Program/Abstract # 423  
**Functional analysis of CSPG in Danio rerio**

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Oligodendrocyte progenitor cells (OPC) have been identified in mammalian central nervous system by the expression of a chondroitin sulfate proteoglycan (designated CSPG4 in the human genome and NG2 in other vertebrates). Although several myelin genes have been identified in Danio rerio, NG-2 positive OPCs have yet to be identified in the adult teleost brain. In order to identify these cells BLAST analysis with a rat CSPG-4 protein sequence was conducted using the Ensemble Blast site of the Danio rerio genome sequencing project at Sanger. CSPG-4 was identified and syntenic to the human CSPG4. The entire sequence of the NG-2 cDNA has been confirmed through RACE analysis. Expression of CSPG4 was confirmed in Zebrafish adult brain using RT-PCR and in situ hybridization. Adult Zebrafish brains were positive for CSPG4 gene expression in a subset of cells suggesting that OPCs similar to mammalian OPCs are present in the Zebrafish brain. Additionally in situ hybridization demonstrated expression of CSPG-4 between 18 and 24hpf embryos in more distinct regions of somites. CSPG-4 gene expression has also been detected in mesenchymal condensations of developing chondrocytes within the jaw at 48hpf. By 4days post fertilization, CSPG-4 gene expression is downregulated in chondrocytes. In order to determine a function for CSPG-4 in developing chondrocytes and oligodendrocyte progenitors, morpholinos targeted to the CSPG-4 gene were injected into one-celled embryos. The morphology of the cranial skeleton was not altered by the morpholino knockdowns suggesting redundancy or nonessential function for CSPG-4 in Zebrafish chondrogenesis.

**doi:** 10.1016/j.ydbio.2009.05.449

Program/Abstract # 424  
**Knockdown of PAPSS1 expression in an inducible RNAi mouse model demonstrates the requirement for sulfation in the developing neocortex**

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Sulfation of macromolecules is known to modulate a number of cellular processes from protein-protein interactions to growth factor signaling. The universal sulfate donor in the cell, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), is synthesized by two isoenzymes, PAPSS1 and PAPSS2, which are each expressed in a tissue- and temporal-specific manner. PAPSS1 is the predominant isoenzyme expressed in the developing brain; therefore, to determine the importance of cellular sulfation during normal brain development, we developed a single-vector inducible RNAi construct containing the H1 promoter regulated by the tetracycline repressor flanked with loxP sites. Generation of transgenic mice expressing this construct and subsequent crossing with Nestin-Cre mice generated mice which express an RNAi hairpin directed to PAPSS1 in Nestin-expressing cells. Radiometric activity assays of whole brain lysates from these mice at E15.5 revealed an 85 reduction in PAPS synthetase activity over control mice, indicating the successful in vivo knockdown of PAPSS1. Phenotypically, mice with reduced PAPSS1 expression have smaller brains, with a marked reduction in the size of the neocortex. This difference in size is readily apparent by E14.5 and persists into adulthood. Our preliminary analysis indicates that the overall patterning of the cortex is largely normal, and that the reduction in cortical size is not due to increased cell death, suggesting a function for sulfation in controlling progenitor cell division and/or differentiation.

**doi:** 10.1016/j.ydbio.2009.05.450

Program/Abstract # 425  
**Apical and basal Ascl1 (Mash1) progenitors in the dorsal neural tube contribute differentially to inhibitory and excitatory neuronal populations in the spinal cord**

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The dorsal spinal cord comprises a network of inhibitory and excitatory neurons that are required for relaying somatosensory information. The majority of these neurons (dILA, inhibitory and dILB, excitatory) arise at a relatively late phase of neurogenesis from a common progenitor domain defined by the neural bHLH transcription factor, Ascl1 (Mash1). Here we redefine this Ascl1 progenitor domain into at least two subdomains (apical and basal) in the neural tube and show that these two spatially separable Ascl1 lineages contribute differentially to inhibitory and excitatory neuronal populations. To reveal this novel lineage relationship, we generated two distinct inducible Ascl1-CreER transgenic mouse lines. In a line where CreER^T2 was knocked into the Ascl1 locus, expression of CreER^T2 recapitulates a complete Ascl1 pattern. Whereas, a BAC transgenic line with CreERTM replacing Ascl1 in the BAC preferentially marks a basal subdomain at the lateral edge of the ventricular zone. Lineage analysis from these two Cre lines reveals that inhibitory neurons are generated from the apical subdomain of the Ascl1 progenitor pool whereas excitatory neurons are generated from both the apical and basal subdomains. In addition, Lsm1, a marker for symmetrically dividing neuronal progenitors, is restricted to the basal subdomain. Together these observations suggest distinct neuronal subtypes arise from progenitors with distinct cell division modes. Functional analysis of Ascl1 in these two distinct lineages is being examined using a conditional mutant allele of Ascl1.

**doi:** 10.1016/j.ydbio.2009.05.451

Program/Abstract # 426  
**LMO4 controls the balance between excitatory and inhibitory spinal V2 interneurons**

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Multiple excitatory and inhibitory interneurons form the motor circuit with motor neurons in the ventral spinal cord. Notch signaling initiates the diversification of immature V2-interneurons into excitatory V2a-interneurons and inhibitory V2b-interneurons. Here we provide a transcriptional regulatory mechanism underlying their balanced production. LIM-only protein LMO4 controls this binary cell fate choice by regulating the activity of V2a- and V2b-specific LIM-complexes inversely. In the spinal cord, LMO4 induces GABAergic V2b-interneurons in collaboration with SCL and inhibits Lhx3 from...
generating glutamatergic V2a-interneurons. In LMO4;SCL compound mutant embryos, V2a-interneurons increase markedly at the expense of V2b-interneurons. We further demonstrate that LMO4 nucleates the assembly of a novel LIM-complex containing SCL, Gata2 and NLI. This complex activates specific enhancers in V2b-genes consisting of binding sites for SCL and Gata2, thereby promoting V2b-interneuron fate. Thus, LMO4 plays essential roles in directing a balanced generation of inhibitory and excitatory neurons in the ventral spinal cord.

