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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. Cell behaviors during gastrulation

During embryogenesis, the arrangement of multicellular tissue is dramatically changed to establish properly shaped embryos. These movements of groups of cells are often highly organized and collective. Investigating the mechanism of collective cell migration is therefore essential for understanding embryogenesis. *Xenopus* leading edge mesoderm (LEM) is one of the suitable models for studying this morphogenetic movement, because of easy micromanipulation of tissue excised from the embryo. During *Xenopus* gastrulation, LEM moves into the blastocoel ahead of the axial mesoderm, which forms the notochord and muscles in the future. In our previous studies, we have shown that LEM generates the driving force of mesodermal migration, and measured the physical value of this force with the explant. To address how each single LEM cell generates the force for collective migration in the explant, we have introduced Traction Force Microscopy (TFM) for *Xenopus* LEM explant migration and found that traction force was generated by the cells, which were located at the anterior part of explants. Particularly in the first few rows of cells of LEM explants, frequent intracellular Ca^{2+} transients were observed. We have also been able to demonstrate that this spatiotemporally controlled Ca^{2+} dynamics is essential for the anterior-directed tissue migration through the regulation of the asymmetric lamellipodia formation.

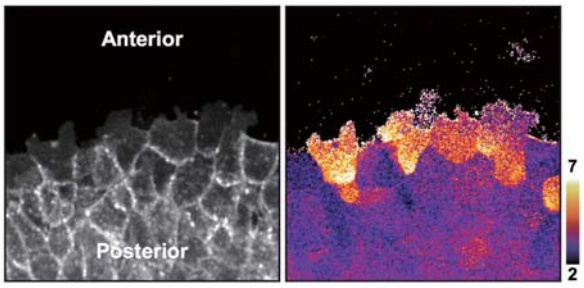

Figure 1. A snapshot of intracellular Ca^{2+} transients in a migrating LEM explant of *Xenopus laevis*. Ca^{2+} dynamics were visualized with a FRET probe YC-Nano2. Inhibition of these Ca^{2+} transients with Ca inhibitors perturbs the tissue migration.

II. Mechanotransduction in *Xenopus* embryonic cells

During early embryogenesis, a variety of dynamic morphogenetic movements occur, which include convergent extension of the axial mesoderm, epiboly of the ectoderm, and neural tube formation. These movements must generate physical forces at the levels of cells and tissues. However, it is still not fully understood how these forces influence morphogenetic processes. This project is to elucidate the mechanisms of sensing and responding to mechanical stimuli in *Xenopus* embryos.

Cells are known to sense mechanical stresses in several ways, for example, with TRP channels, F-actin, cadherins, and focal adhesions. Physical stimuli sensed by these molecules are converted to intracellular chemical signals, which in turn induce cellular response. Protein phosphorylation may be one of the earliest responses to mechanical stresses. Therefore, we are attempting to profile protein phosphorylation upon mechanical stimuli and identify target proteins in *Xenopus* embryos. In order to comprehensively analyze levels of protein phosphorylation, we took a phosphoproteomic approach using mass spectrometry. So far, we found that a mechanical stress applied to *Xenopus* embryos changes phosphorylation levels of many proteins related to cytoskeleton and cell adhesion. Consistently, we observed that mechanical stimulation induced cytoskeletal remodeling. Furthermore, some protein kinases, including ERK2, were activated in response to a mechanical force. As shown in Figure 2, ERK2 was phosphorylated by the mechanical force and its phosphorylation was rapidly attenuated after release from the force. We concluded that *Xenopus* embryos have a system to sense and respond to mechanical stimulation, which activates protein kinase signaling pathways and regulates cytoskeletal organization.

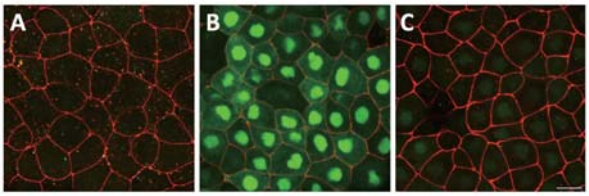

Figure 2. ERK2 is activated by a mechanical force in *Xenopus* embryonic cells.
A mechanical force was applied to *Xenopus* gastrula embryos by centrifugation. Embryos were stained with anti phoshorylated ERK2 antibody (green) and an actin-binding compound, phalloidin (red). Superficial cells around the animal pole were observed. (A) control (not centrifuged). (B) centifuged at 450 x g for 15 min. (C) centrifuged as in B and then incubated for 15 min. Bar = 25 μm

