Program/Abstract # 234

Genomic approaches to understanding atrial septation
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Atrial septation is a critical step in separating the systemic and pulmonary circulations in tetrapods and atrial septal defects are among the most common forms of human congenital heart disease. The canonical view of atrial septation is based on intra-cardiac morphogenetic events, however, recent studies highlight the critical role played by Hedgehog signaling in the second heart field. Here we aim to define the Hedgehog-mediated molecular networks required for atrial septation in the developing mouse embryo using combined genomic approaches. We have developed and validated a microdissection procedure to isolate posterior second heart field (pSHF) tissue. To define Shh dependent transcripts, we have performed transcriptional profiling of pSHFs in wildtype and Shh mutant embryos at E9.5. 146 up-regulated and 124 down-regulated transcripts have been identified, of which 40 have been validated by RT-PCR. Initial expression analysis suggests 4 discrete domains of gene expression in the pSHF: medial mesenchymal, ventral mesenchymal, lateral endodermal, and lateral mesothelial. We interpret these patterns with respect to previous identified functions of the genes analyzed and propose a testable model in which a lateral proliferating population of cells in the posterior SHF are required for atrial septation. To complement the microarray expression data, we have developed an in-vivo ChIP-seq protocol to identify direct targets of Hh signaling in the pSHF. Expressing the Flag-tagged Gli1 or Gli3 transcription factor in the SHF Me2c lineage, our preliminary Gli:Flag-ChIP data show 8-fold enrichment for a known Gli tagger Mbc, active Rac1 and F-actin using a combination of functional analysis and time-lapse imaging in the embryo as well as protein localization in primary myoblasts. These studies demonstrate, first, that Mbc is essential in the FCMs but that the founder cells are capable of fusing with FCMs in its absence. Functional studies also show that activated Rac1 in the FCMs rescues the mbc loss of function phenotype, confirming that the role of Mbc is to activate Rac1. Our data further show that Mbc, active Rac1 and F-actin foci are highly enriched, if not exclusive, to the FCMs at their contact sites with founder cells and developing myotubes. We have confirmed the localization of F-actin during fusion by live imaging in embryos. Of note, we also observe Mbc, active Rac1 and F-actin in Sns:Kirre associated projections that emanate from the FCMs and appear to push into the myotube.

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Program/Abstract # 235

Mbc, active Rac1 and F-actin foci localize to points of cell contact in fusion-competent myoblasts, where they drive fusion with founder cells and myotubes
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Myoblast fusion in the Drosophila embryo initially occurs between founder cells, which pattern the musculature, and fusion competent myoblasts (FCMs). This precursor continues to fuse with additional FCMs until the final myotube size is achieved. Cell surface proteins Sns and Kirre mediate recognition between these two cell types, and are critical in the FCMs and founder cells/developing myotubes, respectively. In contrast to this initial asymmetry, current models for myoblast fusion predict that the bipartite guanine nucleotide exchange factor Mbc and its target Rac1 function symmetrically in the fusion process. Such models have also inferred that actin foci are also symmetric at the fusion site. Our results demonstrate that the site of fusion is quite asymmetric, however, with respect to these proteins and the process of fusion. We have analyzed Mbc, activated Rac1 and F-actin using a combination of functional analysis and time-lapse imaging in the embryo as well as protein localization in primary myoblasts. These studies demonstrate, first, that Mbc is essential in the FCMs but that the founder cells are capable of fusing with FCMs in its absence. Functional studies also show that activated Rac1 in the FCMs rescues the mbc loss of function phenotype, confirming that the role of Mbc is to activate Rac1. Our data further show that Mbc, active Rac1 and F-actin foci are highly enriched, if not exclusive, to the FCMs at their contact sites with founder cells and developing myotubes. We have confirmed the localization of F-actin during fusion by live imaging in embryos. Of note, we also observe Mbc, active Rac1 and F-actin in Sns:Kirre associated projections that emanate from the FCMs and appear to push into the myotube.

