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The complex morphogenesis of organisms is achieved by dynamic rearrangements of tissues during embryogenesis, in which change in cellular morphology as well as orchestrated cell movements are involved. For cells to know how they should change their shape and where they should move, information called "cell polarity" is essential. How then is the cell polarity established within cells? Is it intrinsically formed within the cells or triggered by extracellular cues? Furthermore, little is known as to how coordinated and complex cell movements are controlled in time and space. We attempt to understand the mechanisms underlying these events using several model animals, including frogs, fish, mice and ascidians, taking physical parameters such as force in consideration, in addition to conventional molecular and cellular biology.

I. Biological significance of cell polarity

Cell polarity is required for proper embryogenesis and organogenesis. It is well understood that there are a number of genes essential for establishing cell polarity; they include planar cell polarity (PCP) genes. In early embryogenesis, gastrulation is one of the best studied models in which cell polarization regulated by PCP genes is thought to play an essential role. Although several signaling pathways required for PCP have been identified, they seem to play permissive rather than instructive roles. To identify the trigger of cell polarization, we are currently investigating the output of cell-cell interactions that lead to cell polarization and recently found that transient intracellular Ca^{2+} is one of the earliest events that occurs prior to cell polarity formation (Shindo et al.). Furthermore, we believe that force is generated by tissue-tissue interactions and therefore mechanical stress may play an essential role in the triggering of cell polarity. To test this possibility, we are artificially applying mechanical stresses to embryonic tissues and examining cellular responses from various aspects.

Apparently, in mammals, cell polarity is also important for embryogenesis even earlier than the gastrulation stage when the primitive streak is formed. We knocked out one of two *prickle*-related genes *mpk1* that belongs to the PCP gene family and found that *mpk1*$^{-/-}$ mutants are early embryonic lethal and die between E5.5 and E6.5 mainly due to defects in cell polarization of the epiblast.

In mice, Prickle1 and Prickle2 proteins are highly expressed in the brain and they seem to have important roles for the development of higher order structures and brain functions. Recently, we also knocked out the other *prickle* gene *mpk2* encoding Prickle2. Homozygous *mpk2*$^{-/-}$ embryos developed to adult and were fertile. Interestingly, however, they exhibited lowered thresholds to the development of epilepsy. We reason that this epileptic phenotype is caused by an aberrant development of the neural network, most likely due to the disruption of neuronal cell polarity. Neurons are highly polarized cells that have dendrites and an axon. Interestingly, at least Prickle1 is localized in the post-synapse of hippocampus neurons (Figure 1). Therefore, it is possible that Prickle proteins contribute to the establishment of synaptic connection and the improper connection of neurons in *prickle* mutants might affect neuronal functions causing the epilepsy-like phenotype. To understand the molecular and cellular mechanisms for the regulation of neuronal cell polarity and the regulation of neural activity, we are currently investigating the cellular pathology of brain tissues and cellular morphology of hippocampus neurons derived from *mpk2*$^{-/-}$ embryos.

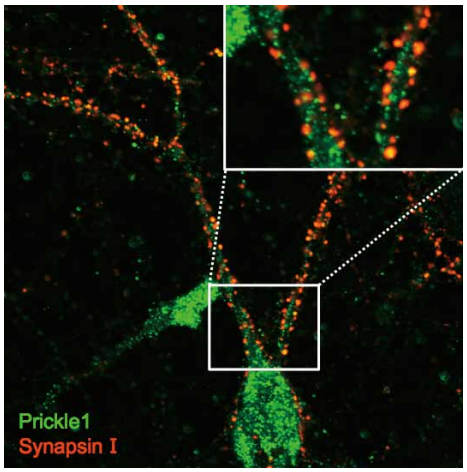

Figure 1. Localization of mouse Prickle1 in hippocampus neurons in primary culture. Prickle1-specific antibody stains punctate Prickle1 proteins adjacent to the pre-synaptic marker Synapsin I.

II. Protein ubiquitylation system involved in the Wnt/PCP pathway

Although Wnt/PCP signaling has been shown to play an essential role in the regulation of gastrulation movements, the molecular mechanisms of how Wnt signals intracellularly and how it regulates tissue movements remain elusive. We have shown that Wnt/PCP signaling activates the protein ubiquitylation/degradation system, which is essential for cell motility during *Xenopus* gastrulation. In order to clarify how the ubiquitylation system is involved in the Wnt/PCP pathway, we focused on β-TrCP (transducin-repeat containing protein), a component of E3 ubiquitin ligase

