porter, Althea</td>
<td>383</td>
</tr>
<tr>
<td>Portman, Doug</td>
<td>138, 383</td>
</tr>
<tr>
<td>Postlethwait, John</td>
<td>536</td>
</tr>
<tr>
<td>Postlethwait, Johnathan</td>
<td>264</td>
</tr>
<tr>
<td>Potter, Steve</td>
<td>348</td>
</tr>
<tr>
<td>Pourquie, Olivier</td>
<td>267</td>
</tr>
<tr>
<td>Powell, Davalyn</td>
<td>440</td>
</tr>
<tr>
<td>Powell, Marian</td>
<td>292</td>
</tr>
<tr>
<td>Powell-Coffman, Jo Anne</td>
<td>70</td>
</tr>
<tr>
<td>Praitis, Vida</td>
<td>451</td>
</tr>
<tr>
<td>Prakash, Manu</td>
<td>548</td>
</tr>
<tr>
<td>Premkumar, Devika Sharanya</td>
<td>238</td>
</tr>
<tr>
<td>Preston, Elicia</td>
<td>15, 426</td>
</tr>
<tr>
<td>Price, Kari</td>
<td>503</td>
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<tr>
<td>Prill, Kendal</td>
<td>327</td>
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<tr>
<td>Prince, Vicky</td>
<td>58</td>
</tr>
<tr>
<td>Prince, Victoria E</td>
<td>355</td>
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<tr>
<td>Prioleau, Brittany</td>
<td>539</td>
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<td>Prosser, Haydn</td>
<td>306</td>
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<td>Purzner, James</td>
<td>294</td>
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<td>Quina, Lely</td>
<td>275</td>
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<td>Quintanilla, Carlo G.</td>
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<td>65</td>
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<td>Raible, David</td>
<td>109, 464</td>
</tr>
<tr>
<td>Raines, Anna</td>
<td>348</td>
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<tr>
<td>Rajderkar, Sudha</td>
<td>193</td>
</tr>
<tr>
<td>Ramahi, Joseph</td>
<td>200</td>
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<tr>
<td>Ramaker, Jenna</td>
<td>27</td>
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<tr>
<td>Ramakrishnan, Siddharth</td>
<td>280</td>
</tr>
<tr>
<td>Ramirez, PhD, Julio</td>
<td>437</td>
</tr>
<tr>
<td>Ramirez-Bergeron, Diana</td>
<td>281</td>
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<tr>
<td>Name</td>
<td>Page</td>
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<td>Robertson, Morgan</td>
<td>107</td>
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<td>Robitaille, Aaron</td>
<td>65</td>
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<tr>
<td>Rodrigues, Gabriel</td>
<td>378</td>
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<td>Rodrigues, Paulo</td>
<td>378</td>
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<tr>
<td>Rodriguez, Adriana</td>
<td>486</td>