doi:10.1016/j.ydbio.2009.05.453

Program/Abstract # 427
Epibranchial placodal cells are required non-cell autonomously for the formation of neural crest-derived otic ganglion
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Neural crest cells (NCCs) give rise to all parasympathetic motor ganglia. Several trophic factors have been identified for survival of parasympathetic ganglia, but the detailed source of these factors remains unclear. To address this problem, we focused on the development of the NCC-derived otic ganglion. Using the Hoxa3-Cre/ROSA-EYFP genetic lineage reporter, which labels rhombomere 6 (r6)-derived NCCs, we found that the otic ganglion originates from r6. The otic precursors initially migrate toward the third branchial arch (ba3), and pass through the epibranchial placode-derived cells (petrosal ganglion) in ba3 before migrating into the more rostral ba2. This NCC-placode interaction suggests that placodal cells may be important for the migration, differentiation, and/or survival of NCC-derived otic precursors. To test this hypothesis, we analyzed Neurog2+/− embryos, which show a significant decrease in neurons of the petrosal ganglion. We found that this placodal defect caused an 80% reduction in the number of neural precursors in the otic ganglion. To elucidate the underlying cause of this defect in Neurog2+/− embryos, we placed the Hoxa3 lineage reporter in the background of Neurog2−/− embryos and followed the fate of r6-derived NCCs. We show that NCCs delaminate normally from the dorsal r6, but undergo apoptosis midway to the petrosal ganglion, thus failing to reach their proper target area in ba2. These results suggest that placodal cells provide non-cell autonomous signals required for survival and/or migration of neural crest-derived parasympathetic motor ganglia.

doi:10.1016/j.ydbio.2009.05.454

Program/Abstract # 428
The Atoh1-expressing cell lineage develops into both hair cells and supporting cells
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Auditory function depends on the formation of a highly ordered mosaic of hair cells (HC) and supporting cells (SC) in the cochlea. During development, formation of the prosensory domain first distinguishes HC and SC progenitors from other epithelial cells in the cochlear duct. Expression of the transcription factor Atoh1 is thought to be the earliest determinant of HC fate; absence of Atoh1 results in a complete loss of both HCs and SCs, while misexpression of Atoh1 in nonsensory cells induces ectopic HCs. Early cochlear expression of an Atoh1-LacZ knock-in reporter is broad, but at later stages, expression is found only in HCs. Consequently, it is not clear whether Atoh1 is ever expressed in SC progenitors, or is exclusively in HC progenitors. To discriminate between these possibilities, we used an inducible Cre-PR to permanently mark Atoh1-expressing cells with YFP, and have found that a significant percentage of cells from this lineage do develop as SCs. Inhibition of Notch signaling, necessary for the formation of the HC-SC mosaic, increases the total number of YFP-labeled cells, but decreases the percentage that develop as supporting cells. Our results suggest that the Atoh1/HC lineage is established early in cochlear development, but that expression of Atoh1 in prosensory cells does not absolutely result in commitment to a HC fate. Instead, our data indicate that Atoh1 is neither expressed in all prosensory cells, nor is restricted only to HCs, suggesting that the pattern of Atoh1 expression in the developing cochlea may be more complex than previously supposed.

doi:10.1016/j.ydbio.2009.05.455

Program/Abstract # 429
Molecular factors that regulate neuronal cell fate determination within the developing inner ear
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During mammalian inner ear development, the generation of prosensory, proneural and nonsensory lineages from multipotent progenitor cells located within the otocyst requires distinct molecular programs that are spatio-temporally coordinated to give rise to mechanosensory hair cells, neurons of the cochleovestibular ganglion (VIII cranial nerve), and nonsensory cells respectively. While several of the genes that specify the hair cell lineage have been identified, factors that are sufficient to induce a neuronal fate are unknown. Here we show that nonsensory epithelial cells within the cochlea can be induced to develop as neurons through ectopic expression of the proneural basic helix-loop-helix transcription factors Neurog1 or NeuroD1. Moreover, since the high-mobility-group type transcription factor Sox2 is expressed in otocyst neuronal cells and, later, in cochlear ganglion neurons, we utilized both gain- and loss-of-function studies to examine the role of Sox2 in cochlear neuron formation. We show that ectopic expression of Sox2 is sufficient to induce a neuronal fate in nonsensory epithelial cells. Moreover, cochlear ganglion neurons were absent in Sox2-deficient cochleae, indicating that Sox2 is also required for neuronal formation. Our data indicate that Sox2, Neurog1 and Neurod1 are all sufficient to induce a neuronal fate in otocyst-derived cells, demonstrating that nonsensory regions of the developing cochlea retain neurogenic capacity at least through the early postnatal period. These results suggest that spatio-temporal regulation of transcription factor expression plays a key role in the development and patterning of the inner ear.

doi:10.1016/j.ydbio.2009.05.456

Program/Abstract # 430
Wnt/β-catenin signaling promotes hair cell formation in the avian cochlea
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Canonical Wnt signaling has been implicated in multiple developmental events including the regulation of cell fate, differentiation and pattern formation. Activation of the canonical Wnt/β-catenin pathway leads to the down-regulation of GSK3β, which normally targets β-catenin for ubiquitin-dependent proteolysis, resulting in the accumulation of β-catenin in the cytoplasm. β-catenin migrates to