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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. The roles of PCP core components in mouse development

In epithelia, the roles of planar cell polarity (PCP) have been extensively studied, whereas in non-epithelia, they have yet to be fully understood. We are exploring the roles of PCP in the mesenchymal tissues by using mouse genetics. Recently, we generated a hypomorphic allele of mouse *Prickle1*, one of the core PCP factors. We previously reported (Tao, H. *et al*. Proc. Natl. Acad. Sci., USA, 2009) *Prickle1* null/null mice die around E6.0 in gestation due to the failure of gastrulation. In contrast, the *Prickle1* hypomorphic mutant mice we generated by a partial deletion of the gene survived to P0. Interestingly, the mutant mice had shortened noses. Detailed analyses at the cell level suggested that Prickle1 governs convergent extension of nasal cartilage cells which is required for the lengthening of the nose. Wnt5, a ligand that activates the PCP pathway was found to be expressed forming a concentration gradient of the transcripts from the distal tip of developing nose. As ubiquitous overexpression of Wnt5 caused short noses, the concentration gradient of Wnt5 may be important for proper nose elongation. Our findings further suggests that PCP signaling employed multiple times at different places during development may be one of the universal mechanisms of organ morphogenesis, especially for organ elongation. We hope that in combination with comparative genomic analysis, these PCP mutant mice will serve as good models that can explain morphological variations in mammals.

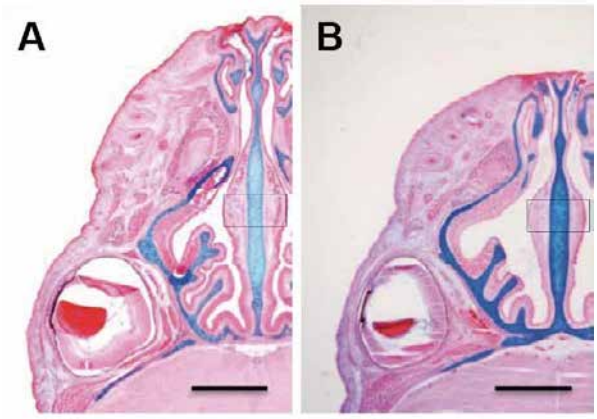

Figure 1. Nose phenotype of mouse *Prickle1* mutant.
The hypomorph allele (B) shows shortened nose cartilage stained with alcian blue due to insufficient convergent extension compared to that of wild type (A).

II. Regulation of cell adhesion by the ubiquitin system during gastrulation

During gastrulation, dorsal mesoderm cells migrate toward the midline and align along the antero-posterior (A-P) axis to form the notochord. In this process, cells repeatedly undergo cell adhesion and detachment, and thus, cell-to-cell interaction must be tightly regulated. We found that one of the cadherin superfamily cell adhesion molecules, paraxial protocadherin (PAPC), plays an essential role in this process. The PAPC transcript first appears in the dorsal marginal zone at the early gastrula stage and is subsequently restricted to the paraxial mesoderm in *Xenopus* and zebrafish. Using *Xenopus* embryos, we demonstrated that PAPC is also regulated at the protein level and is degraded and excluded from the plasma membrane in the axial mesoderm by the late gastrula stage. This regulation requires phosphorylation-dependent poly-ubiquitination. PAPC is phosphorylated by GSK3 in the evolutionarily conserved cytoplasmic domain, and this in turn is necessary for poly-ubiquitination by an E3 ubiquitin ligase β-TrCP. We also show that precise control of PAPC by phosphorylation/ubiquitination is essential for normal *Xenopus* gastrulation cell movements. Taken together, our findings unveil a novel mechanism of regulation of a cell adhesion protein and show that this system plays a crucial role in vertebrate embryogenesis. (Kai, M. *et al*., PLoS One *in press*).

III. Cellular behavior during neural tube closure

During the formation of the neural tube, an anlage of the central nervous system, neural progenitor cells emerge in a broad region on the dorsal side and gradually change their

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

positions toward the midline. However, what kind of cellular and molecular dynamics occur during this process is not clear, probably because analyses at the single cell level with high spatial and temporal resolutions have not been conducted. We analyzed zebrafish neurulation at single cell resolution, and found that progenitor cells showed non-muscle myosin II-dependent asynchronous and periodic movements. We also found that actomyosin cytoskeleton showed an isotropic, periodic remodeling in the cell cortex, while it showed directionaly constant movements in peripheral cellular protrusions. Careful examination revealed that F-actin remodeling was temporarily correlated with a deformation of cell morphology, suggesting that the periodic actomyosin contractility contributes to the convergence movements. We further found that components of the planar cell polarity (PCP) pathway were required for the periodic F-actin remodeling, suggesting that the PCP/noncanonical Wnt pathway controls the periodicity of the actomyosin. Our findings unveil a repertoire of cellular movements based on periodic actomyosin contractility that contributes to the convergence movements of multicellular organisms.