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<tr>
<td>Rodriguez, Marita</td>
<td>115</td>
</tr>
<tr>
<td>Rodriguez-Larrain, Gisella</td>
<td>406</td>
</tr>
<tr>
<td>Roeske, Maxwell</td>
<td>254</td>
</tr>
<tr>
<td>Roffers-Agarwal, Julaine</td>
<td>471</td>
</tr>
<tr>
<td>Rogers, Katherine</td>
<td>511</td>
</tr>
<tr>
<td>Rogers, William</td>
<td>254, 257</td>
</tr>
<tr>
<td>Roh, Minna</td>
<td>452</td>
</tr>
<tr>
<td>Rohacek, Alex M.</td>
<td>189</td>
</tr>
<tr>
<td>Rohner, Nicolas</td>
<td>20</td>
</tr>
<tr>
<td>Rojas-Benitez, Diego</td>
<td>393</td>
</tr>
<tr>
<td>Rokhsar, Daniel</td>
<td>44</td>
</tr>
<tr>
<td>Romero-Carvajal, Andres</td>
<td>544</td>
</tr>
<tr>
<td>Rong, Xiaozhi</td>
<td>204</td>
</tr>
<tr>
<td>Ros, Marian</td>
<td>420</td>
</tr>
<tr>
<td>Rose, Lesilee</td>
<td>502, 504</td>
</tr>
<tr>
<td>Rose, Lesilee S.</td>
<td>524</td>
</tr>
<tr>
<td>Roselló-Diez, Alberto</td>
<td>374</td>
</tr>
<tr>
<td>Rosen, Mitchell</td>
<td>89</td>
</tr>
<tr>
<td>Rosenblum, Norman D.</td>
<td>488</td>
</tr>
<tr>
<td>Rosin, Jessica</td>
<td>297</td>
</tr>
<tr>
<td>Ross, Kelly G.</td>
<td>83</td>
</tr>
<tr>
<td>Rossant, Janet</td>
<td>51</td>
</tr>
<tr>
<td>Rossi, Christy Cortez</td>
<td>441</td>
</tr>
<tr>
<td>Rot, Chagai</td>
<td>199</td>
</tr>
<tr>
<td>Rothschild, Sarah C.</td>
<td>119</td>
</tr>
<tr>
<td>Rouaux, Caroline</td>
<td>29</td>
</tr>
<tr>
<td>Rougvie, Ann</td>
<td>490</td>
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<tr>
<td>Row, Richard</td>
<td>478</td>
</tr>
<tr>
<td>Royall, Joshua</td>
<td>290</td>
</tr>
<tr>
<td>Rubinstein, Dustin</td>
<td>541</td>
</tr>
<tr>
<td>Rudiño, Enrique</td>
<td>427</td>
</tr>
<tr>
<td>Ruzzo, Walter</td>
<td>481</td>
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<tr>
<td>Ryan, Aimee</td>
<td>128</td>
</tr>
<tr>
<td>Ryan, Michael</td>
<td>250</td>
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<tr>
<td>Ryckebüsch, Lucile</td>
<td>153</td>
</tr>
<tr>
<td>Rydeen, Ariel</td>
<td>509</td>
</tr>
<tr>
<td>Sagerstrom, Charles</td>
<td>407</td>
</tr>
<tr>
<td>Saijoh, Yukio</td>
<td>485</td>
</tr>
<tr>
<td>Saint-Jeannet, Jean-Pierre</td>
<td>468</td>
</tr>
<tr>
<td>Sakuma, Tetsushi</td>
<td>324</td>
</tr>
<tr>
<td>Salanga, Cristy</td>
<td>78</td>
</tr>
<tr>
<td>Salanga, Matthew</td>
<td>487</td>
</tr>
<tr>
<td>Saleh, Maya</td>
<td>191</td>
</tr>
<tr>
<td>Salinas-Saavedra, Miguel</td>
<td>234</td>
</tr>
<tr>
<td>Sam, Racette</td>
<td>138</td>
</tr>
<tr>
<td>San Roman, Adrianna</td>
<td>93</td>
</tr>
<tr>
<td>Sánchez Alvarado, Alejandro</td>
<td>79, 81</td>
</tr>
<tr>
<td>Sánchez Rubio, Mario Eduardo</td>
<td>101</td>
</tr>
<tr>
<td>Sandell, Lisa L.</td>
