Concurrent Session 5: Cellular Contact in Growth and Differentiation

Program/Abstract # 32
Regulation of adherens junctions components during chick neural crest cell migration
Lisa A. Taneyhill, Sharon Jhingory, Chyong-Yi Wu
Department of Animal Sciences, University of Maryland, College Park, MD, USA

The neural crest, a population of migratory cells derived from the future central nervous system in the developing vertebrate embryo, gives rise to a diverse range of cell types, including most of the peripheral nervous system, melanocytes, and the craniofacial skeleton. Initially existing as adherent epithelial cells (the premigratory neural crest), these cells undergo an epithelial-to-mesenchymal transition (EMT) characterized by the loss of intercellular contacts to facilitate their emigration from the dorsal neural tube. This EMT is mediated, in part, by the regulatory activity of the Snail2 transcriptional repressor. We have identified components of premigratory neural crest cell adherens junctions (cadherin6B and alpha-N-catenin) to be Snail2 targets that play important roles in proper neural crest cell migration. Knock-down and overexpression of both adherens junctions components enhances and inhibits, respectively, neural crest cell migration in vivo. Furthermore, alpha-N-catenin regulates the appropriate detachment of neural crest cells and their movement away from the neural tube through a mechanism that requires changes in Cadherin protein levels. Snail2 directly represses transcription of both adherens junctions components, and loss of either in a Snail2-depleted background rescues the neural crest cell migration defects normally observed in chick embryos with reduced Snail2. Collectively, our results point to the importance of dismantling adherens junctions to facilitate the proper migration of neural crest cells during the development of the vertebrate embryo.

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Program/Abstract # 33
Stability control of ATF4 protein is involved in the promotion of neural crest EMT
Yoshio Wakamatsu, Takashi Suzuki, Noriko Osumi
Div. of Dev. Neurosci., Tohoku Univ. Grad. Sch. of Med., Sendai, Japan

Neural crest, the 4th germ layer in vertebrate development, provides platforms of studying several biological phenomena, such as embryonic patterning, directed migration, cell differentiation etc. Epithelial–mesenchymal transition (EMT) is one of them, and it allows neural crest cells to delaminate from the epithelial ectoderm, and to migrate extensively in the embryonic environment. In this study, we have identified ATF4, a basic-leucine-zipper transcription factor, as one of the neural crest EMT regulators. Although ATF4 alone was not sufficient to drive formation of migratory neural crest cells, ATF4 cooperated with Sox9 to induce neural crest EMT by controlling the expression of cell–cell and cell–extracellular matrix adhesion molecules. This was likely, at least in part, by inducing the expression of Foxd3, which encodes another neural crest transcription factor. We also found that ATF4 protein level was strictly regulated by proteasomal degradation and p300-mediated stabilization, allowing ATF4 protein to rapidly accumulate in the nuclei of neural crest cells undergoing EMT. Thus, our results emphasize the importance of the spatio-temporal control of protein stability in the neural crest EMT.

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Program/Abstract # 34
Control of skin pattern formation by gap junction in zebrafish
Masakatsu Watanabe, Shigeru Kondo
FBS, Osaka Univ, Japan

Skin pattern is one of the most intriguing aspects of animals. Zebrafish has stripe pattern on its body which consists of melanophore, black pigment cell, and xanthophore, yellow pigment cell. Developments and differentiations of these pigment cells have been well studied, however it still remains unclear how spatial pattern of pigment cells is determined on fish skin. We have proposed that the skin pattern is formed cell-autonomously and cell–cell interaction among the chromatophores is a key factor for the pattern formation. Furthermore we also have proposed that connexin41.8 (cx41.8), a responsible gene for leopard fish is a key molecule for the pattern formation although the function of cx41.8 for pattern formation we generated transgenic fish cx41.8 works as an activator for melanophore development and inhibitor for xanthophore development which lead to the stripe pattern formation of zebrafish. We also provide evidence that gap junction protein has a potential to contribute pattern variation of animals.

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Program/Abstract # 35
Cytoskeletal polarity mediates localized induction of the heart precursor lineage
Brad Davidsonab, James Cooleya, Stacia Whitakerab, Sarah Sweeneyab
a MCB, University of Arizona, Tucson, AZ, USA
bKeck Imaging Center, Cal-Tech, Pasadena, CA, USA

Embryonic cells must make appropriate fate decisions within a complex and dynamic environment. In vitro studies suggest that the cytoskeleton acts as an integrative platform for this environmental...
input, modulating intrinsic mechanical properties in response to signal transduction and reciprocally modulating signal transduction in accordance with these mechanical properties. However, in vivo evidence that the cytoskeleton carries out similar functions during embryonic cell fate specification remains limited. Here we show a critical, in vivo role for the cytoskeleton in modulating heart precursor cell specification. In the basalmost chordate *Ciona intestinalis*, heart founder cells divide asymmetrically. The smaller daughters undergo differential induction to form the heart precursor lineage. Through staged dissociations, we show that cell–cell contact mediates differential induction at a specific developmental timepoint. At this time, heart founder cells form a polarized, highly invasive membrane which penetrates the underlying epidermis. Through targeted manipulations of Cdc42 activity and actin dynamics we demonstrate that these polarized protrusions serve to spatially restrict inductive signaling. These findings illustrate the importance of bi-directional interactions between intercellular signaling and the cytoskeleton during embryonic development. These studies also highlight the potential for dynamic cytoskeletal changes to refine cell fate specification in response to crude morphogen gradients.