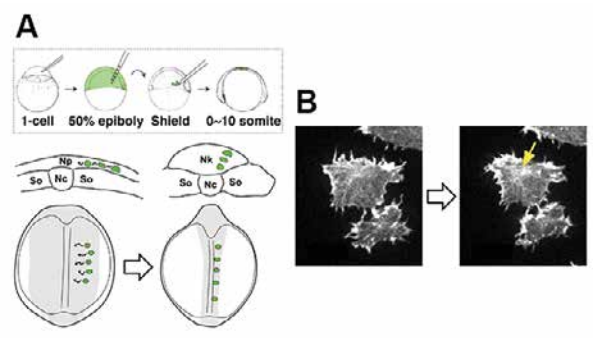

Figure 2. Live-cell imaging analyses at single cell resolution.
(A) A small number of presumptive neural cells expressing fluorescent proteins were transplanted to non-labeled host embryos. Then, cellular movements in the neural plate during convergence were observed at short time intervals. Np, neural plate; Nk, neural keel; So, somite; Nc, notochord. (B) Cortical F-actin was distributed as a meshwork-like structure and periodically concentrated. The time interval of the periodicity corresponds to that of the convergence movements.

IV. A novel plasma membrane structure capturing centrosome determines the orientation of cell division

The orientation of the mitotic spindle has been proposed to control cell fate choices, tissue morphogenesis and architecture, thus playing an important role in shaping embryonic forms. It is already reported that most cells divide along the A-P axis at the last division in ascidain epidermis.

With live imaging observation, we found a novel membrane structure invaginating along A-P polarity toward the centrosome in the epidermal last cell division cycle. Live-imaging observation showed the invagination toward the centrosome. Observation using Serial block face Scanning Electron Microscopy (SBF-SEM) confirmed this invagination reached the centrosome. The result of UV laser ablation indicated that mechanical tension, which was generated between the centrosome and plasma membrane, might form this invagination. With cell cycle progression, the length of invagination became short and the centrosome was pulled to the posterior side. Thus, we hypothesize that these membrane invaginations are involved in spindle orientation along the A-P axis. We would like to propose a novel template of spindle orientation with membrane invagination capturing the centrosome.

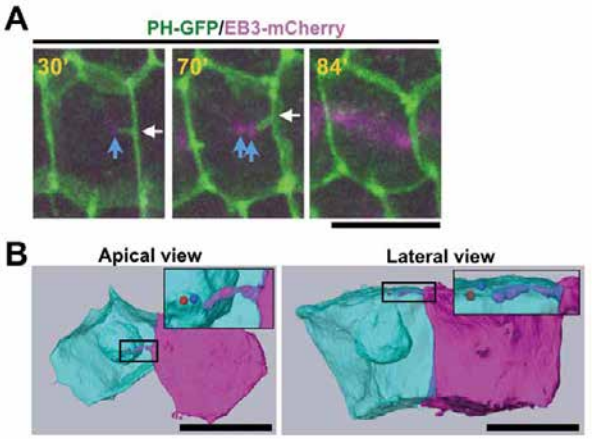

Figure 3. Novel membrane invagination structure in ascidian epidermal cell.
(A) Live imaging of membrane invagination toward microtubule organizing center (MTOC), seemingly, centrosome. PH-GFP (green), plasma membrane probe; EB3-mCherry (magenta), MTOC probe; numbers (orange), recording time in minutes. Anterior membrane invaginates (white arrow) toward MTOC (blue arrow) (30'), centrosomes are aligned (70') and eventually spindle is formed (84') along the A-P axis. (B) 3D images reconstructed from SBF-SEM data. Centrosomes are indicated as red and blue balls. The black-lined squares are enlarged in upper right panels. All bars show 10 μm.