III. Statistical analysis of neural tube formation based on pulsed apical constriction

During early development of the central nervous system, neuroepithelial cells constrict their apices by actomyosin contractility. This cell shape change is called apical constriction, which makes the neural plate into a tubular structure, called the neural tube. We previously showed that two types of intracellular Ca^{2+}-concentration changes, a single-cell and a multicellular wave-like fluctuation occurred in the *Xenopus* neural plate and modulate the patterns of apical constriction during neural tube formation. In this study, to further investigate the role of the Ca^{2+}-induced pulsed apical constriction, we performed statistical analysis in which we fitted a linear mixed model with single-cell and multicellular Ca^{2+} transients as explanatory variables and the amount of the closing movement of the neural tube as a response variable to data of wild-type embryos, and obtained estimated contributions of single-cell Ca^{2+} transients and multicellular Ca^{2+} transients. We found that the coefficient of single-cell Ca^{2+} transients was significantly larger than that of multicellular Ca^{2+} transients. These results suggest that the Ca^{2+} fluctuations at the single-cell level more effectively accelerate apical constriction and neural tube formation than do multicellular Ca^{2+} fluctuations *in vivo*.

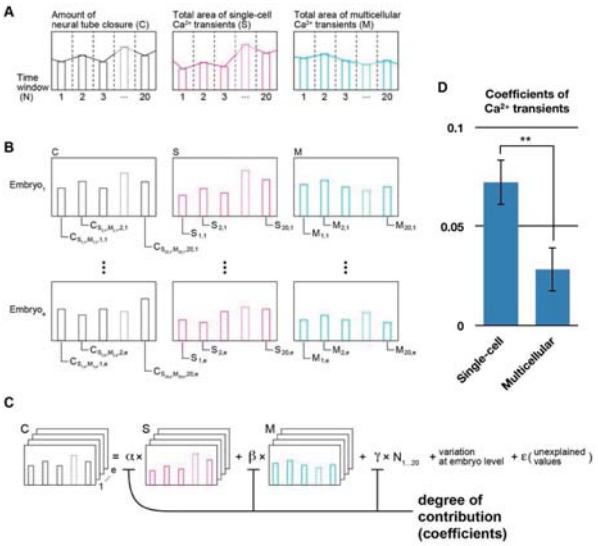

Figure 3. Schematic workflow and coefficients of the statistical model.
(A) Temporal profiles of closing movement, the total area of single-cell Ca^{2+} transients, and multicellular Ca^{2+} transients during neural tube formation. (B) Generation of data set for statistical analysis by dividing temporal profiles into time windows. (C) Fitting mixed linear model to the data set to estimate contributions. (D) Statistics for fixed effects.

IV. A novel membrane structure orchestrates centrosome positioning and the orientation of cell division axis

The positioning of the centrosome is critical for the polarity of cilia and the orientation of the mitotic spindle, which are important events during morphogenesis. Previously, in ascidian, *Ciona intestinalis* embryos, we found a unique centrosome-targeting of the membrane structure (membrane invagination) in epidermal cells, which divide stereotypically along the anterior-posterior (A–P) axis. We also reported that membrane invaginations showed the A–P polarity and a tensile force toward the posterior end of cell. By injection of antisense morpholino oligo (MO), we revealed that Dishevelled (Dsh), a core component of Planar Cell Polarity (PCP) pathway was involved in the A–P polarity of membrane invaginations. In the Dsh-depleted embryos, the direction of the invagination as well as mitotic spindle orientation was randomized. In addition, epidermal cilia normally localized in the posterior side of the cell was anteriorized. These results support our hypothesis that the membrane invagination controls the direction of cell division as well as ciliary positioning via the tethering centrosome at the posterior side of the epidermal cell and highlight physiological importance of this unique structure.

