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The complex morphogenesis of organisms is achieved by consecutive cell-to-cell interactions during development. Recent studies suggest that growth factors play crucial roles in controlling such intercellular communications in a variety of organisms. In addition to secretory factors that trigger intracellular signaling, transcription factors that act in the nucleus to regulate gene expression are thought to be essential for the determination of cell fates. Our main interest is to understand how pattern formation and morphogenesis during development is regulated by these growth and transcription factors. We address this problem using several model animals, including frogs, mice and ascidians, and by employing embryology, genetics, molecular and cellular biology, and biochemistry.

Ⅰ. Establishment of cell polarity during vertebrate embryogenesis

Gastrulation is one of the most important processes during the morphogenesis of early embryos, involving dynamic cell migration and change in embryo shape. In spite of its importance, the mechanism underlying the event has just begun to be studied at the molecular level. During *Xenopus* gastrulation, mesodermal cells migrate to the inside of the embryo and move on the blastocoel roof. One of the important mechanisms for this process is the cell movement called “convergent extension (CE)”. As convergent extension begins, cells are polarized and aligned mediolaterally, followed by the mutual intercalation of the cells that acquired planar cell polarity (PCP). In the regulation of vertebrate convergent extension, Wnt/PCP pathway is implicated.

To understand the role of one of the core components of the PCP pathway, we knocked out one of two *prickle*-related genes *mpk1* in mice. We found that the *mpk1*$^{-/-}$ mutants die in early embryogenesis between E5.5 and E6.5. The mutants showed arrested development with failure of primitive streak and mesoderm formation and failure of distal visceral endoderm migration. At the cellular level, the *mpk1*$^{-/-}$ epiblast tissue is disorganized, with a clear defect in cell polarization. Furthermore, we showed *mpk1* genetically interacts with another PCP gene *Vangl2/stbm* in epiblast polarization, indicating that PCP pathway components *mpk1* and *Vangl2/stbm* are essential for early cell polarity, particularly the apical-basal polarity of the epiblast.

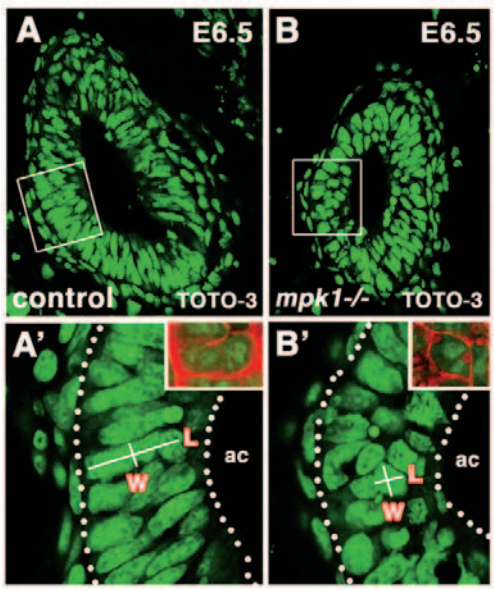

Figure 1. Fluorescent images of nuclei (stained by TOTO-3, green) in transverse sections of wild type (A, A’) and *mpk1*$^{-/-}$ mutant (B, B’) embryos. Insets are higher-magnification images of the respective epiblast cells indicated by F-actin (red) and nuclei (green). Arrowheads indicate the boundary between the extraembryonic and embryonic regions.

We also studied how PCP is established within the cells using explants of *Xenopus* embryonic tissues and found that heterogenous combination cultures of tissues such as mesoderm and ectoderm trigger the cell polarity as revealed by the live-imaging analysis of microtubule growth orientation. We are currently investigating whether physical force generated at the interface of two cell populations is involved in the initiation of the cell polarity.

Ⅱ. Protein ubiquitination and membrane trafficking involved in the Wnt/PC pathway

The Wnt/PCP signaling pathway has been shown to play an essential role in the regulation of gastrulation movements. However, the molecular mechanisms of how Wnt signals intracellularly and how it regulates the tissue movements remain elusive. In order to clarify the Wnt signal transduction mechanism, we searched for the proteins essential for this signaling pathway and implicated protein ubiquitination and membrane trafficking in the Wnt signal transduction. Dishevelled is a cytoplasmic protein, which plays a pivotal role in the Wnt pathway. When the Wnt pathway is activated, Dishevelled is translocated to the plasma membrane. This translocation activates downstream signaling components such as Rho GTPases, and regulates actin cytoskeleton and cell polarity. We have identified a novel E3 ubiquitin ligase complex that binds to Dishevelled.