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Program/Abstract # 236

Mapping and phenotypic characterization of the dead elvis (del) mutation in zebrafish
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During embryonic development, subsets of cells differentiate into discrete muscle tissues. This process forms tissues that allow the embryo to propel itself, contract the heart muscle to drive blood throughout the organism, and perform other functions necessary for survival. Our project is focused on a specific zebrafish mutant, dead elvis (del), which was discovered through a novel screening methodology in Dr. Lessman’s laboratory at the University of Memphis. The dead elvis (del) mutation manifests a non-motile, homozygous phenotype around 20 h after fertilization. The dead elvis mutant has obvious myotome defects and a lack of sarcomere organization. This project involves the genetic mapping and phenotypic characterization of the dead elvis mutation. Using a whole genome screen, we localized the mutation to LG9, and isolated candidate genes. Utilizing immunohistochemistry and confocal microscopy techniques, we have observed muscle formation and sarcomeric assemblage differences in the dead elvis mutant. Further study of this mutation may aid in the understanding of myotome development and lead to more insight into human neuromuscular disease conditions.

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Program/Abstract # 237

Elucidating the circadian-controlled gene xNocturnin's expression and function in somitogenesis
Nicole Johnson, Kristen Curran

Modeling approaches to understanding atrial septation
Andrew Hoffmann, Joshua Bosman, Michael Herriges, Ivan Moskowitz
University of Chicago, Chicago, IL, USA

Atrial septation is a critical step in separating the systemic and pulmonary circulations in tetrapods and atrial septal defects are among the most common forms of human congenital heart disease. The canonical view of atrial septation is based on intra-cardiac morphogenetic events, however, recent studies highlight the critical role played by Hedgehog signaling in the second heart field. Here we aim to define the Hedgehog-mediated molecular networks required for atrial septation in the developing mouse embryo using combined genomic approaches. We have developed and validated a microdissection procedure to isolate posterior second heart field (pSHF) tissue. To define Shh dependent transcripts, we have performed transcriptional profiling of pSHFs in wildtype and Shh mutant embryos at E9.5. 146 up-regulated and 124 down-regulated transcripts have been identified, of which 40 have been validated by RT-PCR. Initial expression analysis suggests 4 discrete domains of gene expression in the pSHF: medial mesenchymal, ventral mesenchymal, lateral endodermal, and lateral mesothelial. We interpret these patterns with respect to previous identified functions of the genes analyzed and propose a testable model in which a lateral proliferating population of cells in the posterior SHF are required for atrial septation. To complement the microarray expression data, we have developed an in-vivo ChIP-seq protocol to identify direct targets of Hh signaling in the pSHF. Expressing the Flag-tagged Gli1 or Gli3 transcription factor in the SHF Me2c lineage, our preliminary Gli:Flag-ChIP data show 8-fold enrichment for a known Gli binding region in the Ptc1 locus. Integrating our gene expression and Gli-binding results will elucidate the Hh-dependent SHF molecular pathways required for atrial septation.

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Program/Abstract # 237

Elucidating the circadian-controlled gene xNocturnin’s expression and function in somitogenesis
Nicole Johnson, Kristen Curran
Formation of somites during vertebrate development involves ultradian oscillations (from 1 to 2 h) in gene expression. We hypothesized that circadian-controlled genes may play a role in the core timekeeping mechanism governing somitogenesis. Past studies in our lab revealed a circadian-controlled output gene, xNocturnin, is highly expressed in somites. To investigate xNocturnin’s role in somite segmentation and/or its timing, morpholinos (MOs) were utilized to decrease xNocturnin protein expression on one side of the embryo. *Xenopus laevis* embryos were injected in one cell at the 2-cell stage with either a MO that binds the translation start site of xNocturnin mRNA or a control MO. Injected embryos were cultured for 24 h, fixed, and assayed for muscle actin (a marker of somites) using whole-mount immunohistochemistry. We found that injecting xNocturnin MO into one cell of a 2-cell stage embryo decreases the amount of detectable somites on the injected side compared to the uninjected side. Weakened segmentation and/or fewer numbers of somites suggest that xNocturnin may be involved in the timing or differentiation of somites. Data from other studies suggest that cellular events following an ultradian rhythm could be modulated or governed by circadian genes, and it revealed genes involved in somitogenesis (i.e. hairy2a) have both a circadian rhythm and somite segmentation specific rhythm. If this is true, then a novel, pleiotropic function for xNocturnin has been discovered.

