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, 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 mouse lines for live imaging

Currently, it is common to observe the behavior of cells and gene expression in living embryos to understand the mechanisms underlying embryonic development and morphogenesis. 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 mammalian species. We have previously established a transgenic mouse line ubiquitously expressing EGFP (Enhanced Green Fluorescent Protein) fused with histone H2B by homologous recombination in the Rosa26 locus, and embryos obtained from this mouse line have been used for timelapse live imaging to analyze cell behavior. It would be useful to visualize nuclei with other colors of fluorescent proteins and visualize cell shapes, cytoskeleton or other organelles in a similar way to observe cells and cell behaviors in living embryos. Prof. Fujimori, as a visiting scientist, has been participating in a project producing a series of mouse reporter lines to express fluorescent markers, which is taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CDB. This year we have established 17 lines of these mice. In each mouse, cDNA encoding fusion protein with fluorescent protein and a localization sequence was 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 called 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

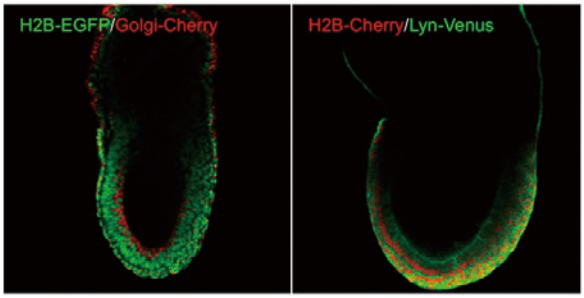

Figure 1. Examples of 7.5 day embryos obtained after crossing of two reporter mouse lines. Nuclei and golgi apparatus and cell membrane and nuclei are visualized with green and red fluorescent proteins genetically introduced as transgenes.

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. These mice will provide useful materials for live imaging of cells in early embryos as well as cells in the tissues of developing and adult animals. In addition to the establishment of mouse lines, we have been trying to improve optical systems for live imaging, and we could improve systems to culture in better conditions during live imaging. The culture conditions of peri-implantation mouse embryos have also improved. We are planning to observe and reveal cell shape, morphogenesis, cell lineage, gene expression and cell differentiation by combining these techniques.

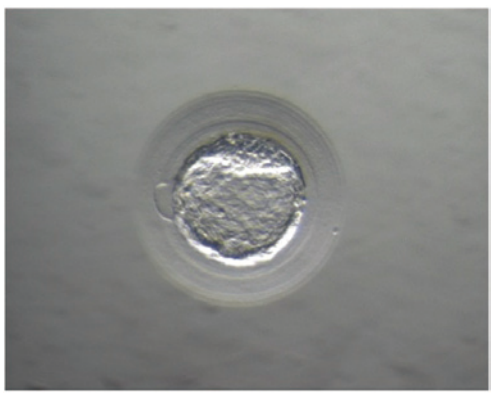

Figure 2. A 4.5day rabbit embryo. The shape is very similar to other mammalian blastocysts at this stage. However, development from slightly after this stage is different from corresponding stages of mouse embryo development.

II. Studying early development of rabbit 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 started examination of morphological changes and gene expression during early stages of development until the peri-implantation stage. We will focus especially on the formation of body axes, and compare with corresponding stages in mice.

III. Formation, maintenance of ell 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, wherefertilization 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. The cilia on the surface of ovarian epithelial cells were observed with a high-speed CCD camera and these movements were recorded. These cilia moved 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. The importance of the directional ciliary beating for the transport of ova is supported by the experiments shown in the figure 3. An ovum was put at the opening end of a longitudinally opened oviduct, and this ovum was moved along the fold running parallel to the ovary-uterus axis of the oviduct without muscle contraction. 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 “Planner Cell Polarity (PCP)”. We have been studying how PCP is established during development, and how this polarity is maintained for a long period in later stages. Ciliary movement is one good indicator of PCP, therefore we established a system to analyze ciliary beats with automated image processing, and found ciliary beat frequency was slightly different between estrus cycles.

We are now focusing on several genes involved in PCP formation. 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 known also in mammalian species, and some of them have shown to be playing 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 would like to reveal mechanisms including the molecular functions, cellar shape, tissue morphology and involvement of mechanical forces in this system.

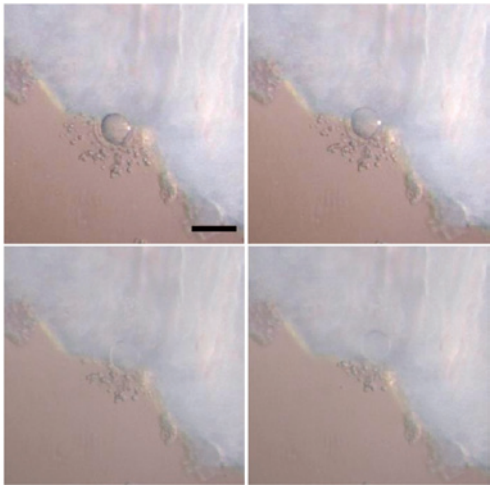

Figure 3. Transport of an ovum via the movements of cilia on the oviduct epithelial cells. Timelaplse images of movement of an ova on the longitudinally opened preparation of the mouse oviduct.

Publication List

〔Original papers〕

- Abe, T., Kiyonari, H., Shioi, G., Inoue, K., Nakao, K., Aizawa, S., and Fujimori, T. (2011). Establishment of conditional reporter mouse lines at ROSA26 locus for live cell imaging. Genesis *49*, 579-590.
- Nakagawa, T., Izumino, K., Ishii, Y., Oya, T., Hamashima, T., Jie, S., Tomoda, F., Fujimori, T., Nabeshima, Y., Inoue, H., and Sasahara, M. (2011). Roles of PDGF receptor-beta in the structure and function of postnatal kidney glomerulus. Nephrol Dial. Transplant. *26*, 458-468.
- Shi, D., Komatsu, K., Uemura, T., and Fujimori, T. (2011). Analysis of ciliary beat frequency and ovum transport ability in the mouse oviduct. Genes to Cells *16*, 282-290.
- Shioi, G., Kiyonari, H., Abe, T., Nakao, K., Fujimori, T., Jang, C., Huang, C., Akiyama, H., Behringer, R.R., and Aizawa, S. (2011). A mouse reporter line to conditionally mark nuclei and cell membranes for in vivo live-imaging. Genesis *49*, 570-578.