complex. β-TrCP has been shown to regulate the canonical Wnt pathway by ubiquitinating β-catenin, but it is not known whether β-TrCP is involved in the Wnt/PCP pathway. We found that expression of the dominant negative form of β-TrCP (β-TrCPΔF) impaired gastrulation movements. β-TrCPΔF significantly reduced cell-cell adhesion. In addition, β-TrCP ubiquitylates the cell adhesion molecule cadherin. It localizes to the plasma membrane and bridges cell-cell contacts through its homophilic binding. When β-TrCPΔF was expressed, cadherin was internalized and formed large aggregates in the cytoplasm (Figure 2). This result suggests that β-TrCP is required for maintaining cadherin on the plasma membrane and this function is essential for normal gastrulation. We also found that β-TrCP binds to one of the core PCP proteins, prickle. It recruits β-TrCP to the plasma membrane, and affects ubiquitylation of cadherin by β-TrCP. We are currently investigating the role of prickle in the regulation of β-TrCP activity to demonstrate the molecular mechanism of how Wnt/PCP signaling regulates gastrulation movements.

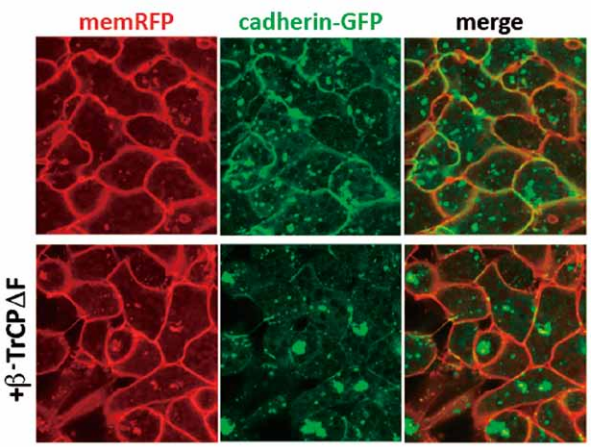

Figure 2. β-TrCP regulates cadherin localization. Membrane-tethered RFP (memRFP) and GFP-tagged cadherin were expressed in *Xenopus* embryos. Cadherin localized mainly to the plasma membrane. When the dominant negative β-TrCP (β-TrCPΔF) was expressed, cadherin was internalized and formed aggregates in the cytoplasm. This suggests that β-TrCP regulates cadherin localization and affects cell adhesion during gastrulation.

III. Cellular morphogenesis during neural tube formation

Neural tube formation is one of the prominent morphogenetic processes during embryogenesis, by which the central nervous system such as the brain and spinal cord are established. In this process, cells in the neural ectoderm undergo cell-cell intercalation, cell elongation, and apical constriction, which allow the neural tissue to form a groove along the anterior-posterior axis and converge toward the midline. Although cytoskeletal elements are responsible for such cellular behaviors, how these are regulated in this process *in vivo* is unknown.

To discern the role of the actomyosin network in neural tube formation, using zebrafish embryos, we analyzed the spatio-temporal dynamics of non-muscle myosin II, focusing on its regulatory right chain. By combining immunohistological and live-imaging analyses, we found that non-muscle myosin II was initially activated as puncta within the motile cells. These puncta were progressively incorporated to the apical region, and colocalized with the adhesive molecule. Furthermore, inhibition of non-muscle myosin II caused defects in cellular motility and morphogenesis, suggesting that non-muscle myosin II acts as the physical linkage between motile cells during zebrafish neural tube formation.

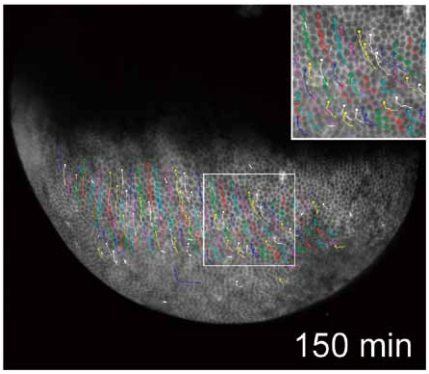

Figure 3. DSLM time-lapse images of ventral non-neural ectoderm from mid (150 min) neurula stages of a *Xenopus* embryo. The trajectories of cell movements (colored dots and lines) and cell division orientations (white short lines) were manually traced. Cells in the middle of the anterior-posterior axis moved rapidly toward the midline. Dorsal is upper side and anterior right. Insets are magnified views of the boxed areas in each image.

We also investigated the physical contribution of the non-neural ectoderm to neural tube formation using *Xenopus* embryos, since dynamic cellular behaviors occur not only in the neural ectoderm but also in the non-neural ectoderm. We tested a role of cell division using chemical inhibitors and found that cell division is not a major driving force in neural tube formation. We are currently examining other cellular mechanisms, including surface expansion, rearrangement, and radial intercalation of non-neural ectoderm cells, using digital scanned laser light sheet microscopy (DSLM) as a powerful tool. DSLM time-lapse images of neurula embryos revealed that cells in the middle of the anterior-posterior axis are highly motile, migrating toward the midline (Figure 3), implying that mechanical properties of these cells may contribute to proper neural tube formation.