This E3 ligase complex is essential for the translocation of Dishevelled. In addition, we have shown that Dishevelled and other PCP signaling components are ubiquitinated. This result suggests that protein ubiquitination is important for Wnt/PCP signal transduction. We also identified a novel SNARE family protein, xSynt2, which colocalizes with Dishevelled and is essential for its translocation to the plasma membrane. This SNARE protein is also shown to be essential for the gastrulation movements. SNARE family proteins are known to regulate membrane fusion processes. These findings show that the ubiquitin system and membrane trafficking regulate gastrulation movements in *Xenopus* embryos.

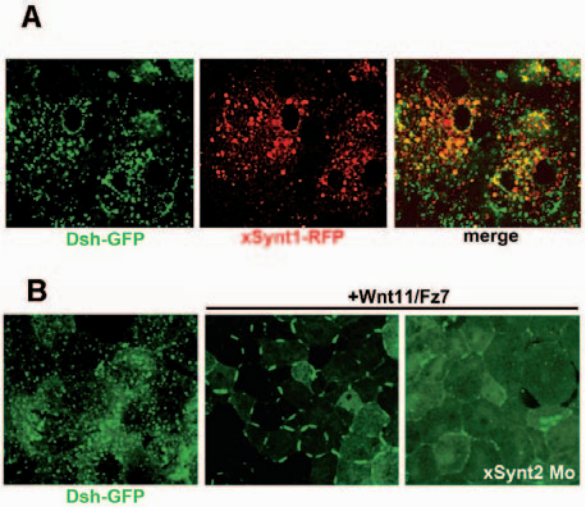

Figure 2. Membrane trafficking regulates the localization of Dishevelled. (A) Dishevelled shows punctate localization (green), and is colocalized with the SNARE protein xSynt1 (red). (B) Localization of Dshevelled-GFP (green). Wnt signaling translocates Dishevelled to the plasma membrane, which is inhibited by antisense Morpholino against xSynt2.

Ⅲ. Cellular morphogenesis during neural tube formation

Neural tube formation is one of the most dynamic morphogenetic processes during early embryogenesis and its failure is known to cause malformations known as neural tube defects. To understand cellular and molecular mechanisms regulating neural tube formation, we focused on MIDLINE1 (MID1) and a paralogous gene MID2. MID1 is a conserved microtubule-associated protein and responsible for Opitz G/BBB syndrome. This syndrome is associated with midline abnormalities such as hypertelorism, hypospadias, and heart defects, suggesting the developmental functions of MID1. We found that MID1 colocalized with microtubule in embryonic cells in *Xenopus*, and the combined depletion of MID1 and MID2 induced neural tube defects. Histological and live-imaging analyses also revealed that neuroepithelial cells in affected embryos became rounded and failed apical constriction and cell elongation. In addition, these defects are tightly correlated with the disorganization of the microtubule network along the apicobasal axis, which should be required for cell shape change and epithelial integrity. Theseese data suggest that the regulation of the microtubule network by MIDs is required for correct neural tube closure and that similar mechanisms also underlie the development of other organs affected in Opitz G/BBB syndrome patients.

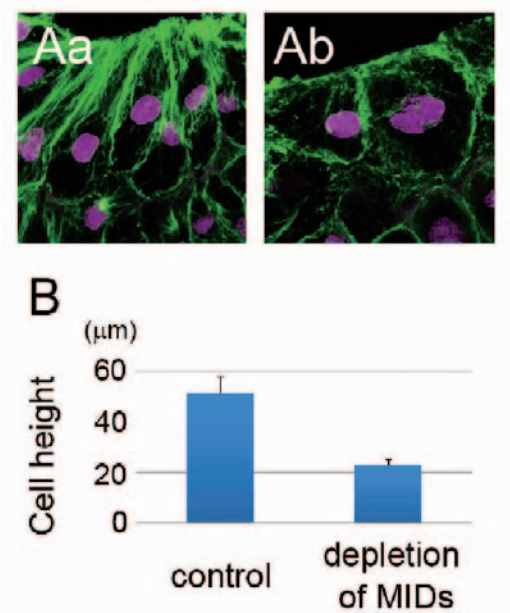

Figure 3. The changes of cell shape and the microtubule network during neural tube closure. (Aa) In control, neuroepithelial cells elongated their shape and developed microtubule along apicobasal axis (green). (Ab) In MIDs-depleted embryo, neuroepithelial cells became rounded and failed microtubule reorganization. (B) Quantitative data of cell height in superficial layer.

