Program/Abstract # 141
A genetic screen for situs abnormalities
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The initially bilaterally symmetrical embryo breaks symmetry in a consistent manner to give the wild type situs solitus embryo. While inversion of the left–right (L–R) axis is completely viable, incomplete conversion has serious health implications often resulting in congenital heart disease. Indeed it is increasingly obvious that low level L–R patterning defects underlie a proportion of human congenital heart defects. Work from many groups has revealed a basic L–R determination pathway comprising a cilia driven leftward flow of liquid in the embryonic node, subsequent activation of the left sided Nodal pathway and in turn activation of left sided expression of Pitx2. Various gaps however, are apparent in this model. Using a forward genetic approach we have been isolating mouse mutants affecting L–R development. An initial screen, analysing 135 pedigrees identified 10 single gene defects affecting L–R patterning. This frequency suggests that approximately 64–128 genes control L–R patterning in the mouse. We identified 5 lines that had, in addition to abnormal L–R patterning, complex phenotypes including pulmonary agenesis, exencephaly, polydactyly, ocular and craniofacial malformations. These complex abnormalities are present in human disease syndromes such as VACTERL and SLO syndromes. We present our ongoing analysis of mutations affecting L–R development.

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Program/Abstract # 142
A potential link between fetal exposure to deet and birth defects in chick development
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Teratogens are chemical or environmental agents that cause birth abnormalities in a developing embryo. Deet is a widespread pesticide, which is an active ingredient in most tick and insect repellents. Since embryos have the potential of being exposed to deet, testing the insecticide on developing embryos is of extreme importance. This research project focused on the study of prenatal exposure of deet in chick embryos. The effect of deet on chick development was studied at the physiological level by noting birth weight and abnormal external structures of 7 and 15 day old chick embryos exposed to varying concentrations of the insecticide. The results suggest that even low doses of deet cause physiological defects including microthalmia and hemorrhage as compared to control embryos. Acetylcholinesterase (AChE) is the enzyme that catalyzes the reaction for the neurotransmitter acetylcholine to be broken down into choline and acetic acid at neuromuscular junctions. This enzyme functions to return an activated neuron to its resting state by inhibiting acetylcholine. If AChE activity is suppressed by an inhibitor such as a pesticide, the concentration of acetylcholine increases in the synapse causing the over stimulation of neurons. Expression of AChE in mammalian skeletal muscles has been shown to be controlled at the mRNA stability level. Chick embryos treated with deet will be further evaluated by histochemical procedure and copper thiocholine staining to locate areas of AChE activity in skeletal muscles. Following this analysis, the presence of acetylcholinesterase mRNA in skeletal muscles will be determined using RT PCR.

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Program/Abstract # 143
Analysis of Fgf gene expression patterns in the ear-forming region of the chick
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We are interested in the role of FGF signaling in induction and patterning of the middle ear and tissue interactions within the ear-forming region. These tissues include the epithelium of the inner ear, notochord, neural tube, menenchyme (neural crest and mesoderm derived), surface ectoderm and pharyngeal endoderm. We wanted to determine the spatial and temporal FGF signaling in this region prior to the morphological appearance of the middle ear bone (columella condensation) at 96 h of development. The gene expression patterns of Fgf family members have not previously been analyzed systematically in the chick ear-forming region. We have used in situ hybridization analysis of all the available Fgf ligands at stages 8/9, 14 and 18. Our results demonstrate that Fgf8, 13 and 19 are expressed at stages 8/9. Fgf10, 16 and 18 have expression by stage 14 and within 24 h Fgf 2, 3, 9, 14 and 20 have also turned on expression. Fgfs are expressed in specific tissues within the ear-forming region suggesting that FGF signaling is required for regional cell identity. We are currently analyzing the spatio-temporal expression of the Fgf receptors and undertaking tissue recombination experiments to determine which tissues and FGFs are required for columella condensation.

