DIVISION OF EMBRYOLOGY

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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 in other animals. This is mainly due to difficulties in approaching the developing embryo in 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 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. It is useful to visualize nuclei, cell shapes, cytoskeleton or other organelles to observe cells and cell behaviors in living mouse embryos. We have established a series of transgenic mouse lines for live imaging, which is a part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CDB. In each mouse, cDNA encoding fusion protein with fluorescent protein and a localization sequence were inserted into Rosa26 locus. The sequence for the fusion protein was following stop sequences that are surrounded by loxP sites on both sides. These loxP sites can be recognized by an enzyme, Cre recombinase, that catalyzes recombination between two loxP sites to remove the stop sequences. Thus, when Cre recombinase is activated in a spatial-temporally specific manner, the following reporter fluorescent proteins are expressed in a specific way. And once this irreversible reaction is induced in the germ line, the derived offspring possess the transgene without the stop sequence and express the reporter fusion protein ubiquitously.

We are also 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 enhancer/promoter region of important gene encoding factors regulating cell differentiation in these stages. Combining these two types of reporter transgenic mouse lines, we have started analysis of behaviors of cells comparing gene expression properties at the single cell level. This year, we have reported establishment of mouse lines to monitor the cell cycle.

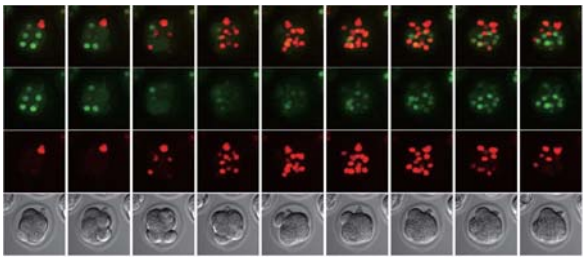

Figure 1. Examples of a morula expressing fucci2, cell cycle markers. The G(1) and S/G(2)/M phases are distinguished by mCherry(red) and mVenus (green) signals respectively.

For the live imaging of early mammalian embryos, a combined microscope and incubation system is an important tool. Conventional CO2 incubators provide better conditions compared with 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 are also trying to observe and reveal aspects of cell shape, morphogenesis, cell lineage, gene expression and cell differentiation by combining these techniques.

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 mice 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 the images of the embryos within the uteruses are 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.

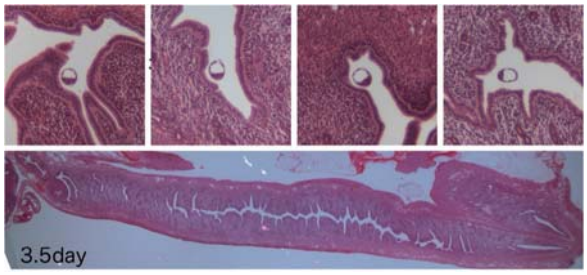

Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

III. Studying early development of rabbits as a new model of mammalian embryogenesis

Mice have been the main experimental animal used for the study of mammalian developmental biology because genetics and genetic engineering including transgenesis and targeted mutagenesis are applicable for mice. We also have been mainly studying mouse embryogenesis. However, rodent embryos do not necessarily provide ideas common in mammalian embryogenesis because the styles of early embryonic development differ between species, and rodent embryos do not provide a typical style when compared with other animals. We searched for animals that are suitable for the study of early embryogenesis comparing possible candidates, and decided to use rabbits. We are studying morphological changes and gene expression during early stages of development until the peri-implantation stage. We are focusing especially on the formation of germ layers and body axes, and compare with corresponding stages in mice.

IV. Formation, maintenance of cell polarity and tissue morphogenesis in the mouse oviduct

The oviducts (fallopian tubes) are tubes connecting the periovarian spaces and the uterine horns. The ova released from the ovary are transported through the oviduct, where fertilization occurs with the spermatozoon moving from the uterus. We are focusing on the region of the oviduct close to the opening to the ovary. In this region many multi-ciliated cells exist. These cilia move in one direction along the ovary-uterus axis. This directional movement of multi-cilia might play a major role in the transportation of ovum from periovarian space, although muscle contractions also play roles in the region close to the uterus. This suggested that the ovum was transported by the directionally beating cilia. This directional beating of cilia was based on the polarity of the microtubule assembly in the cilia. Skeletal microtubules in the cilia are arranged in a “9 +2”array, and the central two bundles are aligned facing the same direction in each cell. This suggests that oviduct epithelial cells possess polarity along the cell surface parallel to the longitudinal axis of the oviduct; this type of cellular polarity is called “Planer Cell Polarity (PCP)”. We have been studying how PCP is established during development, and how this polarity is maintained over a long period in later stages.

