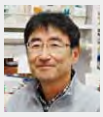
DIVISION OF MORPHOGENESIS

Professor
UENO, Naoto



Associate Professor
KINOSHITA, Noriyuki

Assistant Professor:	*TAKAHASHI, Hiroki*
	SUZUKI, Makoto
Technical Staff:	*TAKAGI, Chiyo*
NIBB Research Fellow:	*YAMAGUCHI, Takeshi*
Postdoctoral Fellow:	*NEGISHI, Takefumi*
	SUZUKI, Miho
	NINOMIYA, Hiromasa
SOKENDAI Graduate Student:	*MIYAGI, Asuka**
	HAYASHI, Kentaro
	TOMINAGA, Hitoshi
Technical Assistant:	*YAMAMOTO, Takamasa*
	MURAKAMI, Michiyo
	MIYAGI, Asuka
	YASUE, Naoko
	WATANABE, Mika
Secretary:	*MIYAKE, Satoko*
	TSUGE, Toyoko

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. A novel signaling pathway for head formation

The head is formed in the most anterior part of the neural tube which is the anlage of the brain and spinal cord. How is the head region selected in its particular region of the tube? Patterning of the anterior-posterior axis is controlled by secreted molecules known as growth factors whose effects are mediated by their downstream mediators which facilitate differentiation of the brain and eyes. It was previously shown that the inhibition of the Wnt/β-catenin signaling pathway by various factors is critical for the anterior specification of head formation. We have revealed that Flop1 and Flop2 (Flop1/2) G protein-coupled receptors contribute to the regulation of head formation by inhibiting Wnt/β-catenin signaling in the amphibian *Xenopus*. Both the overexpression and knockdown of Flop1/2 resulted in abnormal head formation. More interestingly, overexpression of Flop1/2 in the absence of bone morphogenetic protein (BMP) signaling which is inhibitory to general neural differentiation, resulted in ectopic head formation in the ventral side. We have also found that Flop1/2 inhibits Wnt/β-catenin signaling by promoting β-catenin degradation. Although the ligand for the Flops is not identified, our findings suggest that Flop1 and Flop2 are novel inhibitory components of the Wnt/β-catenin signaling pathway and essential for proper head formation.

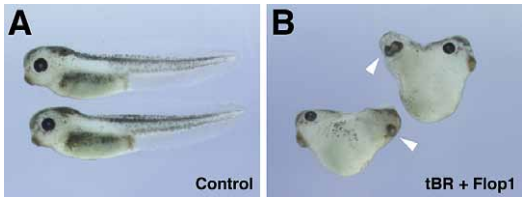


Figure 1. Ectopic head formation by Flop1 overexpression and BMP inhibition.
(A) Control larvae showing normal head and tail structures.
(B) Abnormal larvae in which Flop1 is overexpressed in the absence of BMP signals show duplicated heads with eyes at the expense of trunk and tail.

II. Mechanotransduction in *Xenopus* embryonic cells

During early vertebrate development, many dynamic morphogenetic movements occur, which include convergent extension of the axial mesoderm, collective migration of mesendoderm cells, epiboly of the ectoderm, and neural tube formation. These movements must generate physical forces between cells and tissues. There is accumulating evidence that forces generated by moving cells and tissues affect morphogenetic processes. The purpose of this project is to elucidate mechanisms that sense and respond to mechanical force in *Xenopus* embryonic cells, and to clarify the roles of mechanotransduction systems during development.

It is known that cells can sense mechanical stresses in several ways, for example, TRP channels, cell surface glycoproteins, flow-sensing cilia, cadherins, and focal adhesions. Physical stimuli sensed by these molecules may be converted to intracellular signaling and induce cellular response. Several studies using mammalian tissue culture cells have revealed that mechanical stresses induce activation of some protein kinases. Therefore we examined whether mechanical force might change protein phosphorylation in *Xenopus* embryonic cells. For this purpose, we used the proteomic approach to comprehensively analyze protein phosphorylation profiles. We have found that mechanical stress applied to *Xenopus* embryos changes phosphorylation states of several proteins, suggesting that *Xenopus* embryonic cells have a mechanism to sense mechanical forces, which involves protein phosphorylation/dephosphorylation.