V. Notochord and evolution of chordates

Recently, much more attention has been paid to cephalochordate amphioxus to answer an important question of metazoan evolution, namely the origin and evolution of chordates. The phylum Chordata consists of three subphyla, Cephalochrdata, Urochordata and Vertebrata. A long debate on whether cephalocordates or urochordates are an early divergence among chordates has recently reached a consensus in which cephalochordates are believed to be more ancestral, leaving urochordates and vertebrates as a sister group. While the amphioxus develop well-organized somites, their neural tube lacks a brain-like structure and the notochord contains myofibrils. In order to characterize the three organs of adult amphioxus, we examined their differential gene expression profiles by RNA-seq analysis. The analysis of differential expression profiles of genes highly expressed in the notochord, somite muscle, and neural tube, revealed a molecular affinity between the notochord and the somite muscle, as previous studies suggested. In addition, we could identify 335 genes that are preferentially expressed in the notochord, 411 genes in the somite muscle, and 514 in the neural tube. These genes play roles in the formation, maintenance and function of the three organs. The repertoires of genes provide a molecular basis for further studies of amphioxus.

VI. Subfunctionalization of duplicated MyoD genes in polyploid *Xenopus laevis*

Polyploid organisms offer a model for understanding the evolutionary effects of gene duplication. The African clawed frog *Xenopus laevis* has experienced recent allopolyploidization, which gave rise to the pseudotetraploid genome. We found that the duplicated *MyoD* genes (*XlMyoDM* and *XlMyoDZ*) are under subfunctionalization as indicated by their differential expression patterns. The combined expression pattern of *XlMyoDM* and *XlMyoDZ* corresponds to that of the unduplicated *Xenopus tropicalis XtMyoD*. To test the possibility that the ancestral function of MyoD is subdivided between the duplicated copies, we asked whether *XlMyoDM* and *XlMyoDZ* regulate different sets of target genes. An overexpression experiment of either *XlMyoD* gene followed by RT-qPCR of a selected MyoD target genes showed that both *XlMyoDM* and *XlMyoDZ* genes possess comparable activity of inducing the target genes we examined. To find the differential gene regulation by *XlMyoDM* and *XlMyoDZ* genome-wide, the overexpressed samples were subjected to a transcriptome analysis. By comparing *XlMyoDM*, *XlMyoDZ* and *XtMyoD* target genes, we will answer the important questions whether either *XlMyoD* gene acquired novel functions or lost its original functions during evolution.

VII. Cnidarian-symbiodinium Symbiosis

Corals are declining globally due to a number of stressors. Such stresses can lead to a breakdown of the essential symbiotic relationship between coral and *Symbiodinium*, a process known as coral bleaching. Although the environmental stresses causing this breakdown are largely known, the molecular and cellular mechanisms of symbiosis are still unclear. Corals are not very suitable as laboratory systems, because they are difficult to work with due to their slow growth, long generation times, and calcareous skeletons. To overcome these limitations, we focused on the small sea anemone *Aiptasia* as a novel experimentally tractable cnidarian model organism (Figure 4). *Aiptasia*, just as reef-building corals, establishes a stable but temperature-sensitive symbiosis with *Symbiodinium*. *Aiptasia* can be repeatedly bleached and repopulated with *Symbiodinium*, grows rapidly, and lacks a calcareous skeleton, allowing microscopic and cellular biological analyses. In order to further elucidate the symbiotic mechanisms, it is necessary to establish molecular biological approaches. Therefore, we have attempted to develop a method of gene transfection to *Aiptasia*. Investigating symbiosis using *Aiptasia* should improve our understanding of the symbiotic mechanism.

Figure 4. Aiptasia can be bleached in the laboratory by raising culture temperature.
(left) Symbiotic Aiptasia cultured at 25℃. (right) Aposymbiosis induced by culturing at 30-34℃.

Publication List

〔Original paper〕

- Yajima, H., Suzuki, M., Ochi, H., Ikeda, K., Sato, S., Yamamura, K., Ogino, H., Ueno, N., and Kawakami, K. (2014). Six1 is a key regulator of the developmental and evolutionary architecture of sensory neurons in craniates. BMC Biol. *12*, 40.

〔Review articles〕

- Hashimoto, M., Morita, H., and Ueno, N. (2014). Molecular and cellular mechanisms of development underlying congenital diseases. Congenit. Anom. (Kyoto) *54*, 1-7.
- Satoh, N., Tagawa, K., Lowe, C.J., Yu, J.K., Kawashima, T, Takahashi, H., Ogasawara, M., Kirschner, M., Hisata, K., Su, Y.H., and Gerhart, J. (2014). On a possible evolutionary link of the stomochord of hemichordates to pharyngeal organs of chordates. Genesis *52*, 925-934.