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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 developing embryos 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. Live 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.

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. To monitor cell movement and the status of the trophectoderm (TE) specification pathway in living embryos, we established Cdx2-GFP reporter mice allowing us to visualize the expression of Caudal-type transcriptional factor (Cdx2), a key regulator of the initiation of TE differentiation. Cells localized in an outer position initiated the expression of Cdx2. Subsequently, cells that changed their position from an outer to an inner position down-regulated Cdx2 expression and contributed to the pluripotent inner cell mass (ICM). Our results indicate that cells expressing even high levels of Cdx2 can internalize, deactivate the TE-specification molecular pathway and be integrated into the pluripotent cell population. This suggests that cells expressing Cdx2 exhibit plasticity of specification to TE and ICM lineages through positional changes.

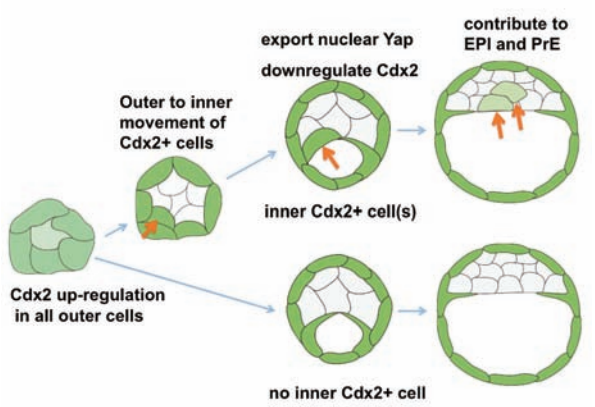

Figure 1. Cdx2 expressing cells in preimplantation mouse embryo exhibit plasticity of specification. Outer positioned cells up-regulate Cdx2 expression, but some internalized cells down-regulate its expression and contribute to both pluripotent epiblast (Epi) and extra-embryonic primitive endoderm (PrE).

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.

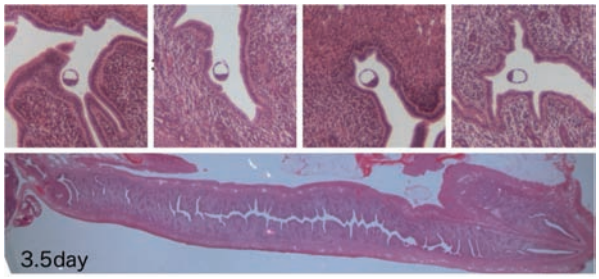

Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

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. To investigate the mechanisms of the epithelial fold pattern formation, we utilized mathematical modeling and simulations. By considering mechanical properties of the epithelial sheets we reproduced the longitudinally aligned folds and the branched folds which are observed in wild-type and the *Celsr1* mutant mice, respectively (Figure 3). Experimental measurements of mechanical tensions in the epithelial sheet were consistent with the tensions predicted from the simulations. Our experimental and mathematical analyses also successfully linked the epithelial tensions to cellular shapes. We are also focusing on some other PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain their polarity.

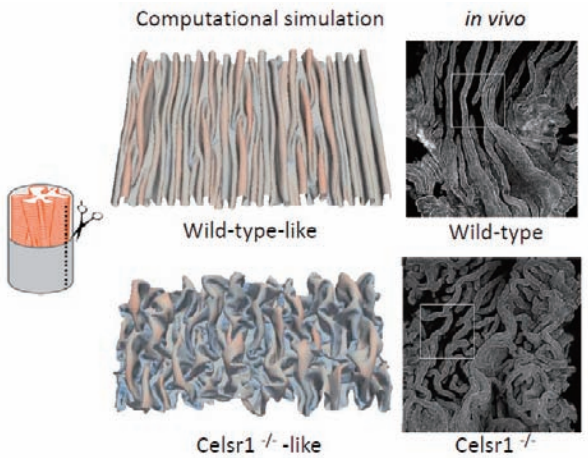

Figure 3. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

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. To understand how mechanical forces contribute to various patterns, measuring cellular and tissue mechanical states are necessary. We developed statistic techniques to infer the mechanical states by using fluorescent microscopic images during morphogenesis (Figure 4). By employing this method, we inferred mechanical forces in multi-cellular systems including MDCK cultured cells, and early embryogenesis in *C. elegans* and mice. Further computational simulations based on the inferred mechanical information reproduced morphological features of the multi-cellular systems. Thus, the mechanical information will be useful to investigate physical mechanisms of early embry-

onic development and morphogenesis during organogenesis in late stages of development.

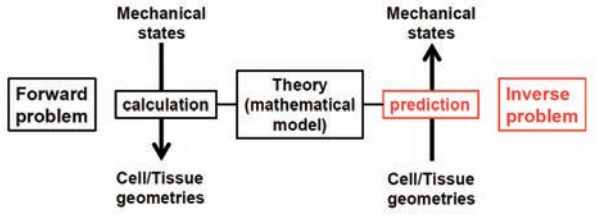

Figure 4. Statistical inference of cellular/tissue mechanical states.

Publication List:

〔Original papers〕

- Koyama, H., Shi, D., Suzuki, M., Ueno, N., Uemura, T., and Fujimori, T. (2016). Mechanical regulation of three-dimensional epithelial fold pattern formation in the mouse oviduct. Biophys. J. *111*, 650-665.
- Shi, D., Usami, F., Komatsu, K., Oka, S., Abe, T., Uemura, T., and Fujimori, T. (2016). Dynamics of planar cell polarity protein Vangl2 in the mouse oviduct epithelium. Mech. Dev. *141*, 78-89.
- Takemoto, T., Abe, T., Kiyonari, H., Nakao, K., Furuta, Y., Suzuki, H., Takada, S., Fujimori, T., and Kondoh, H. (2016). R26-WntVis reporter mice showing graded response to Wnt signal levels. Genes Cells *21*, 661-669.
- Toyooka, Y., Oka, S., and Fujimori, T. (2016). Early preimplantation cells expressing Cdx2 exhibit plasticity of specification to TE and ICM lineages through positional changes. Dev. Biol. *411*, 50-60.

〔Review article〕

- Shi, D., Arata, M., Usui, T., Fujimori, T., and Uemura, T. (2016). Seven-pass transmembrane cadherin CELSRs, Fat4 and Dchs1 cadherins: From planar cell polatiry to three-dimensional organ architecture. In The Cadherin Superfamily, S. Suzuki, and S. Hirano, eds., pp. 251-275.