Division of Embyology

Professor
FUJIMORI, Toshihiko

Assistant Professor:	*TOYOOKA, Yayoi*
	KOYAMA, Hiroshi
Technical Staff:	*OKA, Sanae*
NIBB Research Fellow:	*SHI, Dongbo*
Postdoctoral Fellow:	*DAY, F. Timothy*
	NAKANOH, Shota
SOKENDAI Graduate Student:	*KAMEMIZU, Chizuru*
	ITO, Tomoaki
	USAMI, Fumiko
Technical Assistant:	*HIGUCHI, Yoko*
Secretary:	*KATO, Azusa*

The aim of our research is to understand the events underlying early mammalian development during the period from pre-implantation to establishment of the body axes.

Early events during embryogenesis in mammals are relatively less understood, as compared to other animals. This is mainly due to difficulties in approaching the developing embryo in the oviducts and the uterus of the mother. Another characteristic of mammalian embryos is their highly regulative potential. The pattern of cell division and allocation of cells within an embryo during the early stages vary between embryos. The timing of the earliest specification events that control the future body axes is still under investigation. Functional proteins or other cellular components have not been found that localize asymmetrically in the fertilized egg. We want to provide basic and fundamental information about the specification of embryonic axes, differentiation of cell lineages, behaviors of cells, and the regulation of body shape in early mammalian development. We are also interested in the relationship between embryos and their environment, the oviducts and the uterus.

I. Establishment of a live imaging system for observation of early mouse development

To understand the mechanisms underlying embryonic development and morphogenesis, it has become common to observe the behavior of cells and gene expression in living embryos in a variety of animal species. Progress in GFP technologies, genetic engineering and embryonic manipulation concomitantly with the progress in optics and microscope technologies, including development of highly sensitive photo-detectors, has allowed us to observe developing embryos live, even in mammals. We have established a series of transgenic mouse lines for live imaging, which is part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CLST. These mouse lines have been used to visualize nuclei, cell shapes, the cytoskeleton and other organelles to observe cell behaviors in living mouse embryos in many laboratories over the world. We also established mouse lines to monitor the cell cycle.

We have also been establishing several reporter mouse lines in the lab to study gene expression patterns during the peri-implantation stage of mouse development. In these mice, cDNA encoding fluorescent proteins are connected to the mouse genomic DNA sequences of the enhancer/promoter region of important genes encoding factors regulating cell differentiation in these stages. Combining these two types of reporter transgenic mouse lines, we have been analyzing behaviors of cells comparing gene expression properties at the single cell level.

For the live imaging of early mammalian embryos, a combined microscope and incubation system is an important tool. Conventional CO_2 incubators provide better conditions compared to microscope top incubation chambers, including stability of temperature and humidity, to support embryonic development *in vitro*. Incubation microscopes have also recently become commercially available, however, these are expensive for personal use. We have modified an incubation microscope with wide field fluorescent illumination, which is relatively inexpensive. We added a spinning disc confocal system and sensitive EM-CCD camera for observation of developing mouse embryos with less photo-toxicity and higher spatial resolution.

We have been trying to observe and reveal aspects of cell shape, morphogenesis, cell lineage, gene expression and cell differentiation in developing embryos and in other tissues by combining these techniques. We reported multiple phases in transcriptional regulation of the *Nanog* gene, which is known to be one of the major pluripotent regulators, during the pre-implantation stages of development.

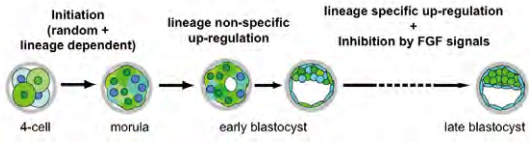

Figure 1. Multiple phases in *Nanog* gene expression during preimplantation mouse development. Weak expression of Nanog starts around the 4-cell stage, and is random. From the 4-cell stage to the morula, the up-regulation of Nanog expression is not lineage specific. Lineage specific differences appear by the early blastocyst stage, and inhibition by FGF signaling becomes evident in the late blastocyst respectively. Images are adapted from Komatsu and Fujimori, 2015.