<td>208</td>
</tr>
<tr>
<td>Sandieson, Leanne</td>
<td>210</td>
</tr>
<tr>
<td>Sandler, Jeremy</td>
<td>514</td>
</tr>
<tr>
<td>Sanger, Thomas</td>
<td>267</td>
</tr>
<tr>
<td>Sapio, Matthew</td>
<td>402</td>
</tr>
<tr>
<td>Sarma, Kavitha</td>
<td>14</td>
</tr>
<tr>
<td>Sauceda, Mario</td>
<td>311</td>
</tr>
<tr>
<td>Sauerwald, Julia</td>
<td>3</td>
</tr>
<tr>
<td>Saund, Ranajeet</td>
<td>485</td>
</tr>
<tr>
<td>Savage-Dunn, Cathy</td>
<td>223, 318</td>
</tr>
<tr>
<td>Sawada, Atsushi</td>
<td>159</td>
</tr>
<tr>
<td>Saxena, Ankur</td>
<td>49</td>
</tr>
<tr>
<td>Schaaf, Marcel</td>
<td>171</td>
</tr>
<tr>
<td>Schacht, Angela</td>
<td>451</td>
</tr>
<tr>
<td>Schaffer, Amos</td>
<td>233</td>
</tr>
<tr>
<td>Schier, Alexander</td>
<td>511</td>
</tr>
<tr>
<td>Schier, Alexander F.</td>
<td>454</td>
</tr>
<tr>
<td>Schindler, Adam</td>
<td>195</td>
</tr>
<tr>
<td>Schindler, Yocheved</td>
<td>53</td>
</tr>
<tr>
<td>Schmidt, Sarah</td>
<td>185</td>
</tr>
<tr>
<td>Schneider, Caitlin</td>
<td>429</td>
</tr>
<tr>
<td>Schneider, Igor</td>
<td>264</td>
</tr>
<tr>
<td>Schoonheim, Peter</td>
<td>171</td>
</tr>
<tr>
<td>Schowalter, Sean</td>
<td>519</td>
</tr>
<tr>
<td>Schramm, Heloisa</td>
<td>192</td>
</tr>
<tr>
<td>Schulze, Sabrina</td>
<td>372</td>
</tr>
<tr>
<td>Schumacher, Linus</td>
<td>439</td>
</tr>
<tr>
<td>Schuster, Keaton</td>
<td>86</td>
</tr>
<tr>
<td>Schwartz, Matthew</td>
<td>306, 308</td>
</tr>
<tr>
<td>Schwartz, Robert J.</td>
<td>269, 270</td>
</tr>
<tr>
<td>Schweickert, Axel</td>
<td>24</td>
</tr>
<tr>
<td>Scimone, M. Lucila</td>
<td>11</td>
</tr>
<tr>
<td>Scott, Ian</td>
<td>35, 332</td>
</tr>
<tr>
<td>Scott, Ian C.</td>
<td>334</td>
</tr>
<tr>
<td>Scott, Matthew</td>
<td>294</td>
</tr>
<tr>
<td>Sears, Karen</td>
<td>19</td>
</tr>
<tr>
<td>Seaver, Elaine C.</td>
<td>545</td>
</tr>
<tr>
<td>Sebe, Joy</td>
<td>109</td>
</tr>
<tr>
<td>Seidel, Chris W.</td>
<td>79</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Semb, Henrik</td>
<td>342, 489</td>
</tr>
<tr>
<td>Semerad, Craig</td>
<td>48</td>
</tr>
<tr>
<td>Sepúlveda, Leonardo A.</td>
<td>155</td>
</tr>
<tr>
<td>Sepulveda, Silvia</td>
<td>157, 235</td>
</tr>
<tr>
<td>Sequarella, Eduardo</td>
<td>127</td>
</tr>
<tr>
<td>Serinagaoglu, Yelda</td>
<td>472</td>
</tr>
<tr>
<td>Sestan, Nad</td>
<td>290</td>
</tr>
<tr>
<td>Severson, David W.</td>
<td>259</td>
</tr>
<tr>
<td>Seydoux, Geraldine</td>
<td>528</td>
</tr>
<tr>
<td>Shafer, Maxwell</td>
<td>191, 345</td>
</tr>
<tr>
<td>Shah, Ankita</td>
<td>201</td>
</tr>
<tr>
<td>Shah, Arish</td>
<td>282, 283, 285</td>
</tr>
<tr>
<td>Shah, Jagesh V.</td>
<td>371</td>
</tr>
<tr>