V. Notochord and evolution of chordates

The notochord is the most prominent organ in chordates in which *Brachyury* (*Bra*) plays pivotal roles in its formation. Since *Bra* is shared by non-chordate animals and expressed in the blastopore it is an intriguing EvoDevo question how *Bra* acquired enhancers that promote its gene expression in the notochord as well as in somatic muscles during the evolution of chordates. In cephalochordate lancelets *Bra* is duplicated. We examined enhancer activity of *Branchiostoma floridae Bra* (*BfBra1* and *BfBra2*) using *Ciona* egg electroporation system. Vista analysis suggested the presence of conserved non-coding sequences not only in 5'-upstream but also 3'-downstream and introgenic regions. The *lacZ* reporter assay showed that (1) the 5'-upstream sequences of both genes promoted reporter expression in muscle cells, (2) the 3'-downstream sequences also have enhancer activity and promoted *lacZ* expression in notochord cells, (3) the introgenic regions of *BfBra2* and *BfBra1* exhibited the activity in muscle and notochord cells, respectively, and (4) the enhancer activity appeared as early as the gastrula stage. These results indicate that the 5', 3' and introgenic regions work together to enhance the *Bra* expression in muscle and notochord cells. The acquisition of enhancer activity of *BfBra* is highly likely to be involved in the formation of muscles and notochord in chordate embryos, which in turn led to the evolution of chordates.

VI. Gene order and direction of transcription contribute to the small genome size in *Ciona intestinalis*

DNA methylation at cytosine residues is an important epigenetic modification found in eukaryotes ranging from plants to humans. Invertebrates offer an interesting model for studying evolutionary changes in the targets and the function of DNA methylation. A marine invertebrate chordate *Ciona intestinalis* has a genome-wide mosaic methylation pattern comprising methylated and unmethylated genes. It has been observed that DNA methylation is targeted to the transcribed region of ubiquitously expressed genes, and a constant targeting of "gene body methylation" irrespective of cell types. To reveal the function of gene body methylation in gene transcription, we analyzed newly synthesized RNA from *C. intestinalis* embryos. By using 4sU labeling and sequencing methods, revealing global RNA processing kinetics at nucleotide resolution, we obtained snapshots of active transcription. Significant differences were seen in co-transcriptional splicing efficiency, in connection with methylation status of exons and introns. The splicing efficiency and DNA methylation status were also correlated to nucleosomal positions, suggesting that epigenetic states in the bodies of transcribed genes control the pre-mRNA processing through nucleosomal positioning.

VII. Effects of light on larval swimming behavior in scleractinian corals

Many reef-building corals form a symbiotic relationship with dinoflagellate algae of the genus Symbiodinium. Corals mostly depend on photosynthetic products from these symbionts for their energy source, and thus light conditions in habitats can influence post-settlement survival. Several previous studies reported that light environments play an essential role in larval habitat selection. However, due to lack of basic photobiological studies in corals, how they sense light and how light affects biological tasks remain largely unexplored. The purpose of this study is to reveal the mechanism of phtoreception and clearly understand the effects of light on reproduction and behavior in corals.

For this purpose, we focused on the effects of light on larval swimming behavior of Acropora corals. We analyzed the larval swimming activity with different light stimuli, and found that larvae paused their swimming behavior immidiately after a change in intensity and spectral composition of light. In addition to the behavioral assay, we surveyed the genome of coral, *Acropora digitifera* and *A. tenuis*, for detecting photoreceptor opsin genes using a molecular phylogenetic analysis. Five opsin gene orthologs were found on the genome and two of the five genes were transcribed in the coral larvae. These results suggested that coral larvae sense light with an opsin-based phototranduction cascade and change their behavior responding to the rapid change in light environments.

Figure 4. A view of stony corals around Orpheus Island, Queensland Australia, November 2017

Publication List:

〔Original papers〕

- Murakami, F., Ando, Y., Miyagi, A., Sugita, S., Ueno, N., and Matsumoto, T. (2017). Measurement of surface topography and stiffness distribution on cross-section of Xenopus laevis tailbud for estimation of mechanical environment in embryo. Dev. Growth Differ. *59*, 343-443.
- Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Cambell, R.E., and Ueno, N. (2017). Distinct intracellular Ca2+ dynamics regulate apical constriction and differentially contribute to neural tube closure. Development *144*, 1307-1316.
- Tanaka, T., Ochi, H., Takahashi, S., Ueno, N., and Taira, M. (2017). Genes coding for cyclin-dependent kinase inhibitors are fragile in Xenopus. Dev. Biol. *426*, 291-300.
- Tokue, M., Ikami, K., Mizuno, S., Takagi, C., Miyagi, A., Takada, R., Noda, C., Kitadate, Y., Hara, K., Mizuguchi, H., Sato, T., Taketo, M.M., Sugiyama, F., Ogawa, T., Kobayashi, S., Ueno, N., Takahashi, S., Takeda, S., and Yoshida, S. SHISA6 confers resistance to differentiation-promoting Wnt/β-catenin signaling in mouse spermatogenic stem cells. Stem Cell Rep. *8*, 561-575.