III. Mathematical analysis of neural tube closure

During early development of the central nervous system, neuroepithelial cells decrease their apical surface area by actomyosin contractility. This cell shape change is called apical constriction, which converts the neural plate into a tubular structure, called the neural tube. We previously showed that intracellular calcium ion (Ca^{2+}) dynamically fluctuated throughout the *Xenopus* neural plate from the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).

single-cell to whole-tissue levels with distinct patterns. Live-imaging revealed that the Ca^{2+} fluctuations correlated with a remodeling of F-actin and the apical constriction, suggesting that the dynamic Ca^{2+} signaling positively regulates the neural tube closure. In this study, to further investigate the role of the Ca^{2+} fluctuations, we developed a two-dimensional vertex model, in which the natures of the apical constriction and the pulsed contraction of the apical junctions were introduced. Computational simulation of our model shows that the pulsed contraction accelerates the apical constriction and decrease of the tissue size independent of its frequency. However, although dense rather than sparse pulsation induces rapid tissue deformation, its overall effect throughout the entire course of the simulation is not effective when the total number of the pulses is constant. These data suggest that the distinct patterns of Ca^{2+}-induced local contraction always contribute to tissue deformation.

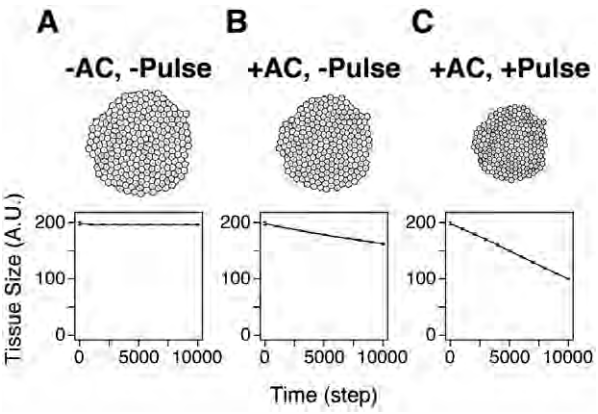


Figure 2. Effects of apical constriction (AC) and pulsed contraction (Pulse) on modeled epithelial sheets.
Representative images of modeled epithelial sheets at the end of each simulation (top) and average tissue sizes during the time course of the simulations (bottom) without the apical constriction and the pulsed contraction (A), with the apical constriction (B), and with the apical constriction and the pulsed contraction (C). Error bars depict s.e.m.

IV. Membrane invagination toward centrosome links the posterior ciliary positioning to spindle orientation in ascidian epidermal cells.

The role of the centrosome changes during cell cycle progression. Centrosomes form the basal bodies of cilia in interphase and the pole of the spindle in mitotic phase. Then, the positioning of the centrosome is important for the polarity of cilia and the orientation of the spindle.

During the last mitotic division of epidermal cells of the ascidian embryo, it is already reported that most cells divide along the anterior-posterior (A-P) axis. We found a unique filamentous membrane structure, which we call "invagination" elongated toward the centrosome in this division cycle. We have proposed a model, in which this novel membrane invagination maintains the position of the centrosome to the posterior of the cell with tensile force and is involved in spindle orientation along the A-P axis. Interestingly, we also found the formation of cilia on epidermal cells during the last division cycle. Therefore, we confirmed the position of cilia located to the posterior of the cell and its basal body was captured by the invagination with live-imaging analysis and Serial block face Scanning Electron Microscopy (SBF-SEM) observation. Posteriorly localized cilia are conserved among several organisms including ascidians for establishing the left – right (L-R) axis. Our investigations will provide a new model of not only spindle orientation, but also ciliary positioning.

