ment of dystroglycan in mediating by RhoA function during gastrulation EMT.

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Program/Abstract # 78
Novel retinotectal projection pathway in deeper laminae of the developing chick optic tectum
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The optic tectum is a visual center of the lower vertebrates and receives retinal axons in a retinotopic manner. After invading the tectum, retinal fibers run through the superficial layer of the tectum, make a right turn, and enter deeper laminae to form terminal arborization in the specific retinorecipient laminae. Previous studies have shown that the terminal arborizations are formed in the upper laminae (above lamina g in SGFS). It has been accepted that retinal axons never enter deeper laminae. We developed high sensitive tracing system in which gene of fluorescent reporter protein is integrated in the genome and is expressed stably in long term (Sato et al., 2007; Harada et al., 2008), and re-examined the projection pattern of retinal axons in the tectal laminae of developing chick embryos. Surprisingly, we found a bundle of retinal fibers that run in deeper laminae than SGFS. These fibers run on distinct pathway from known ones. After invading the optic tectum, these fibers run on the dorsal margin of the tectum, make a right-angled turn, then extend in deeper laminae to the lateral side without entering the superficial layer where known retinal fibers run. As development proceeds, these fibers decrease but still remain after hatching. We also found that some of known retinal fibers running in the superficial layer transiently pass through lamina g and invade deeper laminae. High sensitive tracing system has elucidated novel tract of retinal fibers in the deeper optic tectum. We are trying to elucidate the origin and terminals of the fibers.

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Program/Abstract # 79
Elucidating the role of Hoxa-5 in development of the chick axial skeleton
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In the developing axial skeleton, hox proteins act combinatorially to govern the identity of vertebral segments, and to regulate cartilage differentiation later in development. Here, we test the role of Hoxa-5 in development of the avian axial skeleton. Hoxa-5 patterns segments around the cervical-thoracic transition in mice, but it is unknown how it regulates cartilage differentiation pathways in axial tissues, and, given differences in Hox expression patterns between mice and chicks, whether Hoxa-5 specifies the same segmental identities in the two lineages. We find that overexpression or knockdown of Hoxa-5 in chick pre-somitic mesoderm alters vertebral morphologies, but these changes do not appear to be homeotic transformations in segmental identity. Further examination is aimed at elucidating the molecular changes associated with altered Hoxa-5 expression during cartilage differentiation.

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Program/Abstract # 80
Functional analysis of Klf2 during embryonic skeletal development
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Endochondral ossification is the process by which the majority of the bones of the body are formed. It occurs via the differentiation of mesenchymal cells into chondrocytes to produce a cartilage template of the skeleton (chondrogenesis), followed by replacement of this template with bone (osteogenesis). This complex process is incompletely understood. Kruppel-like factor 2 (Klf2) is a zinc finger transcription factor upregulated 30-fold during chondrogenesis. With known roles in regulating blood vessel tone, T-cell and smooth muscle cell migration, it as-yet has no known role in skeletal development. Here we provide evidence for the functional significance of Klf2 in limb development. Retroviral-driven misexpression of Klf2 in chick embryos results in reduction of overall bone length, transformations of digit identity, and an alteration of bone morphology coined the 'web of bone'. We are currently using a viral construct containing a tissue-specific promoter to assess if the web of bone is due to a disruption of signalling from the cartilage itself, or from the surrounding perichondrial cell layer. In addition, in situ hybridisation analysis is being used to examine gene expression alterations in response to Klf2 misexpression, to identify the network in which Klf2 functions and elucidate its mode of action in chondrogenesis and osteogenesis.

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Program/Abstract # 81
Where'd my tail go?
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The Araucana chicken breed lacks all caudal tail structures, due to an unidentified autosomal dominant rumpless mutation, and is reminiscent of the human caudal agenesis phenotype. Morphological analysis reveals a variable number of missing vertebrae and associated structures from the lower synsacrum and tail region. Extension is compromised as early as the tailbud organizer stage. We have investigated the role of apoptosis, cell migration and cell proliferation as possible mechanisms. Migration through the ventral ectodermal ridge is unaffected, as determined by Laminin/E-Cadherin double immunostaining and DiI fate mapping. TUNEL staining reveals increased levels of apoptosis in the ventral tailbud region. Cell proliferation studies using EdU show the expected lack of proliferation in the tailbud. However, cells situated rostro-ventrally show reduced proliferation, shrinking the population available for caudal tail extension. Together these data demonstrate a reduction in the number of ventral cells contributing to the tailbud, which accounts in part for the Araucana phenotype.

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Program/Abstract # 82
Cellular aspects of LR asymmetric morphogenesis in early heart development
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