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The aim of our research is to understand the events underlying early mammalian development. One of the characteristics of mammalian embryonic development is that embryogenesis proceeds in the oviducts and the uterus of the mother, and interaction between an embryo and maternal tissue is essential. Another characteristic is the highly regulative potential of embryos. 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. 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 and tissue morphology 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 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 found that cells expressing Cdx2 exhibit plasticity of specification to TE and ICM lineages through positional changes.

II. Histological observation of mouse embryos developing in the uterus

Mammalian 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, and images of the embryos within the uteruses were captured to make high resolution three-dimensional re-constructions. We are preparing samples obtained from various developmental stages. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic development have been examined using these images. We are identifying molecules involved in the interaction between embryo proper and uterine cells, which may play major roles in embryonic development.

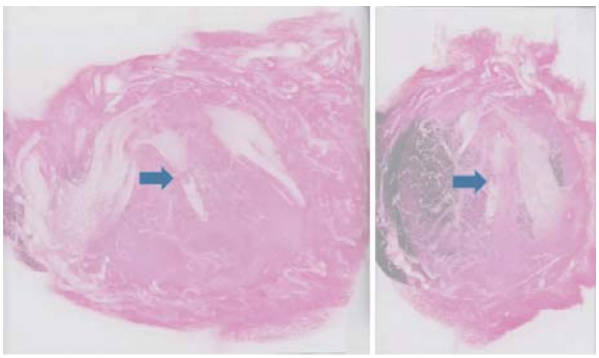

Figure 1. Three-dimensional image reconstruction of the pregnant uterus. Uterus of 5 days after fertilization were sectioned and images were digitized by image scanner, and reconstructed three-dimensionally to observe the relationship between embryo and the maternal tissue. Right panel represents the pseudo 3D image observed from the lateral side of the left image. Arrows indicate the embryo in the decidual tissue.

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. The epithelium of the mouse oviduct consists of multi-ciliated cells and secretory cells. Ovulated oocytes are transported in oviducts by unidirectional motility of multicilia 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 a variety of 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, 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 2). 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

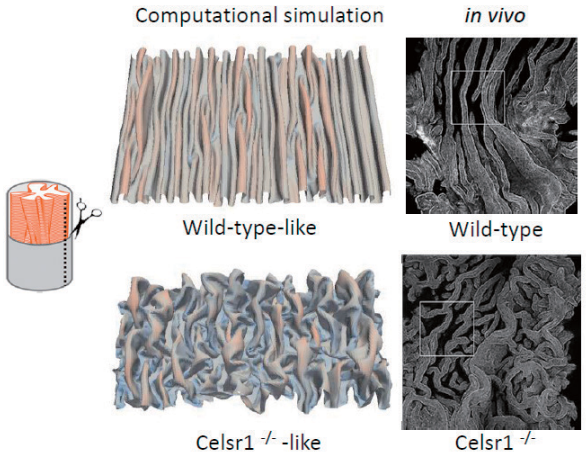

Figure 2. Epithelial fold patterns in oviduct and the reproduction of the patterns by computational simulations.

trying to understand how oviduct epithelial cells establish and maintain their polarity.

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 of morphogenesis, measuring cellular and tissue mechanical states is necessary. We developed statistic techniques to infer mechanical states using fluorescent microscopic images during morphogenesis (Figure 3). By employing this method, we inferred mechanical forces in multi-cellular systems including cultured epithelial cells, and early embryogenesis in *C. elegans* and mice. Further computational simulations based on the inferred mechanical information reproduced morphological features of the multi-

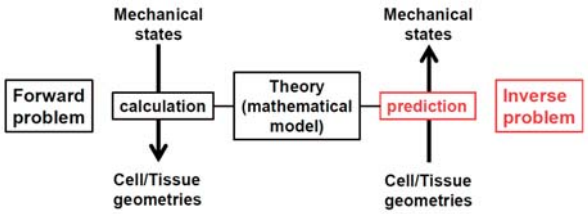

Figure 3. Theoretical inference of cellular/tissue mechanical states. Schematic illustration of inference.

cellular systems. Thus, the mechanical information will be useful to investigate physical mechanisms of early embryonic development and morphogenesis during organogenesis in late stages of development.

V. Mammalian tissue morphogenesis requiring mechanosensor channel Piezos

Several examples have shown that mechanical stimuli can work as key components for tissue or organ development. However, our knowledge about involvement of mechanotransduction in biological phenomena or their precise mechanisms is still limited. It is partially because key mechanosensors are not yet identified in many cell types. Piezos are recently identified mechanically activated cation channels functioning in mammalian cells (Figure 4). They are activated when mechanical forces are applied on the cell membrane. Series of data show that Piezo2 serves as the main mechanosensor in sensory neurons for light touch sensation, proprioception and breathing. We found that Piezos are also required for proper vascular morphogenesis. To further elucidate how Piezo-mediated mechanotransduction is involved in vascular development, we have been developing systems to manipulate mechanical stimuli and monitor

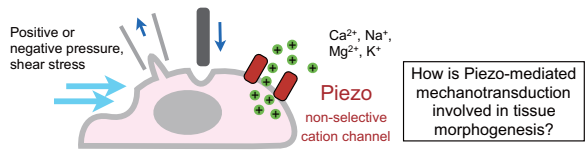

Figure 4. Schematic illustration of Piezo mechanically activated non-selective cation channel and the key question of this study.

Piezo activity in vitro and in vivo. Analyses utilizing these systems and mouse lines deficient in Piezos will clarify the relationship among mechanical forces, Piezo activation, cellular responses, and tissue morphogenesis, this may provide a basis to understand the roles of mechanotransduction in tissue morphogenesis.

VI. Mechanics of cell population patterning during development

During development, cells actively and/or passively move, resulting in various patterns of cell distribution. We investigated the effect of both active and passive cell movements. First, we mathematically modeled the process of neural tube closure in *Xenopus laevis*, and found that active cell contractility and its frequency are critical parameters for the tissue shrinkage. Second, we theoretically investigated the effect of passive cell movements provoked by frictional forces from surrounding tissues. The passive movements generated various patterns, such as an elongated cell cluster, multiple cell clusters, etc. The former situation is actually observed during elongation of the notochord in mice. This theoretical framework would be widely applicable to developmental processes.

Publication List:

〔Original papers〕

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- Nonomura, K., Woo, S.H., Chang, R.B., Gillich, A., Qiu, Z., Francisco, A.G., Ranade, S.S., Liberles, S.D., and Patapoutian, A. (2017). Piezo2 senses airway stretch and mediates lung inflation-induced apnoea. Nature *541*, 176-181.
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- Suzuki, M., Sato, M., Koyama, H., Hara, Y., Hayashi, K., Yasue, N., Imamura, H., Fujimori, T., Nagai, T., Campbell, RE., and Ueno, N. (2017). Distinct intracellular Ca^{2+} dynamics regulate apical constriction and differentially contribute to neural tube closure. Development *144*, 1307-1316.