We have been focusing on several genes involved in PCP formation. The molecular basis of PCP formation has been genetically studied, particularly using fruit fly, and several genes have been shown to be involved in the regulation of PCP formation. Homologues of these genes are also known in mammalian species, and some of them have been shown to play similar roles. Disruption of functions of some of these genes resulted in abnormal PCP formation in skin, inner ear, and early embryos of mice. We are also focusing on some of the PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain polarity. We have been trying to reveal mechanisms including the molecular functions, cellar shape, tissue morphology and involvement of mechanical forces in this system.

V. Analysis of mechanical properties of cells during embryonic development

Mechanics is one of the essential components for biological processes including cell shape transformation, and tissue morphogenesis etc. However, how mechanical properties such as force and material stiffness regulate these processes is poorly understood. To approach this problem, measuring cellular geometric information and mechanical properties are necessary. We developed an image processing based technique to measure cellular geometric information from fluorescent microscopic images and a framework to theoretically estimate the mechanical properties. The image processing technique enabled us to robustly detect cell contour from images with high noise and non-uniform illumination, although the algorithm of the technique is quite simple. By employing the image processing technique, we successfully extracted geometric information of early embryonic cells during cytokinesis in *C. elegans* and of mouse cells in cell sheets. In the framework for estimating mechanical properties, geometric information was combined with a mechanical simulation, which was technically based on the data assimilation (Figure 3). We spatio-temporally

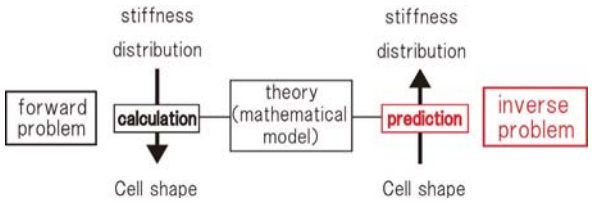

Figure 3. Theoretical estimation of cell surface stiffness. Schematic illustration of estimation.

estimated cell surface stiffness during cytokinesis by systematically fitting the *in vivo* cell shape to the mechanical simulation. We found that cell polar and equatorial regions were stiffer and softer, respectively. Further theoretical modeling showed that the relative difference of stiffness between the two regions could be a primary determinant for cleavage furrow ingression during cytokinesis. We speculated that the relative contributions of cell surface stiffness and the contractile ring could explain the contractile ring dependent and independent cytokinesis.

Publication List

〔Original papers〕

- Abe, T., Sakaue-Sawano, A., Kiyonari, H., Shioi, G., Inoue, K., Horiuchi, T., Nakao, K., Miyawaki, A., Aizawa, S., and Fujimori, T. (2013). Visualization of cell cycle in mouse embryos with Fucci2 reporter directed by Rosa26 promoter. Development *140*, 237-246.
- Okamoto, M., Namba, T., Shinoda, T., Kondo, T., Watanabe, T., Inoue, Y., Takeuchi, K., Enomoto, Y., Ota, K., Oda, K., *et al*. (2013). TAG-1-assisted progenitor elongation streamlines nuclear migration to optimize subapical crowding. Nature Neurosci. *16*, 1556-1566.
- Xu, G., Shen, J., Ishii, Y., Fukuchi, M., Dang, T.C., Zheng, Y., Hamashima, T., Fujimori, T., Tsuda, M., Funa, K., *et al*. (2013). Functional analysis of platelet-derived growth factor receptor-beta in neural stem/progenitor cells. Neuroscience *238*, 195-208.

〔Review articles〕

- Abe, T., Aizawa, S., and Fujimori, T. (2013). Live imaging of early mouse embryos using fluorescently labeled transgenic mice. Methods Mol. Biol. *1052*, 101-108.
- Abe, T., and Fujimori, T. (2013). Reporter mouse lines for fluorescence imaging. Develop. Growth Differ. *55*, 390-405.