

phila has not been identified yet. Indeed, the formin we identified as Drok substrate genetically interacts with Drok and its knock-down causes PCP phenotypes.

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Program/Abstract # 205

The roles of beta-catenin pathway in the chick dermomyotome and myotome

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Cell-cell signaling through extracellular signals is essential for development. Wnts are a family of secreted proteins important for their roles in proliferation and differentiation in both vertebrates and invertebrates. We have previously shown that β -catenin dependent Wnt signaling (via Wnt3a) causes an expansion of the dermomyotome and myotome. In these studies, we showed that tissue proliferation in the dermomyotome coupled with cell hypertrophy in the myotome contributed to the expansion of the myotome. However, we did not assay the requirement of β -catenin dependent Wnt signaling in myogenesis. We hypothesized that β -catenin signaling is required for proper development of the dermomyotome and myotome. To test our hypothesis, we overexpressed Dickkopf1 (Dkk1), a known inhibitor of the Wnt/ β -catenin pathway in the neural tube of developing chick embryos and assayed the effects on the dermomyotome and myotome. Our results showed that over expression of Dkk1 reduced the area of the dermomyotome and myotome, as compared to controls. Many mechanisms could explain the reduction in size of the myotome. To determine if myogenesis was delayed by Dkk1, we performed whole mount immunostaining of electroporated embryos with antibodies against Myosin Heavy Chain (MHC). Our preliminary results indicated that MHC expression on the electroporated side is delayed by ~ 1.1 somites compared to the control side ($n=8$, p -value <0.05). We are currently working to distinguish whether this is an effect on specification and/or differentiation.

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Program/Abstract # 206

Role of Ror1 in the developing chick neural tube and somites

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Many congenital diseases occur due to defects in neural tube (NT) and somite development. One family of secreted proteins that plays a major role in these processes are Wnts. Though Wnts usually signal via Frizzled receptors, Ror1 and Ror2 tyrosine kinase receptors have also been shown to play important roles in Wnt signaling. They have been shown to activate both the β -catenin dependent and independent pathways. For example, Wnt5a signaling via Ror1/2 inhibits the β -catenin dependent pathway while Wnt3a signaling via Ror2 activates the β -catenin dependent pathway. This implies that depending on which Wnt is bound to the receptor, different pathways can be excited. Our data shows Ror1 expression in the dorsal NT and throughout the dermomyotome of developing chick embryos. Together, these data led us to hypothesize that Ror1 has a key role in the NT and somites. Our research goal is to determine the role of

Ror1 and to distinguish whether it acts via a β -catenin dependent or independent pathway. To test the role of Ror1, we knocked it down in the NT and somites by electroporating an RNAi construct. Because the area of the NT and somites was noticeably reduced on the electroporated side as compared to controls, we assayed for apoptosis using a TUNEL assay. Cell death was seen on the electroporated side of the NT but not in controls. By contrast, no increase in cell death was observed in electroporated somites. We are currently testing whether Ror1 acts via a β -catenin dependent or independent pathway in the NT.

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Program/Abstract # 207

Restricting cell movement: The role of Tspan18 in neural crest migration

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Unlike typical neuroepithelial cells in the developing central nervous system, neural crest cells undergo an epithelial to mesenchymal transition (EMT) and migrate great distances to give rise to diverse structures, such as the peripheral nervous system, melanocytes and facial bone. Anomalies of the neural crest lead to several cancers including neuroblastoma and melanoma, and metastatic cells undergo an EMT that resembles predictable events during neural crest delamination, making the neural crest a unique model to study cancer progression. Despite its fundamental importance, neural crest delamination and migration are poorly understood. Tetraspanin 18 (Tspan18), a member of the tetraspanin family of transmembrane proteins whose activity has been implicated in cell signaling, motility and adhesion, is abundantly and specifically expressed in premigratory chick neural crest cells. Interestingly, Tspan18 expression is down-regulated when neural crest cells migrate, suggesting that Tspan18 negatively regulates neural crest migration. Interfering with Tspan18 function promotes, while overexpression inhibits neural crest cell migration. These data suggest that Tspan18 may play a vital role in neural crest emigration from the neural tube, regulating the proper developmental timing of neural crest migration. Gaining insight into Tspan18 function in neural crest cells will give us a better understanding of how dynamic interactions at the cell surface mediate cell migration, and may provide clues to understanding how cancers like neuroblastoma become metastatic.

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Program/Abstract # 208

The Twist-Slug-Snail regulated gene sizzled's role in mesoderm and neural crest formation

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The secreted Wnt and Tollid proteinase inhibitor Sizzled (Salic et al., 1997. *Development* **124**:4739; Lee et al., 2006. *Cell* **124**:147) is an immediate-early target of regulation of the transcription factors Slug/Snail2 and Twist in the early *Xenopus* embryo. Similarly, the level of *sizzled* RNA increases in Snail1 morphant embryos. An in situ hybridization analysis indicates that in addition to its previously described expression in the ventral regions of blastula stage embryos, *sizzled* expression is present at the anterior (cement gland) and posterior (proctodeum) domains of neurula stage embryos, and can

be detected within the neural plate and the neural crest. Morpholino-based studies indicate that the loss of Sizzled function leads to the loss of mesoderm (Xbra expression and myotome formation) and neural crest (sox9). We will present a more complete characterization of the late stage sizzled expression pattern, as well as the morphant phenotype.