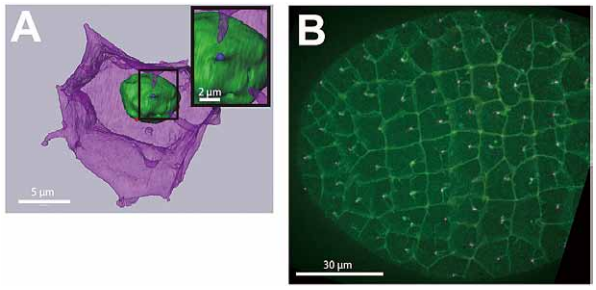


Figure 3. Membrane invagination associated with the epidermal cilia of acidian embryo.
(A) 3D image reconstructed from SBF-SEM data shows that tips of invagination approach the basal body. The basal body and daughter centriole are indicated as blue and red balls, respectively. The black-lined square is enlarged in upper right panel. Anterior is left. (B) A frame of a time lapse video of membrane invaginations reaching cilia. PH-tdTomato (green), plasma membrane probe; Arl13b-GFP (magenta), cilia probe. Anterior is left.

V. Notochord and evolution of chordates

The expression profile of *Brachyury* is highly conserved in metazoans from cnidarian to vertebrates. Specifically, *Brachyury* is expressed in the blastopore region of early embryos, and functions in invagination of the endomesodermal germ layer through the blastopore into the embryo. This "primary" function of *Brachyury* is associated with morphogenesis.

Comparison of *Brachyury* expression between nonchordate invertebrates and chordates clearly shows an additional expression domain seen only in chordates, namely, the dorsal midline of the blastopore, an embryonic region associated with the formation of the notochord. This "secondary" function arose during the evolution of chordates.

Therefore, it is now evident that the evolutionary origin of the notochord is essentially the question of the molecular mechanisms underlying how *Brachyury* acquired its secondary expression domain at the mid-dorsal region of the blastopore. To know how chordate ancestors gained the new expression domains, we used Amphioxus, the most ancestral chordates. We functionally analyzed the Amphioxus *Brachyury* promoter regions by reporter assay, using *Ciona* embryos.

VI. The biological function of invertebrate DNA methylation in pre-mRNA processing

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 the "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. 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 cell 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.

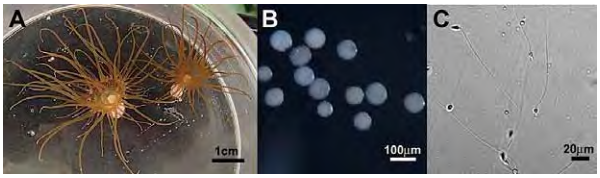


Figure 4. *Aiptasia* spawning can be induced under controlled laboratory conditions
(A) *Aiptasia* mature female (left) and male (right). (B and C) *Aiptasia* eggs and sperms, respectively.

Publication List:

〔Original papers〕

- Kai, M., Ueno, N., and Kinoshita, N. (2015). Phosphorylation-dependent ubiquitination of paraxial protocadherin (PAPC) controls gastrulation cell movements. PLoS One *10*, e0115111.
- Miyagi, A., Negishi, T., Yamamoto, T.S., and Ueno, N. (2015). G protein-coupled receptors Flop1 and Flop2 inhibit Wnt/β-catenin signaling and are essential for head formation in Xenopus. Dev. Biol. *407*, 131-144.
- Negishi, T., and Yasuo, H. (2015). Distinct modes of mitotic spindle orientation align cells in the dorsal midline of ascidian embryos. Dev. Biol. *408*, 66-78.
- Sakamaki, K., Iwabe, N., Iwata, H., Imai, K., Takagi, C., Chiba, K., Shukunami, C., Tomii, K., and Ueno, N. (2015). Conservation of structure and function in vertebrate c-FLIP proteins despite rapid evolutionary change. Biochem. Biophys. Rep. *3*, 175-189.
- Uno, Y., Nishida, C., Takagi, C., Igawa, T., Ueno, N., Sumida, M., and Matsuda, Y. (2015). Extraordinary diversity in the origins of sex chromosomes in anurans inferred from comparative gene mapping. Cytogenet. Genome Res. *145*, 218-229.

〔Original paper (E-publication ahead of print)〕

- Sekiguchi, T., Kuwasako, K., Ogasawara, M., Takahashi, H., Matsubara, S., Osugi, T., Muramatsu, I., Sasayama, Y., Suzuki, N., and Satake, H. Evidence for conservation of the calcitonin superfamily and activity-regulating mechanisms in the basal chordate *Branchiostoma floridae*: insights into the molecular and functional evolution in chordates. J. Biol. Chem. 2015 Dec 7.