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**Program/Abstract # 238**

Reduced tendon differentiation in the *Irx1* knockout mouse

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*Irx1* (Iroquois-related homeobox-like-1) is a newly identified homeodomain-containing transcription factor that belongs to the TALE superclass of atypical homeodomain protein. During embryonic development, *Irx1* is expressed in the progenitors of skeletal muscle, tendon and cartilage in somites and limb buds, suggesting its role in the musculoskeletal system development. To investigate the roles of *Irx1* in the formation of the musculoskeletal system in mammals, we generated mice carrying a null allele of the *Irx1* gene. *Irx1* homozygous mice presented with a kinky tail and all the *Irx1* knockout mice examined showed reduced tendon formation throughout the body including the head, trunk, limb and tail. Bone and skeletal muscle morphology in the *Irx1* knockout mice appeared normal. We next examined the effect of the *Irx1* deficiency on musculoskeletal system development. During early differentiation of tendon progenitors, the expression patterns of somite compartment markers including Sox9, Myogenin, Pax9 and tendon lineage marker Scleraxis were indistinguishable between the homoyzogotes and wildtype embryos. On the other hand, at the later tendon maturation stages, expressions of collagens and several tendon marker genes, such as tenomodulin and fibromodulin, were reduced in the tendon precursors of the *Irx1* homozygous mutant embryos. These results suggest that *Irx1* is dispensable for the initial step of the tendon progenitor differentiation, and instead required for the later maturation stages of the tendon formation during the embryonic development.

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**Program/Abstract # 239**

Coordination of growth factor signaling and cell death during vertebrate rib development

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Normal development requires a balance between cell death and proliferation. Using a genetic approach in the mouse, we are studying how Apaf1, a programmed cell death (PCD) factor, and Shh, a growth factor, coordinate to pattern the thoracic skeleton. Animals lacking Apaf1 develop normal skeletons while those null for Shh lack vertebrae and vertebral ribs but form sternal ribs and a sternum. When Apaf1 and Shh (DKO) are both removed, however, the axial skeleton fails to develop. This was surprising because removal of a PCD gene would be expected to rescue rib formation in the Shh KO. Upon examining PCD in the different genotypes, a normal pattern is observed in wild-type embryos but is absent in the Apaf1 KO. Shh KO embryos exhibit an increase in PCD concentrated in the sclerotome. The combined absence of Shh and Apaf1 eliminates this increase even though the Shh KO rib phenotype is not rescued. This suggests that in the DKO, the rib progenitors die by another mechanism or fail to proliferate. To determine which progenitors give rise to specific parts of the thoracic skeleton, gene expression patterns were analyzed. No difference was observed between wild-type and Apaf1 KO embryos. Shh KO and DKO embryos do not express sclerotome markers, Sox9 and Pax9, and lack epaxial but not hypaxial muscle progenitors. These results imply an unexpected requirement for Apaf1 during development and suggest that the vertebral and sternal ribs are established by different mechanisms. These studies pave the way for future investigations into the molecular control of cell proliferation, death and cell fate changes during mammalian development and hopefully our observations will direct therapeutic methods to prevent cell death due to injury.

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**Program/Abstract # 240**

Role of Syndecan-4 in mouse development

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The Non-Canonical Wnt/Planar Cell Polarity (PCP) pathway in mammals regulates cell polarity, convergent extension (CE) movements and oriented cell division. In all this cases, PCP signaling has the same final effect of lengthening and narrowing tissues during development. Analyses of mice mutants for PCP core genes have revealed a growing list of new PCP phenotypes as diverse as: neural tube formation, polarization of the sensory epithelia within the inner ear and convergence and extension of the cochlea, midtut formation, cardiac and nephron arrangement and cell polarity of growth plate chondrocytes. In our laboratory we have demonstrated that *Xenopus* Syndecan-4 (*xSdc4*), a cell-surface heparan sulfate proteoglycan and focal adhesion component, regulates Non-Canonical Wnt signaling and is essential for CE and neural tube closure in *Xenopus* embryos. This evidence suggests a possible interaction between *xSdc4* and components of the PCP pathway. Our aim is to study the role of Syndecan-4 (*Sdc4*) and its relation with the PCP pathway during mouse development. Importantly, no role for *Sdc4* in mouse