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Morphogenesis

Program/Abstract # 75
Expression pattern of E-Cad, Ocln and ZO-1 in cleavage-stage Monodelphis domestica embryos
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During blastocyst formation in the laboratory opossum, M. domestica, each blastomere adheres to the zona pellucida, flattens against the zona, and later forms tight junctions with its neighbors. This contrasts greatly with mouse blastocyst formation, wherein cells adhere to each other and later form tight junctions, at no time adhering to the zona. We hypothesized that the spatio-temporal expression of the proteins encoded by E-Cad, Ocln and ZO-1 may explain the difference between eutherian (mouse) and marsupial (opossum) blastocyst formation. Confocal microscopy revealed that E-cadherin is found in both cytoplasm and embryonic coats (zona pellucida, mucoid layer). Its presence in the cytoplasm declined as blastocyst formation became imminent. The tight-junction proteins occludin and zona occludens were expressed similarly in the cytoplasm, although occludin later appeared in the perivitelline space, eventually becoming contained in the blastocoel. Both occludin and zona occludens were detected in zygotic nuclei. Occludin reappeared in larger amounts in cell nuclei during blastocyst formation. The presence of these cell-membrane proteins in nuclei warrants further investigation. Although the antibodies used in this investigation were molecularly similar only E-cadherin, not occludin or zona occludens, was detected in extracellular coats. It is thus unlikely that this E-cadherin expression pattern is artifactual. Rather, its presence in embryonic coats as well as cell surfaces (as occurs in mouse) suggests that it may facilitate blastomere-zona adhesion well in advance of blastomere-blastomere adhesion. (Support: NSF Grant IOS - 0718404)

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Program/Abstract # 76
Nodal signal is required for morphogenetic movements of epiblast cells in pre-streak chick blastoderm
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During axis formation in amniotes, posterior and lateral epiblast cells in the area pellucida undergo a counter-rotating movement along the midline to form primitive streak (PS) (Polonaise movements). Using prestreak chick blastoderms, we investigated the signaling involved in such morphogenetic movements in epithelial-epiblast. In whole embryo cultured blastoderm, inhibition of Nodal signal by Lefty1 suppressed the movement of DiI-marked epiblast cells at early to late blastula stages. In Lefty1-treated embryos, PS formation and Brachyury expression were affected. However, in some Lefty1-treated embryos without PS, Brachyury was expressed in the posterior epiblast with patchy staining, suggesting that prospective mesoderm cells failed to move for PS formation but expressed Brachyury. SU5402 did not affect epiblast cell movements and PS formation. In cultured posterior blastoderm on chamber slide, either Lefty1 or Cerberus-S inhibited the migration of explants. Multicellular rosette, which is thought to be involved in morphogenetic movement of developing epithelium, was found predominantly in the posterior epiblast, where poly-ingression of epiblast cells and Polonaise movements occur. In whole embryo cultured blastoderm, Lefty1, but not SU5402, inhibited the formation of rosettes. Three-dimensional reconstruction of the rosettes showed that there were at least two types of rosette, one with a ventral hollow and the other with a protruding cell. Results suggest that Nodal signal plays a central role in the morphogenetic cellular movements of epithelial-epiblast before and at the onset of PS formation.

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Program/Abstract # 77
Involvement of Dystroglycan in Epithelial-Mesenchymal Transition during chicken gastrulation
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Regulated disruption of basement membrane (BM) is a critical step of Epithelial–Mesenchymal Transition (EMT) in development and disease. Using chick gastrulation as a model, we have demonstrated that the loss of basally localized small GTPase RhoA activity and destabilization of basal microtubules in epiblast cells lead to disruption of epitheliall cell–BM interaction and subsequently breakdown of the BM during EMT. However, precise molecular mechanisms controlling the interaction between the BM and the basal membrane of epithelial cells and its subsequent disruption during EMT are still poorly understood. In this study, we analyzed the role of dystroglycan (Dg) in this process. Dg is a basal membrane protein and reported to play a role in maintaining epithelial architecture. Dg mRNA is restricted to epiblast and its expression is down-regulated in cells undergoing gastrulation EMT. Beta-Dg protein is localized to the basolateral membrane in the epiblast cells and the basal localization is lost in cells undergoing EMT. Interestingly, the subcellular localization of Dg is affected by microtubule destabilization. Disruption of microtubules leads to the loss of basal membrane beta-Dg localization and of BM protein laminin expression. We also show that failure of RhoA downregulation during EMT results in the retention of basal beta Dg expression. Our data support a role of Dg in linking the BM with microtubules. Furthermore, these data suggest an involve-

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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