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Program/Abstract # 209

Mesodermal Wnt signaling organizes the neural plate via Meis3

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In vertebrates, canonical Wnt-signaling controls posterior-neural cell lineage specification. Although Wnt-signaling to the neural plate is sufficient for posterior identity, the source and timing of this activity remain uncertain. Furthermore, critical molecular targets of this activity have not been defined. We utilized the advantages of the *Xenopus* embryo system to identify the endogenous Wnt activity and its role in controlling a critical downstream transcription factor, Meis3. Wnt3a is expressed in a specialized mesodermal domain: the paraxial dorsal-lateral mesoderm, which signals to overlying neuroectoderm. We show that Wnt3a is required in this region to activate Meis3 expression in adjacent neuroectoderm cells. Loss of zygotic Wnt3a in this region blocks Meis3 expression, triggering the subsequent loss of posterior neural fates. Over-expression of Meis3 protein is sufficient to rescue this phenotype. Moreover, neural caudalizing Wnt3a morphogenic activity requires functional Meis3 in the neural plate. ChIP and transgenic promoter analyses show that Meis3 is a direct target of Wnt β -catenin signaling. At later neurula stages, we show that Wnt3a and Meis3 act in a feedback loop in the neural plate, which is auto-regulated by Meis3. This suggests a new model for neural anterior-posterior patterning, in which Wnt3a from the paraxial mesoderm induces posterior cell fates via direct activation of a critical transcription factor in the overlying neural plate. This work reveals a core Wnt-Meis-Hox gene regulatory network controlling hindbrain formation. This mode of action is conserved in various developing systems throughout the Bilateria super phylum.

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Program/Abstract # 210

The role of pkd2 in zebrafish embryonic nodal patterning

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Pkd2 is a gene implicated in human autosomal dominant polycystic kidney disease. In addition to kidney defects, mutations and knockdowns of *pkd2* in mouse and zebrafish disrupt normal left-sided nodal expression in the lateral plate mesoderm. Although *pkd2* has been shown to be important in creation and initial restriction of nodal expression domains to the left lateral plate mesoderm, the link between *pkd2* and the initiation of nodal expression is unknown. *Pkd2* mutants and morphants also display altered organ size that may be a direct effect of reduced mesendoderm patterning. To probe the functions of Polycystin-2 in embryonic patterning, we are generating an antibody against the zebrafish protein to examine its cellular location. We are also performing epistasis experiments to determine whether *pkd2* is likely to play a cell autonomous role in the nodal

signaling pathway or a non-cell autonomous role involving nodal-lefty regulation.

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Program/Abstract # 211

MicroRNA-221 regulates chondrogenic differentiation through promoting proteosomal degradation of slug by targeting mdm2

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MicroRNAs (miRNAs) are small RNAs with diverse function as negative gene expression regulators. In the present study, we investigated miRNA role during chondrogenic differentiation of chick limb mesenchymal cells. We found that the expression of miR-221 increased upon chondrogenic inhibition. miR-221 blockade via PNA-based antisense oligonucleotides (ASOs) recovered the chondro-inhibitory actions of JNK inhibitor on proliferation, migration of chondrogenic progenitors and the formation of precartilaginous condensation. We determined that one relevant target of miR-221 during chondrogenesis was mdm2 since miR-221 was necessary and sufficient to down-modulate its expression. Mdm2 modulation by miR-221 had significant functional consequences; indeed, down-modulation of mdm2 by miR-221 prevents slug degradation and resulted in up-regulation of slug, a negative regulator for proliferation of chondroprogenitors. These results indicate that miR-221 contributes to the regulation of cell proliferation by inhibiting slug degradation through negative regulation of Mdm2 during chondrogenesis of chick limb mesenchymal cells.

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Program/Abstract # 212

Early embryonic expression of ionotropic and metabotropic GABA receptors in *Xenopus laevis*

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Neurotransmitter specification is a key component of determining the ultimate fate of neurons in the central nervous system. While the role of signaling cascades and transcription factor networks are well understood, little is known regarding the role of activity dependent mechanisms. It has been suggested that GABA may have a function in activity dependence that is distinctly different than classic synaptic signaling. Recent literature has shown that GABA is present prior to synapse formation and that blocking GABA signaling during these stages results in an increased number of excitatory neurons. A prediction emerging from these results is that metabotropic GABA_B receptors are activated leading to activity dependent mechanisms of neurotransmitter specification. We have therefore cloned and characterized the expression patterns of both GABA_B receptors (Gabbr1 and Gabbr2) as well as four of the GABA_A alpha subunits (Gabra1, Gabra2, Gabra3 and Gabra5) in *X. laevis* embryos. *In situ* hybridization was performed on embryos from Nieuwkoop and Faber stages 15-40. Results thus far indicate that metabotropic GABA_B receptors begin expression around stage 15 in the brain while Gabra2 expression does not appear until stage 29/30. Both steadily become more abundant throughout the brain and spinal cord in spatially and temporally specific patterns. Real time PCR experiments are currently underway to detect low expression levels of the receptor subunits.