II. Histological observation of mouse embryos developing in the uterus

One of the characteristics of mammalian development is that embryos develop in the uterus after implantation. Direct interaction between embryos and the cells of the uterus is important. In studies on developmental biology of mammalian embryos, embryos are usually removed from the uterus, and isolated embryos are analyzed. We have been analyzing early embryonic development of mouse comparing the changes in the uterine epithelium. To observe the morphogenesis of embryos within the uterus, serial sections of implanted uteruses were made, stained with hematoxylin and eosin, and images of the embryos within the uteruses were captured to make high resolution three-dimensional re-constructions. Figure 2 shows an example of a section. We are preparing samples obtained from various developmental stages. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic

development will be examined using these images. To obtain three dimensional images of embryos within the uterus, we have been developing a system to automatically extract regions of the uterus where embryos localize by utilizing image analysis after images of serial sections are captured using a slide scanner.

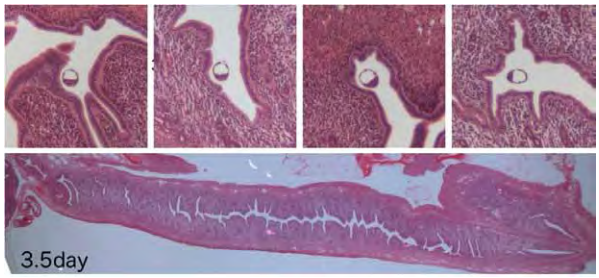

Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

III. Polarity formation in the mouse oviduct epithelium

The oviduct (Fallopian tube) is the organ connecting the periovarian space and the uterine horn. The ova released from the ovary are transported through the oviduct, where the ova are fertilized by spermatozoon entering from the uterus. We are focusing on the region of the oviduct close to the opening to the ovary.

The epithelium of the mouse oviduct consists of multi-ciliated cells and secretory cells. Ovulated oocytes are transported in oviducts by unidirectional motility of multi-cilia and the resultant secretory fluid flow from ovary to uterus. This cellular orientation in the oviduct is one example of planar cell polarity (PCP). PCP is seen in many animals and tissues, where cells sense global axes of the tissue to which they belong and orient themselves to fulfill specialized functions.

Many evolutionally conserved genes are required for the formation of PCP, and several of those encode proteins that are localized in polarized manners within cells. We found that Celsr1, a PCP core member, was strongly concentrated only at the specific domains of cell boundaries which are perpendicular to the ovary-uterus axis and that this polarized localization appeared to precede the directional movement of cilia.

In *Celsr1*-deficient mutant oviducts, cilia were generated and those within each cell appeared to beat as in the wild type oviduct. However, the beating direction was not coordinated along the ovary-uterus axis throughout the epithelia; some cells were oriented orthogonally to the axis, while some others exhibited reverse polarity. In addition to the ciliary polarity phenotype, cellular shape polarity was also impaired in the *Celsr1*-deficient mice. We found a new feature of cellular polarity in the wild type oviduct, e.g. that the apical surface of epithelial cells was elongated along the ovary-uterus axis. In *Celsr1*-deficient mice, epithelial cells showed less elongation and randomized orientation.

Furthermore, we also found that the three-dimensional architecture of oviductal epithelia was dramatically disrupted. In adult wild-type mice, the epithelial cell sheet is bent multiple times, which forms around twenty longitudinal folds parallel to the organ axis. However, in the mutant oviducts, epithelial folds no longer run parallel to the organ axis. The direction of the folds was randomized, and ectopic branches of the folds were observed. This suggests that Celsr1 is important for the formation of multi-scale polarities in the mouse oviduct ranging from the cellular to the tissue scale.

Currently, we have been trying to reveal the mechanisms of oviduct epithelial morphogenesis by integrating the molecular functions of PCP factors, cellular shape changes, tissue morphology, and involvement of mechanical forces. We are also focusing on some other PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain their polarity.

