Program/Abstract # 169
Characterization of cis-regulatory elements that control Pax7 expression during neural crest cell development
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The neural crest is a population of multipotent migratory cells that arises at the border between neural and non-neural ectoderm in vertebrates. It gives rise to many derivatives, including neurons and glia of the peripheral nervous system, melanocytes, and craniofacial cartilage and bone. In the past two decades there has been a surge of efforts toward understanding neural crest induction, with several signaling pathways and tissue interactions being implicated. Recently, the transcription factor Pax7 was identified as an early marker of prospective neural crest cells and its functions shown to be required for neural crest development. The molecular events leading to the specific expression of Pax7, both in early neural crest precursors and as well as in later stages of development are largely unknown. This study focuses on the regulatory elements responsible for Pax7 expression. Phylogenetic footprinting has been used to identify putative regulatory regions upstream of the chicken Pax7 gene. Electroporation of these regions cloned upstream of a reporter construct was used to assay for temporal and spatial expression in chicken embryos. Several regions yielded no reporter signal while others lead to ubiquitous expression of the reporter gene. However, two regions provided specific expression patterns related to endogenous Pax7 expression in early embryos. One in particular seems to be able to trigger expression soon after gastrulation in cells co-expressing Pax7. Deletion analysis of both regions has lead to the identification of novel putative regulators of Pax7 expression early in development.

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Program/Abstract # 170
Sonic hedgehog (SHH) regulates c-myc expression in the developing brain: Direct or indirect link?
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The mitogen Shh participates in the development of the Central Nervous System (CNS), playing a central role in neural progenitor proliferation. C-myc is one of the most potent proto-oncogenes and growth regulators during development. We recently identified c-myc through both microarray and one-hybrid screenings as a novel target gene of the Shh pathway. The research hereby presented attempts to determine whether the Shh signaling pathway directly regulates c-myc expression through Gli transcription factor activity. Through chromatin immunoprecipitation (ChIP) experiments conducted in the C3H10T1/2 mouse fibroblast cell line, we are testing if there is direct binding of Gli proteins to c-myc regulatory regions. In order to study a possible functional link between Shh and c-myc we are performing luciferase assays using a c-myc reporter construct generated in our lab, and evaluating possible variations in c-myc expression levels in gain-of-function (GOF) and LOF conditions. Using in-situ hybridization techniques in both the zebrafish (Danio rerio) and mouse (Mus musculus) models, we are monitoring changes in the c-myc gene expression pattern in the CNS during development. In conclusion, we will present novel evidence that functionally relates c-myc expression with the Shh/Gli signaling pathway during embryonic brain development.

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Program/Abstract # 171
Transcriptional regulation of Ptf1a in the developing nervous system
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Ptf1a, along with an E-protein and Rbpj, forms the transcription factor complex PTF1-J that is essential for proper specification of inhibitory neurons in the spinal cord, retina, and cerebellum. Ptf1a is the tissue specific component of this complex; therefore, understanding how Ptf1a expression is controlled provides critical insight into PTF1-J regulation. Here we show that two highly conserved non-coding genomic regions, a 2.3 kb sequence located 13.4 kb 5′ and a 12.4 kb sequence located immediately 3′ of the Ptf1a coding region, have distinct activity in controlling Ptf1a expression in the neural tube, cerebellum, retina, and diencephalon. The 5′ 2.3 kb sequence functions as an autoregulatory element and directs reporter gene expression to all Ptf1a domains in the developing nervous system. The autoregulatory activity of this element was demonstrated by binding of the PTF1-J complex in vitro, Ptf1a localization to this genomic region in vivo, and the in vivo requirement for Ptf1a for its activity in transgenic mice. In contrast, the 12.4 kb 3′ regulatory region does not contain any conserved PTF1 sites, and its expression in transgenic mice is independent of Ptf1a. Thus, regulatory information for initiation of Ptf1a expression in the developing nervous system is located within the 12.4 kb element. Together, these results identify multiple transcriptional mechanisms that control Ptf1a levels: autoregulation through the PTF1-J complex, and a Ptf1a-independent mechanism for initial activation.

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Program/Abstract # 172
Hoxc13 regulation of Soat1 in the hippocampus and cerebellum
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We recently identified Soat1 (sterol O-acetyltransferase 1) as a regulatory target of Hoxc13 in mouse hair follicles. We provide evidence for direct regulation of Soat1 via multiple Hoxc13 binding sites by immunofluorescence, transient transfection, and chromatin immunoprecipitation assay. As Soat1, a cholesterol esterification protein, has been implicated in atherosclerosis and Alzheimer's disease, we decided to determine whether Hoxc13 was regulating Soat1 in other tissues as well. Here we show that, as in hair follicles, Hoxc13 and Soat1 show distinct co-expression in the hippocampus and cerebellum of the developing and adult brain. We demonstrate reduced Soat1 expression in these brain sections of Hoxc13 null vs. wild-type mice that correspond to histological differences. As behavioral/learning studies suggest that Soat1 is involved in certain types of long term memory formation, we are evaluating cognition in our Hoxc13 null mice and show evidence they share similar deficits with Soat1 null mice. We hypothesize that the