Morphogenesis

Program/Abstract # 101
Secretion of Lunatic fringe is essential for somitogenesis and segmentation clock function
Dustin R. Williams a, Emily T. Shifley b, Susan E. Cole c
 a The Ohio State University Molecular Genetics, Columbus, OH, USA
 b Cincinnati Children’s Hospital, Cincinnati, OH, USA
 c Dept. of Mol. Gen., The Ohio State University, Columbus, OH, USA

During somitogenesis, paired somites bud from the presomitic mesoderm (PSM), giving rise to the axial skeleton and musculature of the back. During somitogenesis, Notch activity levels oscillate as part of a clock that controls the timing of somite formation. Cyclic Notch activation is dependent upon the receptor’s periodic repression by Lunatic fringe (LFG). LFG mRNA levels cycle over a two-hour period in the clock, but for LFNG and Notch activity levels to cycle, there must be mechanisms in place to rapidly eliminate LFNG protein during the short “off” phase of the clock. In vitro, LFNG is rapidly secreted into the extracellular space. We hypothesize that secretion of LFNG is a mechanism for terminating its activity in the clock when it is no longer needed. To test the in vivo relevance of LFNG secretion, we generated knock-in mice expressing a Golgi-tethered LFNG variant that cannot be secreted (Lfg T-LFNG) +/+ heterozygotes have severe skeletal abnormalities, supporting our hypothesis that tethering LFNG in the Golgi generates a hyperactive fringe protein that perturbs the segmentation clock. Somites in these mutants exhibit randomized rostro-caudal patterning and form irregular boundaries. Whole-mount immunohistochemistry confirms that Notch activity is severely downregulated and non-oscillatory in the PSM. Expression of clock genes is perturbed, and endogenous LFNG expression no longer oscillates. These results support our hypothesis that LFNG processing and secretion play important roles in its function in the segmentation clock, and provide further evidence that post-transcriptional regulation of the clock is critical during somitogenesis.

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Program/Abstract # 102
Premature downregulation of Tbx6 within the tailbud alters cell fates and leads to premature termination of axial elongation
Nowlan H. Freese a, Allison Scott b, Susan Chapman b
 a Clemson University Biological Sciences, Clemson, SC, USA
 b Clemson University, Clemson, SC, USA

The process of posterior axial elongation involves ingestion of cells through the primitive streak facilitating secondary neurulation, forming the neural tube and caudal somites. Posterior patterning requires myriad signals within the progenitor pool of cells of the chordoneural hinge and tailbud. The Araucana mutant chicken lacks multiple tail vertebrae. This phenotype results from an autosomal dominant mode of inheritance. Araucana embryos fail to form the final somites, prematurely terminating elongation of the posterior axis. The T-box transcription factor, Tbx6, is expressed in both the tailbud and paraxial mesoderm. Tbx6 is necessary for murine tailbud cells to adopt a mesodermal fate, and has been linked to the cyclical gene expression of the clock and wavefront model through its interaction with Dll1. In Araucana embryos, during tail organizer stages, Tbx6 expression is downregulated at HH15, within the chordoneural hinge and tailbud, which would normally occur at HH25, but expression is still detected in the paraxial mesoderm. Within a few hours expression of the tailbud mesoderm marker Brachyury downregulates, followed by Wnt3a and Fgfl. Cells that normally form paraxial mesoderm appear to adopt a neural fate as witnessed by multiple neural tubes forming. This is followed by cessation of tail extension and upregulation of programmed cell death within the tailbud. These results suggest that Tbx6 plays a role within the chordoneural hinge and tailbud involving cell fate decisions. Disruption of genes necessary for maintenance of the progenitor pool of cells leads to truncation and premature cell death within the tailbud.

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Program/Abstract # 103
Elucidating the role of Hoxa-5 in the development of the chick axial skeleton
Jessica Chen, Soomboal Zahid, Sara Weaver, Meghan Shilts, Jennifer Mansfield
Barnard College, New York, NY, USA

The vertebrate axial skeleton and its associated muscles and connective tissue arise from somites. Each vertebral segment develops with a morphology appropriate to its position along the body axis, and morphologies can be classified into several general types (cervical, thoracic, etc.). Expression of Hox transcription factors, established before somite formation, acts early in development to specify segmental identities, and continues to function later, influencing the differentiation, and thus final morphology, of axial tissues. We are examining the role of Hoxa-5 in the developing avian axial skeleton. Previous loss-of-function analyses in mice showed that Hoxa-5 non-redundantly patterns segments around the cervical–thoracic transition. However, it is unknown how Hoxa-5 interacts with cartilage patterning and differentiation pathways within the somites, or, given differences in Hox group 4 expression between mice and chicks, whether it specifies the same segmental identities in these two lineages. We find that misexpression or RNAi-based knockdown of Hoxa-5 in chick pre-somitic mesoderm alters vertebral morphologies, although it does not transform segmental identities. Hoxa-5 knock-down leads to aberrant cartilage morphology specifically within lateral vertebral elements derived from Hoxa-5 expressing