<td>Shaham, Shai</td>
<td>34</td>
</tr>
<tr>
<td>Shao, Yingyao</td>
<td>394</td>
</tr>
<tr>
<td>Shapiro, Michael</td>
<td>23</td>
</tr>
<tr>
<td>Sharma, Nirmala</td>
<td>347</td>
</tr>
<tr>
<td>Sharma, Richa</td>
<td>345</td>
</tr>
<tr>
<td>Shehane, Richard</td>
<td>330</td>
</tr>
<tr>
<td>Shen, Christine</td>
<td>203</td>
</tr>
<tr>
<td>Shen, Kimberle</td>
<td>402</td>
</tr>
<tr>
<td>Shen, Shanshan</td>
<td>387</td>
</tr>
<tr>
<td>Sheridan, Grace H</td>
<td>455</td>
</tr>
<tr>
<td>Sherratt, Emma</td>
<td>267</td>
</tr>
<tr>
<td>Sherwood, David</td>
<td>47, 148, 195, 450</td>
</tr>
<tr>
<td>Sherwood, David R.</td>
<td>147</td>
</tr>
<tr>
<td>Shewade, Leena</td>
<td>323, 324</td>
</tr>
<tr>
<td>Shi, Lucy</td>
<td>259</td>
</tr>
<tr>
<td>Shi, Weiyang</td>
<td>493</td>
</tr>
<tr>
<td>Shi, Zhong-Dong</td>
<td>114</td>
</tr>
<tr>
<td>Shin, Jeong-Oh</td>
<td>364</td>
</tr>
<tr>
<td>Shin, Jimann</td>
<td>159, 496</td>
</tr>
<tr>
<td>Shinoda, Tomoyasu</td>
<td>163</td>
</tr>
<tr>
<td>Shivdasani, Ramesh</td>
<td>93</td>
</tr>
<tr>
<td>Shubin, Neil</td>
<td>264</td>
</tr>
<tr>
<td>Shuler, Sally</td>
<td>547</td>
</tr>
<tr>
<td>Shuster, Charles</td>
<td>235</td>
</tr>
<tr>
<td>Shuster, Charles B.</td>
<td>157</td>
</tr>
<tr>
<td>Siddiqui, Bilal</td>
<td>137</td>
</tr>
<tr>
<td>Silva Casey, Elena</td>
<td>409</td>
</tr>
<tr>
<td>Simard, Annie</td>
<td>128</td>
</tr>
<tr>
<td>Simske, Jeff</td>
<td>451</td>
</tr>
<tr>
<td>Singh, Anjin</td>
<td>241</td>
</tr>
<tr>
<td>Singh, Pratik</td>
<td>476</td>
</tr>
<tr>
<td>Sinha, Risha</td>
<td>429</td>
</tr>
<tr>
<td>Sinner, Debora</td>
<td>368</td>
</tr>
<tr>
<td>Sittaramane, PhD, Vinoth</td>
<td>444</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Srour, Marissa</td>
<td>56</td>
</tr>
<tr>
<td>Srubek Tomassy, Giulio</td>
<td>29</td>
</tr>
<tr>
<td>Stadt, Harriett</td>
<td>203</td>
</tr>
<tr>
<td>Staebling, Karen</td>
<td>48</td>
</tr>
<tr>
<td>Stafford, Christopher M</td>
<td>455</td>
</tr>
<tr>
<td>Stainier, Didier</td>
<td>107</td>
</tr>
<tr>
<td>Stanga, Daniela</td>
<td>345</td>
</tr>
<tr>
<td>Stankunas, Kryn</td>
<td>105, 336</td>
</tr>
<tr>
<td>Stansbury, Matthew S.</td>
<td>239</td>
</tr>
<tr>
<td>Starz-Gaiano, Michelle</td>
<td>549</td>
</tr>
<tr>
<td>Statopoulos, Angelike</td>
<td>514</td>
</tr>
<tr>
<td>Steele, Robert</td>
<td>18</td>
</tr>
<tr>
<td>Stein, Rachael</td>
<td>108, 429</td>
</tr>
<tr>
<td>Steinbeisser, Herbert</td>
<td>443</td>
</tr>
<tr>
<td>Stenman, Jan</td>
<td>501</td>
</tr>
<tr>
<td>Stepankova, Katerina</td>
<td>180</td>
</tr>
<tr>
<td>Stephan-Backowski, Karl</td>
<td>19</td>
</tr>
<tr>
<td>Stern, Tomer</td>
<td>199</td>
</tr>