IV. Regulation of notochord-specific expression of *Ci-Bra* downstream genes in *Ciona intestinalis* embryos

Brachyury, a T-box transcription factor, is expressed in ascidian embryos exclusively in primordial notochord cells and plays a pivotal role in differentiation of notochord cells. Previously, we identified ~450 genes downstream of *Ciona intestinalis Brachyury* (*Ci-Bra*), and characterized the expression profiles of 45 of these in differentiating notochord cells. We looked for cis-regulatory sequences in minimal

enhancers of 20 *Ci-Bra* downstream genes by electroporating regions within ~3 kb upstream of each gene fused with *lacZ*. Eight of 20 reporters were expressed in notochord cells. The minimal enhancer for each of these eight genes was narrowed to a region ~0.5-1.0-kb long (Figure 4). We also explored the genome-wide and coordinate regulation of 43 *Ci-Bra* downstream genes. When we determined their chromosomal localization, it became evident that they are not clustered in given region of the genome, but rather distributed evenly over 13 of 14 pairs of chromosomes, suggesting that gene clustering does not contribute to coordinate control of *Ci-Bra* downstream gene expression. Our results should provide insights into the molecular mechanisms underlying notochord formation in chordates.

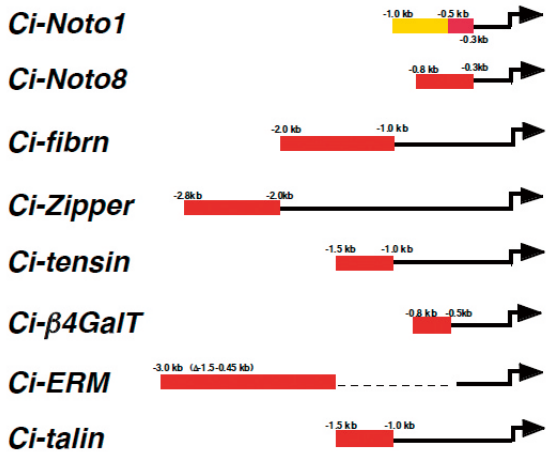

Figure 4. Minimal enhancer region in the 5' upstream sequence of eight Ci-Bra downstream genes that drive reporter expression in notochord cells. The yellow box indicates a possible regulatory region for posterior B-lineage notochord-specific expression of *Ci-Noto1*; the orange box indicates the anterior A-lineage notochord-specific regulatory region of the gene. Red boxes indicate the notochord-specific minimal enhancer region in each of the 5'upstream sequences of *Ci-Bra* downstream genes.

Publication List

〔Original papers〕

- Morita, H., Nandadasa, S., Yamamoto, T.S., Terasaka-Iioka, C., Wylie, C., and Ueno, N. (2010). Nectin-2 and N-cadherin interact through extracellular domains and induce apical accumulation of F-actin in apical constriction of *Xenopus* neural tube morphogenesis. Development *137*, 1315-1325.
- Nojima, J., Kanomata, K., Takada, Y., Fukuda, T., Kokabu, S., Ohte, S., Takada, T., Tsukui, T., Yamamoto, T.S., Sasanuma, H., Yoneyama, K., Ueno, N., Okazaki, Y., Kamijo, R., Yoda, T., and Katagiri, T. (2010). Dual roles of smad proteins in the conversion from myoblasts to osteoblastic cells by bone morphogenetic proteins. J. Biol. Chem. *285*,15577-15586.
- Shindo, A., Hara, Y., Yamamoto, T.S., Ohkura, M., Nakai, J., and Ueno, N. (2010). Tissue-tissue interaction-triggered calcium elevation is required for cell polarization during Xenopus gastrulation. PLos ONE, *5*, e8897.
- Suzuki, M., Hara, Y., Takagi, C., Yamamoto, T.S., and Ueno, N. (2010). *MID1* and *MID2* are required for *Xenopus* neural tube closure through the regulation of microtubule organization. Development *137*, 2329-2339.
- Takahashi, H., Hotta, K., Takagi, C., Ueno, N., Satoh, N., and Shoguchi, E. (2010). Regulation of notochord-specific expression of *Ci-Bra* downstream genes in *Ciona intestinalis* embryos. Zool. Sci. *27*, 110-118.
- Terakubo, H., Nakajima, Y., Sasakura, Y., Horie, T., Konno, A., Takahashi, H., Inaba, K., Hotta, K., and Oka, K. (2010). Network structure of projections extending from peripheral neurons in the tunic of ascidian larva. Dev. Dyn. *239*, 2278-2287.