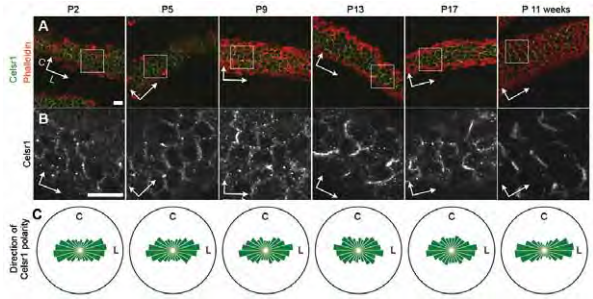

Figure 3. Celsr1 localization in mouse oviduct epithelium. (A-B) Confocal microscopy images of the oviduct epithelium stained for Celsr1 and F-actin (Phalloidin) at several developmental stages. The ovary side is to the left and the uterus side is to the right. Higher magnification of Celsr1 images are shown in (B). P: postnatal days. Arrows indicate the axes; L, longitudinal axis (the ovary-uterus axis); C, circumferential axis. (C) Distribution and orientation of the cellular polarity of Celsr1 localization quantified by image processing. Scale bars: 10 micron. Images are adapted from Shi et al., 2014.

IV. Analysis of mechanical properties of cells and tissues during embryonic development

Mechanics is one of the essential components of biological processes, including cell shape transformation and tissue morphogenesis etc. However, how mechanical states such as force and material stiffness regulate these processes is poorly understood. To approach this problem, measuring cellular and tissue geometric information and mechanical states is necessary. We developed image processing based techniques to measure cellular and tissue geometric information from fluorescent microscopic images and frameworks to theoretically estimate the mechanical states.

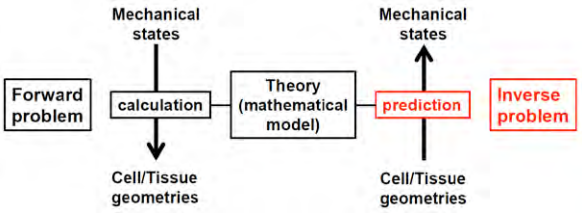

Figure 4. Theoretical estimation of cellular/tissue mechanical states. Schematic illustration of estimation.

By employing image processing techniques, we have extracted geometric information, shapes, and movements of early embryogenesis in *C. elegans* and mice. In the framework for estimating mechanical states, geometric informa-

tion was combined with a mechanical simulation, which was based on data assimilation (Figure 4). We successfully estimated the spatio-temporal dynamics of cellular and tissue mechanical states by systematically fitting the *in vivo* geometric states to the mechanical simulation. The mechanical information will be useful to investigate physical mechanisms of early embryonic development and morphogenesis during organogenesis in late stages in development.

Publication List:

〔**Original papers**〕

- Fujii, S., Nishikawa-Torikai, S., Futatsugi, Y., Toyooka, Y., Yamane, M., Ohtsuka, S., and Niwa, H. (2015). Nr0b1 is a negative regulator of Zscan4c in mouse embryonic stem cells. Sci. Rep. *5*, 9146.
- Horikawa, S., Ishii, Y., Hamashima, T., Yamamoto, S., Mori, H., Fujimori, T., Shen, J., Inoue, R., Nishizono, H., Itoh, H., Majima, M., Abraham, D., Miyawaki, T., and Sasahara, M. (2015). PDGFRα plays a crucial role in connective tissue remodeling. Sci. Rep. *5*, 17948.
- Komatsu, K., and Fujimori, T. (2015). Multiple phases in regulation of Nanog expression during pre-implantation development. Dev. Growth Differ. *57*, 648-656.
- Nakayama, S., Arima, K., Kawai, K., Mohri, K., Inui, C., Sugano, W., Koba, H., Tamada, K., Nakata, Y.J., Kishimoto, K., Arai-Shindo, M., Kojima, C., Matsumoto, T., Fujimori, T., Agata, K., and Funayama, N. (2015). Dynamic transport and cementation of skeletal elements build up the pole-and-beam structured skeleton of sponges. Curr. Biol. *25*, 2549-2554.
- Reyes de Mochel, N.S., Luong, M., Chiang, M., Javier, A.L., Luu, E., Fujimori, T., MacGregor, G.R., Cinquin, O., and Cho, K.W. (2015). BMP signaling is required for cell cleavage in preimplantation-mouse embryos. Dev. Biol. *397*, 45-55.