<tr>
<td>Sternberg, Paul</td>
<td>2</td>
</tr>
<tr>
<td>Stewart, Katherine</td>
<td>191, 345</td>
</tr>
<tr>
<td>Stewart, M. David</td>
<td>269, 270</td>
</tr>
<tr>
<td>Stewart, Scott</td>
<td>105</td>
</tr>
<tr>
<td>Stickney Zimmermann, Heather</td>
<td>464</td>
</tr>
<tr>
<td>Stock, Tyson</td>
<td>451</td>
</tr>
<tr>
<td>Stottmann, Rolf</td>
<td>360</td>
</tr>
<tr>
<td>Stout, Josephine</td>
<td>411</td>
</tr>
<tr>
<td>Strachan, Erin</td>
<td>187</td>
</tr>
<tr>
<td>Strader, Marie</td>
<td>536</td>
</tr>
<tr>
<td>Strobl-Mazzulla, Pablo</td>
<td>295, 309</td>
</tr>
<tr>
<td>Strome, Susan</td>
<td>530</td>
</tr>
<tr>
<td>Stronge, Edward J.</td>
<td>471</td>
</tr>
<tr>
<td>Sturgeon, Kendra</td>
<td>35</td>
</tr>
<tr>
<td>Su, Chen-Ying</td>
<td>465</td>
</tr>
<tr>
<td>Suisse, Annabelle</td>
<td>392</td>
</tr>
<tr>
<td>Sullivan, Charles</td>
<td>547</td>
</tr>
<tr>
<td>Sullivan-Wilson, Alexander</td>
<td>451</td>
</tr>
<tr>
<td>Sun, Dan</td>
<td>385</td>
</tr>
<tr>
<td>Sun, Hua Yu</td>
<td>455</td>
</tr>
<tr>
<td>Sun, Longhua</td>
<td>259</td>
</tr>
<tr>
<td>Sun, Xin</td>
<td>367</td>
</tr>
<tr>
<td>Sun, Yu</td>
<td>35</td>
</tr>
<tr>
<td>Sunkin, Susan</td>
<td>290</td>
</tr>
<tr>
<td>Suriben, Rowena</td>
<td>200</td>
</tr>
<tr>
<td>Suzuki, Ken-ichi</td>
<td>324</td>
</tr>
<tr>
<td>Swalla, Billie</td>
<td>231, 232</td>
</tr>
<tr>
<td>Swalla, Billie J.</td>
<td>88</td>
</tr>
<tr>
<td>Swanson, Tracy</td>
<td>27</td>
</tr>
<tr>
<td>Name</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Tirell, Sara</td>
<td>198</td>
</tr>
<tr>
<td>Titialii, Kayla</td>
<td>97</td>
</tr>
<tr>
<td>Tomchaney, Michael</td>
<td>259</td>
</tr>
<tr>
<td>Tomoyasu, Yoshinori</td>
<td>242, 243, 244</td>
</tr>
<tr>
<td>Topczewski, Jacek</td>
<td>225</td>
</tr>
<tr>
<td>Torii, Keiko</td>
<td>226</td>
</tr>
<tr>
<td>Torii, Keiko U.</td>
<td>543</td>
</tr>
<tr>
<td>Trainor, Paul A.</td>
<td>208</td>
</tr>
<tr>
<td>Tran, Baouyen</td>
<td>200</td>
</tr>
<tr>
<td>Tran, Nam</td>
<td>521</td>
</tr>
<tr>
<td>Treisman, Jessica</td>
<td>392</td>
</tr>
<tr>
<td>Tremblay, Kimberly</td>
<td>352</td>
</tr>
<tr>
<td>Tremblay, Mathieu</td>
<td>345</td>
</tr>
<tr>
<td>Tripuraneni, Nikita</td>
<td>56</td>
</tr>
<tr>
<td>Trisnadi, Nathalie</td>
<td>219</td>
</tr>
<tr>
<td>Tsai, Feng-Chiao</td>
<td>26</td>
</tr>
<tr>
<td>Tsai, Jui-Cheng</td>
<td>90</td>
</tr>
<tr>
<td>Tschopp, Patrick</td>
<td>267</td>
</tr>
<tr>
<td>Tse, Jeffrey</td>
<td>492</td>
</tr>
<tr>
<td>Tsur, Assaf</td>
<td>82</td>
</tr>
<tr>
<td>Tucker, Haley</td>
<td>269, 270</td>
</tr>
<tr>
<td>Turner, Eric</td>
<td>275</td>
</tr>
<tr>
<td>Udvadia, Ava J.</td>
<td>315</td>