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**Program/Abstract # 36**

**Mechanisms of trophoderm fate specification in preimplantation mouse embryos**

Hiroshi Sasaki  
RIKEN Ctr. for Dev. Biol., Kobe, Hyogo, Japan

Preimplantation mouse embryos form two types of cells by the blastocyst stage: trophoderm and inner cell mass. Historically, two models have been proposed for the mechanisms of cell fate specification. One is the Inside–Outside model, in which cell position determines cell fate, and the other is the Polarity model, in which the presence or absence of apico-basal polarity in cells controls cell fate. We previously showed that differential Hippo signaling along the inside–outside axis regulates cell fate by modulating activity of the transcription factor, Tead4. Our current goal is to integrate the molecular mechanism of cell fate regulation by the Hippo pathway with the two historical models. We found that manipulating cell positioning altered Hippo signaling and cell fates. Interestingly, manipulating cell polarity also affected Hippo signaling and cell fates. These results suggest that both Inside–Outside and Polarity models operate in preimplantation embryos, and that both mechanisms control cell fates via Hippo signaling pathway. I will discuss how cell position-dependent and cell polarity-dependent mechanisms operate in preimplantation embryos and how they modulate Hippo signaling to control cell fates.

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**Program/Abstract # 37**

**Expression of Oct4, Cdx2 and Yap1 during blastocyst formation in the marsupial, *Monodelphis domestica***

Yolanda P. Cruz, Jeremy T. Morrison, Niels S. Bantilan  
Dept. of Biol., Oberlin Coll., Oberlin, OH, USA

The marsupial blastocyst first forms as a simple epithelium securely adherent to the zona pellucida. It has no inner cell mass (ICM), which is in the eutherian (mouse) blastocyst contains the embryonic stem cells at this stage. The marsupial ICM equivalent, the pluriblast, is co-planar with the trophoblast. By contrast, the mouse trophoblast encloses the ICM. Oct4, Cdx2 and Yap1 play crucial roles in allocating mouse blastocyst cells to either trophoblast or ICM fates. Because these genes are found in the opossum genome, we hypothesized that they may have a similar role in cell allocation between pluriblast and trophoblast in the opossum blastocyst. In both mouse and opossum, Oct4 is expressed in all embryonic cells prior to blastocyst formation. During mouse blastocyst formation, Cdx2 is upregulated as Oct4 is downregulated in the nascent trophoblast. In the opossum, a patch of cells in the unilaminar blastocyst epithelium (pluriblast) undergoes the same switch in gene expression. In both types of embryos, Yap1 is translocated to the nuclei of putative trophoblast cells but remains in the cytoplasm of cells fated to be ICM or pluriblast. Our results indicate that the roles of Oct4, Cdx2 and Yap1 in allocating cells to the trophoblast lineage are evolutionarily conserved between marsupials and eutherians, despite the topological differences between their blastocysts. Our results also suggest that cell position plays less of a role in mammalian trophoblast differentiation as has been long believed.

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**Program/Abstract # 38**

**Communication between nonadjacent blastomeres in early *Xenopus* embryos**

Michael V. Danilchik, Betsy Brown, Melissa Williams  
Dept Integr Biosci OHSU, Portland, OR, USA

Confocal microscopy of surgically opened *Xenopus* embryos expressing membrane-tethered eGFP unexpectedly revealed hundreds of extremely long, stable membranous processes traversing the entire volume of the blastocoel, linking blastomeres as far as 300 µm apart. Most blastomeres appear to be in long-term contact with a dozen or more adjacent and nonadjacent cells via these long filopodia. The processes are filled with f-actin, and small cytoplasm-bearing blebs translocate bidirectionally within them. They are not cytoplasmic bridges and are not produced during cell division; rather they develop via filopodial extension, exploration and contact. The longest filopodia observed occur before the 512-cell stage, a period critical for embryonic patterning in *Xenopus*. By early blastula stage, protrusive activity abruptly subsides: cells facing the blastocoel display only short protrusions and contact only their immediate neighbors. During this transition, the filopodia settle onto nearby cell surfaces and break up into chains of membrane-bound vesicles. The timing and distribution of these remarkable structures suggest vectorial transport of maternal components between nonadjacent cells. In support of this idea, we observed transfer of lucifer yellow-labeled cytoplasm between nonadjacent blastomeres via engulfment of vesicles budded from the long processes during their breakup. We are presently exploring whether 1) the spatial pattern of traversing filopodia is biased with respect to the primary embryonic axes; 2) early perturbation of dorsal–ventral patterning alters their deployment; or conversely 3) their disruption affects embryonic patterning.  

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