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Program/Abstract # 144
Expression patterns of cadherin-6B in chick limb development
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Cell adhesion molecules play a role in directing migration, cell signaling, and tissue identification necessary during morphogenesis of the embryo. Cadherin-6B (cad-6B) is a cell–cell adhesion protein known to be expressed in the premigratory neural crest, differentiated vascular and visceral smooth muscle cells, optic tectofugal projection neurons, hair cells and spindle-shaped cells of the cochlea, and a subset of the lateral motor column neurons. This study characterizes the mRNA-level expression patterns of cad-6B in comparison to two other type II cadherins, cad-7 and cad-11, in the embryonic development of chicken limbs from Hamburger and Hamilton stages 21 through 34 using whole mount in situ hybridization. Cad-6B was detected first in the posterior proximal mesenchyme and flank region and observed later in the distal anterior and posterior margins of the outgrowing limb. Cad-6B was also found in neurons innervating the limb, in dorsal–ventral muscle precursors, and weakly in the apical ectodermal ridge. Further analysis of sectioned embryos and immunohistochemistry will provide specific tissue identification.

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Program/Abstract # 145
Influence of biomechanical force on joint development marker gene expression in chick embryo limb bud mesenchyme micromass cultures
Peter G. Alexander, Brent E. Bobick, Karen L. Clark, Anisha A. Chandra, Rocky S. Tuan
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We are interested in the role of FGF signaling in induction and patterning of the middle ear and tissue interactions within the ear-forming region. These tissues include the epithelium of the inner ear, notochord, neural tube, menenchyme (neural crest and mesoderm derived), surface ectoderm and pharyngeal endoderm. We wanted to determine the spatial and temporal FGF signaling in this region prior to the morphological appearance of the middle ear bone (columella condensation) at 96 h of development. The gene expression patterns of Fgf family members have not previously been analyzed systematically in the chick ear-forming region. We have used in situ hybridization analysis of all the available Fgf ligands at stages 8/9, 14 and 18. Our results demonstrate that Fgf8, 13 and 19 are expressed at stages 8/9. Fgf10, 16 and 18 have expression by stage 14 and within 24 h Fgf 2, 3, 9, 14 and 20 have also turned on expression. Fgfs are expressed in specific tissues within the ear-forming region suggesting that FGF signaling is required for regional cell identity. We are currently analyzing the spatio-temporal expression of the Fgf receptors and undertaking tissue recombination experiments to determine which tissues and FGFs are required for columella condensation.

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Although many of the molecular mechanisms regulating chondrogenesis have been elucidated, little is known about the genetic regulation of joint formation. Here, we show the development of an in vitro model that may reproduce the cellular interactions and signaling events occurring during joint formation. We show that high-density micromass cultures of embryonic chick limb bud mesenchyme express multiple genes normally expressed during joint formation, including GDF5, Wnt-14, Notch1, Delta1, Pax-1, and CD44, indicating that micromass cultures may serve as a suitable in vitro joint development model. We hypothesize that the chondrogenic nodules and associated internodular, non-chondrogenic regions that form in micromass culture can be used to replicate the molecular events occurring during joint segmentation. Subsequently, we used micromass cultures to investigate changes in joint development-specific gene expression under flexion. Silicone-bottomed FLEXCELL culture plates were modified to simulate uni-axial joint-like bending. Strain testing showed that flexion increases the expression of joint development markers in micromass culture, while decreasing the expression of chondrogenic marker genes. This illustrates that our novel flexed micromass culture system may function as a model to recapitulate and manipulate joint development in vitro. 