</tr>
<tr>
<td>Ukken, Fiona</td>
<td>541</td>
</tr>
<tr>
<td>Ulitsky, Igor</td>
<td>308</td>
</tr>
<tr>
<td>Ulmer, Barbel</td>
<td>173</td>
</tr>
<tr>
<td>Uman, Selen</td>
<td>18</td>
</tr>
<tr>
<td>Umulis, David</td>
<td>7</td>
</tr>
<tr>
<td>Unguez, Graciela</td>
<td>69</td>
</tr>
<tr>
<td>Unguez, Graciela A.</td>
<td>103</td>
</tr>
<tr>
<td>Upadhyaya, Anand</td>
<td>2</td>
</tr>
<tr>
<td>Uribe, Rosa</td>
<td>309, 356</td>
</tr>
<tr>
<td>Urness, Lisa</td>
<td>164, 333</td>
</tr>
<tr>
<td>Uruena, Ana C.</td>
<td>458</td>
</tr>
<tr>
<td>Uygar, Aysu</td>
<td>19, 271</td>
</tr>
<tr>
<td>Valadez Graham, Viviana</td>
<td>427</td>
</tr>
<tr>
<td>Valentin, Guillaume</td>
<td>415</td>
</tr>
<tr>
<td>Valera, Juliana</td>
<td>326</td>
</tr>
<tr>
<td>Van Antwerp, Daniel</td>
<td>167</td>
</tr>
<tr>
<td>Van Maren, Ben</td>
<td>166</td>
</tr>
<tr>
<td>Van Nes, Rachel</td>
<td>400</td>
</tr>
<tr>
<td>VanGompel, Michael J.W.</td>
<td>524</td>
</tr>
<tr>
<td>Varner, Victor</td>
<td>120, 124</td>
</tr>
<tr>
<td>Vasco, Alejandra</td>
<td>22</td>
</tr>
<tr>
<td>Vaughan, Megan</td>
<td>135</td>
</tr>
<tr>
<td>Vázquez, Martha</td>
<td>427</td>
</tr>
<tr>
<td>Veeman, Michael</td>
<td>40, 132</td>
</tr>
<tr>
<td>Veeramani, Swarna</td>
<td>312</td>
</tr>
<tr>
<td>Velez, Carla</td>
<td>429</td>
</tr>
<tr>
<td>Vellky, Jordan</td>
<td>256, 299</td>
</tr>
<tr>
<td>Vichas, Athea</td>
<td>160</td>
</tr>
<tr>
<td>Vidal, Sylvia</td>
<td>345</td>
</tr>
<tr>
<td>Visel, Axel</td>
<td>275</td>
</tr>
<tr>
<td>Visina, Olesya</td>
<td>126</td>
</tr>
<tr>
<td>Voelker, List</td>
<td>282</td>
</tr>
<tr>
<td>Vokes, Martha S.</td>
<td>375</td>
</tr>
<tr>
<td>Vokes, Steven</td>
<td>292</td>
</tr>
<tr>
<td>Vokes, Steven A.</td>
<td>375</td>
</tr>
<tr>
<td>Von Saucken, Victoria</td>
<td>108</td>
</tr>
<tr>
<td>Vora, Siddharth</td>
<td>172</td>
</tr>
<tr>
<td>Voss, Kathleen M</td>
<td>455</td>
</tr>
<tr>
<td>Voss, Randal</td>
<td>442</td>
</tr>
<tr>
<td>Voss, Ulrikke</td>
<td>489</td>
</tr>
<tr>
<td>Vu, Milan</td>
<td>226</td>
</tr>
<tr>
<td>Wagner, Daniel</td>
<td>303, 339</td>
</tr>
<tr>
<td>Wagner, Eileen</td>
<td>408</td>
</tr>
<tr>
<td>Wakimoto, Barbara</td>
<td>520</td>
</tr>
<tr>
<td>Waldron, Lauren</td>
<td>416</td>
</tr>
<tr>
<td>Walentek, Peter</td>
<td>307, 477</td>
</tr>
<tr>
<td>Wallia, Gurjot</td>
<td>10</td>
</tr>
<tr>
<td>Wallace, Andre</td>
<td>59</td>
</tr>
<tr>
<td>Wallingford, John</td>
<td>169, 171</td>
</tr>
<tr>
<td>Wallingford, Mary</td>
<td>338</td>
</tr>
<tr>
<td>Waltman, Michael</td>
<td>319</td>
</tr>
<tr>
<td>Walton, Travis</td>
<td>15, 426</td>
</tr>
<tr>
<td>Wan, Ying</td>
<td>307</td>
</tr>