We also investigated the roles of an adhesion molecule, nectin, which belongs to the immunoglobulin-like cell adhesion molecule in the neural tube formation of *Xenopus*. Depletion of nectin-2 (one of the nectin family members) from early embryo resulted in incomplete neural fold formation. Cellular analyses revealed less accumulation of F-actin at the apical surface, causing an aberrant apical constriction, a cell-shape change that is required for the neural tube folding. Furthermore, we found nectin-2 functionally cooperates with N-cadherin to synergistically enhance apical constriction, highlighting the cooperative action between spatiotemporally upregulated nectin-2 and N-cadherin in the neural plate.

Ⅳ. Brachyury-downstream gene sets in a chordate, *Ciona intestinalis*

In vertebrates, *Brachyury*, a T-box transcription factor gene, seems to have a dual role in the differentiation of axial midline mesoderm cells into notochord and gastrulation cell movements regulated by non-canonical Wnt/planar cell polarity (Wnt/PCP) signaling. To annotate the function of *Brachyury*-downstream genes in chordate embryos, based on subtractive hybridization, dot-blot assays, EST sequences and the expression patterns in whole-mount *in situ* hybridization at embryonic stages, we developed a

knowledge database called "CINOBI: *Ciona* Notochord and *Brachyury*-downstream gene Index" to create comprehensive catalogues of *Brachyury*-downstream gene sets in *Ciona intestinalis*. Combining genome and large-scale cDNA data, we were able to characterize 450 non-redundant *Brachyury*-downstream genes. Twenty-four genes were newly annotated as notochord-expressed genes. Several genes are components of signaling pathways such as Wnt/PCP, NfkB and TGF-beta signaling. We propose that *Brachyury* is linked to these pathways, regulating the expression of each component, and that such a regulatory mechanism might be conserved among chordates.

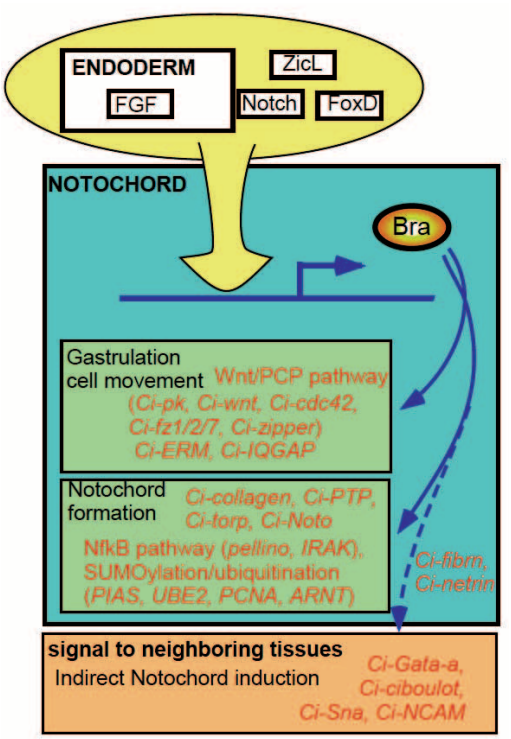

Figure 4. The role of *Brachyury* in the transcriptional network in *Ciona intestinalis*. Upstream positive regulators of *Brachyury* in the endoderm include *FGF* and *ZicL*. Brachyury directly activates *Ci-tropm* and other notochord-specific genes to induce differentiation of the notochord. Cell polarity-related genes such as *Ci-pk* and *Ci-Fz1/2/7* are involved in convergent extension movement in gastrulation. These signaling components are induced directly or indirectly by Brachyury.

Publication List

〔Original papers〕

- Shindo, A., Yamamoto, T.S. and Ueno, N. Coordonation of cell polarity during Xenopus Gastrulation. (2008). PLoS ONE *3*, e1600.
- Hotta, K., Takahashi, H., Satoh, N., and Gojobori, T. (2008). *Brachyury*-downstream gene sets in a chordate, *Ciona intestinalis*: Intrgrating notochord specification, morphogenesis and chordate evolution. Evo. Dev. *10*, 37-51.
- Kawasaki, A., Kumasaka, M., Satoh, A., Suzuki, M., Tamura, K., Goto, T., Asashima, M. and Yamamoto, H. (2008). Mitf contributes to melanosome distribution and melanophore dendricity. Pigment Cell Melanoma Res. *21*, 56-62.
- Gilchrist, M.J., Chiristensen, M.B., Harland, R., Pollet, N., Smith, J.C., Ueno, N. and Papalopulu, N. (2008). Evading the annotation bottleneck: using sequence similarity to search non-sequence gene data. BMC Bioinformatics *9*, 442.