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Program/Abstract # 146
Imaging the epithelial-to-mesenchymal transformation of avian trunk neural crest cells
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The neural crest (nc) is a population of cells that undergoes an epithelial-to-mesenchymal transformation (EMT) in the dorsal neural tube epithelium to produce migratory nc cells. While much has been learned about the nc EMT in fixed tissue, the direct observation of individual neural tube cells undergoing an EMT has the potential to reveal important and novel insights into the mode and mechanisms of the nc EMT. To image the nc EMT at high-resolution, we performed time-lapse confocal microscopy on fluorescently labeled dorsal neural tube cells in avian embryo slice culture. After recording more than 75 different EMT events, we observed that: 1) the trunk nc EMT took place in any dorsal region of the neural tube and not solely at the dorsal–most wedge, 2) there was significant variation in the timing and efficiency of nc cell detachment from the neural tube in relation to the emergence of cells from the neural tube, and, 3) there was no overall correlation between the orientation of the neural tube cell division cleavage plane and the generation of nc cells. To learn more about the behavior of the adherens junctions and the centrosome during the nc EMT, we also performed time-lapse imaging of neural tube cells labeled with either a-catenin-GFP or γ-tubulin-GFP fluorescent proteins. Overall, our observations suggest that there is much more to the nc trunk EMT than simply losing cell–cell adhesion and that there must be additional molecular mechanisms involved that truly “transform” epithelial neural tube cells into mesenchymal nc cells.

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Program/Abstract # 147
Ephrin B2 coordinates the formation of a morphological boundary and cell epithelialization during somite segmentation
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During early development in vertebrates, somitogenesis produces pairs of somites from the presomatic mesoderm (PSM). At the position of a next-forming boundary in the anterior end of PSM, a morphological boundary (fissure) forms, and this event is soon followed by epithelialization of cells that are facing the fissure. We previously reported that the cells posteriorly adjacent to the prospective boundary (posterior border cells) act on the anterior border cells, resulting in the production of a fissure (Sato et al., Development, 2002), and also that suppression of Cdc42 activity is important for the somitic epithelialization (Nakaya et al., Dev. Cell, 2004). We here describe inter- and intracellular signals governing the fissure formation, and how these signals are linked to the epithelialization. We have found that cMeso1, a homolog of mouse MesP2, is capable of inducing the formation of an ectopic boundary when electroporated into chicken PSM using tet-on method (Watanabe et al., Dev. Biol., 2007). cMeso1 upregulates expression of EphA4, which in turn acts on the anterior border cells that express ephrin B2. Whereas EphA4-forward signals are dispensable, ephrin B2-reverse signals are sufficient and also essential to produce a fissure. Interestingly, ephrin B2 also suppresses the activity of Cdc42 through phosphorylation at three sites of the intracellular region of ephrin B2. We present a model wherein ephrin B2 plays dual roles during somitogenesis in the fissure formation and cell epithelialization by bifurcating its intracellular signals toward these different morphogenetic events.

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Program/Abstract # 148
Expression of agrin in the early embryo
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Agrin is a heparan sulfate proteoglycan and has been studied extensively in the late embryonic and postnatal nervous system and muscle. Agrin seems to play a critical role in the mediation of axonal growth and path finding. We studied the expression of agrin by RT-PCR and immunofluorescence in the chick embryo from stages X (morula) to HH17 (29 somites). Agrin mRNA was first detectable at low levels at stage HH1–HH2 (late blastula) and its expression was developmentally regulated. Expression of agrin mRNA was high at stage HH3 (intermediate streak), lower at stage HH4–5 (head process), high at stage HH8–9 (5–6 somites) and lower at stage HH10 (10 somites). Agrin protein fluorescence was strong in the three germ layers at stage HH4 (definitive streak) and was strong in the neuroepithelium at stage HH8 (4 somites). At stage HH11 (13 somites), agrin fluorescence was strong in the neural tube and somites and was intense in the gut roof plate and in the heart primordia. By stage HH17, agrin fluorescence was strong in the neuroepithelium of neural tube and was detected in the laminar surface of the diencephalon and myelencephalon. Agrin expression was strong in the lens and retina in the eye, was strong in the myotome but was not expressed in the dermatome and sclerotome in the somites, was strong in myocardium and endocardium in the heart and in the gut walls. Immunodetection of agrin was intense in neuroepithelium and mesenchymal tissues as they epithelialize and the expression of agrin was developmentally regulated. 

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