<tr>
<td>Wang, Bo</td>
<td>25</td>
</tr>
<tr>
<td>Wang, Chen</td>
<td>317</td>
</tr>
<tr>
<td>Wang, Jianzhong</td>
<td>387</td>
</tr>
<tr>
<td>Wang, Michael</td>
<td>212</td>
</tr>
<tr>
<td>Wang, Shang</td>
<td>510</td>
</tr>
<tr>
<td>Wang, Tsu-Wei</td>
<td>90, 484</td>
</tr>
<tr>
<td>Wang, Wenchao</td>
<td>530</td>
</tr>
<tr>
<td>Wang, Zheng</td>
<td>47</td>
</tr>
<tr>
<td>Wanner, Sarah</td>
<td>58</td>
</tr>
<tr>
<td>Ward, Kelly A.</td>
<td>238</td>
</tr>
<tr>
<td>Wardle, Fiona</td>
<td>415</td>
</tr>
</tbody>
</table>
Waxman, Joshua 329, 479, 509, 537
Weatherbee, Scott 57
Webber, Jemma L. 296
Wei, Chunyao 174
Weideman, Ann Marie 549
Weinstein, Rachel 451
Weissman, Irving 94
Wen, Jun 35
Wen, Wen 187, 188
Wendik, Bjoern 5
Wessel, Gary 18
Whipple, Lauren 383
Whitebirch, Alex 283
Whitener, Amy 314, 497
Whitsett, Jeffrey 368
Whittington, Niteace 409
Widelitz, Randall 278
Wierup, Nils 489
Wilcox, Allison 411
Willett, Ryan 214
Williams, Erin 460
Williams, Jason 440, 441
Williams, Jessica 326
Williams, T.A. 241
Williams, Thomas 254, 255, 256, 257, 299
Williams, Trevor 35
Wills, Airon 171
Wills, Andrea 508
Willson, Jessica 151, 453
Wilson, Stephen 187
Wilson, Steve 464
Windner, Stefanie 415
Winkler, Caitlin 466
Wirgau, Rachel 230
Wistorf, Sabrina 146
Wohnoutka, Paul 290
Wolfhagen-Sand, Fredrik 489
Wolpe, Jacob 87
Womble, Mandy 250, 351
Wong, Andrew C. 277
Wong, Julia 183
Wong, Michael 35
Wong, Siew Fen Lisa 306
Wood, Derek 74
Wray, Susan 32
Wu, Doris K. 362
Wu, Guang 284
Wu, Jia 28
Wu, Ji-Ching 502
Wu, Ping 278
Wu, You 493
Wyler, Steven 31
Wylie, Anika 497
Wylie, Chris 6
Wyrick, Jonathan 399
Wysolmerski, Erin E. 207

X
Xie, Yuansheng 121, 387
Xin, Daisy 57
Xing, Liujing 91
Xu, Jingxia 287
Xu, Jingyue 346, 377
Xu, Ke 54
Xu, Xiang-Xi 379
Xu, Xiangxi 492
Xu, Xiaxia 272
Xue, Lei 394
Xue, Shifeng 301

Y
Yamada, Yoshihiko 420
Yamaguchi, Terry P. 347
Yamaki, Takuo 520
Yamamoto, Takashi 324
Yamanaka, Yojiro 67
Yamashita, Satoshi 161
Yamashita, Yukiko 64
Yan, Connie 196
Yancey, Cole 444
Yang, Cheng-Yao 90
Yang, Jiajia 215
Yang, Xiao 364
Yang, Zhuo 215
Yanowitz, Judith 526, 527
Yao, Qiuming 528
Yao, Yao 189
Yartseva, Valeria 304
Yasuda, Glenn 520
Yatskievych, Tatiana 166, 286
Ye, Lihua 107
Yeh, Chia-Ling 103
Yeh, Tsung-Han 484
Yelon, Deborah 53, 135, 153, 330, 335
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yin, Scott</td>
<td>475</td>
<td>Zhou, Jianfeng</td>
<td>204</td>
</tr>
<tr>
<td>Yin, Yongjun</td>
<td>366</td>
<td>Zhou, Jing</td>
<td>181, 413</td>
</tr>
<tr>
<td>Yin, Zhong</td>
<td>387</td>
<td>Zhou, Pin</td>
<td>204</td>
</tr>
<tr>
<td>Yochem, John</td>
<td>34</td>
<td>Zhou, Yumei</td>
<td>204</td>
</tr>
<tr>
<td>Yokota, Chika</td>
<td>501</td>
<td>Zhu, Zengrong</td>
<td>114</td>
</tr>
<tr>
<td>Yoon, Se-Jin</td>
<td>508</td>
<td>Zhulyn, Olena</td>
<td>423</td>
</tr>
<tr>
<td>Young, Geoffrey</td>
<td>176</td>
<td>Zikmund, Tomas</td>
<td>180</td>
</tr>
<tr>
<td>Yu, Guangwei</td>
<td>535</td>
<td>Zimmerman, Sandra</td>
<td>131</td>
</tr>
<tr>
<td>Yu, Jeffrey</td>
<td>369</td>
<td>Zinski, Joseph</td>
<td>7</td>
</tr>
<tr>
<td>Yu, Jenn-Yah</td>
<td>484</td>
<td>Zitnik, Edward M.</td>
<td>238</td>
</tr>
<tr>
<td>Yu, Kai</td>
<td>532</td>
<td>Zorn, Aaron</td>
<td>365</td>
</tr>
<tr>
<td>Yu, Wei</td>
<td>270</td>
<td>Zueckert-Gaudenz, Karin</td>
<td>48</td>
</tr>
<tr>
<td>Yu, Xiaodan</td>
<td>183</td>
<td>Zuhdi, Nora</td>
<td>438</td>
</tr>
<tr>
<td>Yumoto, Kenji</td>
<td>193</td>
<td>Zuniga, Elizabeth</td>
<td>412</td>
</tr>
<tr>
<td>Yun, Hyo Jung</td>
<td>534</td>
<td>Zuo, Jian</td>
<td>92, 311</td>
</tr>
<tr>
<td>Yun, Kangsun</td>
<td>347</td>
<td>Zurita, Mario</td>
<td>427</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zweig, Jonathan</td>
<td>27</td>
</tr>
</tbody>
</table>

Z

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zacharias, Amanda</td>
<td>15, 426</td>
</tr>
<tr>
<td>Zallen, Jennifer</td>
<td>160</td>
</tr>
<tr>
<td>Zand, Tanya P.</td>
<td>391</td>
</tr>
<tr>
<td>Zannino, Denise</td>
<td>407</td>
</tr>
<tr>
<td>Zayas, Ricardo</td>
<td>80</td>
</tr>
<tr>
<td>Zayas, Ricardo M.</td>
<td>83</td>
</tr>
<tr>
<td>Zeller, Molly</td>
<td>331</td>
</tr>
<tr>
<td>Zeltzer, Sebastian</td>
<td>166</td>
</tr>
<tr>
<td>Zelzer, Elazar</td>
<td>199</td>
</tr>
<tr>
<td>Zeng, Huiqing</td>
<td>389</td>
</tr>
<tr>
<td>Zeng, Lewie</td>
<td>57</td>
</tr>
<tr>
<td>Zeng, Xin-Xin</td>
<td>335</td>
</tr>
<tr>
<td>Zeni, Eliane Cristina</td>
<td>192</td>
</tr>
<tr>
<td>Zhang, Jiakun</td>
<td>320</td>
</tr>
<tr>
<td>Zhang, Jie</td>
<td>296</td>
</tr>
<tr>
<td>Zhang, Shilu</td>
<td>292</td>
</tr>
<tr>
<td>Zhang, Stella</td>
<td>286</td>
</tr>
<tr>
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<td>286</td>
</tr>
<tr>
<td>Zhang, Tao</td>
<td>272</td>
</tr>
<tr>
<td>Zhang, Yan</td>
<td>535</td>
</tr>
<tr>
<td>Zhao, Yaingtao</td>
<td>189</td>
</tr>
<tr>
<td>Zhao, Younjiun</td>
<td>291</td>
</tr>
<tr>
<td>Zhao, Yu</td>
<td>394</td>
</tr>
<tr>
<td>Zheng, Chenguang</td>
<td>272</td>
</tr>
<tr>
<td>Zheng, Liuliu</td>
<td>155</td>
</tr>
<tr>
<td>Zheng, Ning</td>
<td>395</td>
</tr>
<tr>
<td>Zheng, Tao</td>
<td>359</td>
</tr>
<tr>
<td>Zheng, Ying</td>
<td>481</td>
</tr>
<tr>
<td>Zhong, Mei</td>
<td>18</td>
</tr>
<tr>
<td>Zhou, Bin</td>
<td>336</td>
</tr>
</tbody>
</table>