DIVISION OF EMBRYOLOGY

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The aim of our research is to understand the events uThe 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 to 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.

I. Transcriptional regulations of key genes during pre-implantation development.

During the first 4 days after fertilization, the mouse embryo proceeds in its development in the oviduct and uterus, and reaches the blastocyst stage. In the early blastocyst stage, two cell types, namely cells of the inner cell mass (ICM) and cells of the trophectoderm (TE) can be distinguished. TE cells form extra-embryonic tissues including the future placenta. This is the beginning of cell differentiation during mouse development. In the late blastocyst stage, ICM cells form two layers, i.e., the epiblast and the primitive endoderm. Primitive endoderm cells do not contribute to cells of the embryo proper during future development, while the epiblast forms all the parts of the embryo. During these stages, several key factors are known to play important roles in cell differentiation. A few of them are already shown to change their expression levels according to the position of cells within an embryo.

We are currently focusing on Nanog, a protein with homeodomain. Nanog is known as one of the major players in establishing and maintaining pluripotency of ES/iPS cells, and development of epiblast cells in pre-implantation embryos. Expression of Nanog starts at the 4-cell stage and increases later during pre-implantation development. Its expression pattern varies between embryos during blastocyst formation until it is localized to the ICM. At the late blastocyst stage, when the epiblast and primitive endoderm cells are specified in the ICM, Nanog is localized to the epiblast. Although it was suggested that Nanog expression in early stages is stochastic, based on immuno-staining studies of fixed embryos, it remains unclear how Nanog gene expression is regulated and localized to the epiblast during pre-implantation development. To understand actual spatiotemporal changes in Nanog gene expression in each cell, continuous observation of three-dimensional images is necessary. For this purpose, expression of Nanog is visualized by using transgenic mice expressing EGFP under the control of Nanog promoter, which was provided by Dr. Shinya Yamanaka at Kyoto Univ. Embryos of this transgenic mouse line were collected and cultured in vitro. Developmental processes from 2-cell to blastocyst stage were recorded using a laser scan microscope equipped with a CO2 incubator. EGFP signal intensity in each cell was measured correlating with the position of each cell and future cell fate. Transcriptional regulation of Nanog gene expression was separated into three phases. It is initiated at the 4~8-cell stage, and the descendants of cells expressing Nanog higher than others tend to express continuously higher. And in the second phase, it is rather randomly up-regulated during early stages (8~16-cell stage) whereas it may correlate with differentiation status of cells in later stages (16~32-cell stage). Molecular mechanisms underlying these transcriptional regulations are under examination. We are also planning to establish other mouse lines to visualize other key factors by similar approaches.

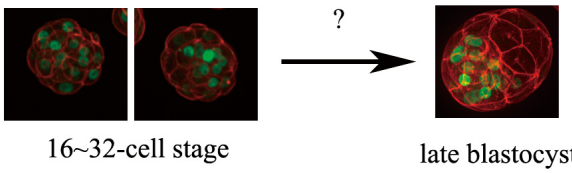

Figure 1. Localization of Nanog protein in pre-implantation embryos. Because protein localization varies between embryos, continuous observation is essential to understand actual behavior of this molecule .

II. Morphological observation of developing mouse embryos 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 decided to analyze early embryonic development of mice comparing the changes in the uterine epithelium. To observe the morphogenesis of embryos within the uterus, a series of sections of implanted uteruses were made, and the morphology of the embryos within the uteruses was observed after hematoxylin-eosin staining. Figure 2 shows an example of a section. At this stage, embryos in a uterus align in the same orientation, and the spaces between neighboring

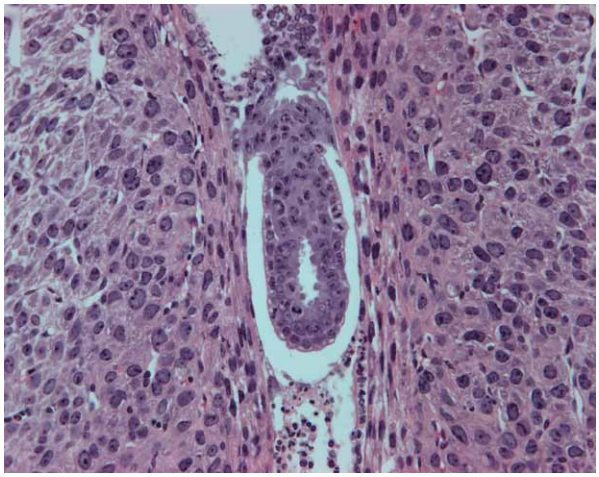

Figure 2. A section of a 5-day mouse embryo in the uterus. Embryos locate to a small gap of uterien epithelium, and align along one axis of the uterus.

embryos are similar. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic development will be examined using these images.

III. Planner cell polarity in the mouse oviduct.

Planar Cell Polarity (PCP) is the asymmetric organization within epithelial cells along the plane of the epithelium in tissues. We are focusing on the epithelial cells of the mouse oviduct. The multiple cilia on the apical cell surface beat back and forth along the axis from the ovary to the uterus, and this directionally controlled beating is believed to transport eggs to the uterus. Although there are many studies in human oviducts, there are few reports on mouse oviductal ciliary movements where we can discern underlying genetic programs. To study ciliary movements in the mouse oviduct, we recorded ciliary beatings with a high speed CCD camera. We calculated the ciliary beat frequency (CBF) by automated image analysis and found that the average CBF was 10.9±3.3 Hz and 8.5±2.5 Hz (± standard deviation) during the diestrus and estrus stages, respectively. Mapping of the CBF to multiple locations in the epithelium showed that the cilia beat regularly at a local level, but have a range of frequencies within the entire plane. We also observed ova with cumulus cells were transported to the uterus side by the opened oviduct at the diestrus and estrus stages. These results suggest that the ciliated cells of the infundibulum can generate unidirectional flows and are able to deliver ova by their ciliary activities despite their discordance in beating periodicity. We also applied this method to analyze the ciliary movements of the ependymal cells.

These ciliated cells may have clear PCP from ovary to uterus. Our aim is to reveal the molecular and cellular mechanisms of regulating PCP in the mouse oviduct. We are preparing experiments to uncover molecular mechanisms underlying PCP formation in the oviducts, mainly by focusing on the involvement of PCP core group genes and their products.

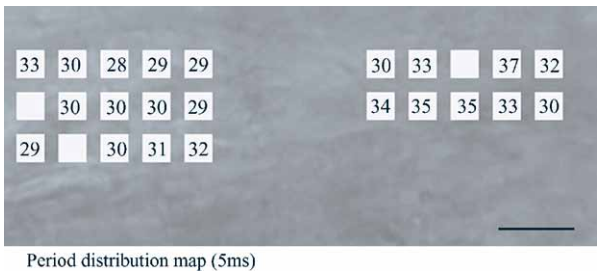

Figure 3. Cilia beat regularly at a local level with a range of frequency in the entire plane. CBF of 25 ROIs that were visualized on the oviductal epithelium (white squares). The number in each white square indicates the n translation when the autocorrelation value was at the first peak, and is equal to the period of beatings (1/200 second). White squares with no number indicate that the CBF measurement at those ROIs was invalid because the autocorrelation value at the first peak was very low. Bar = 10 μm.

Publication List

〔Original papers〕

- Tissir, F., Qu, Y., Montcouquiol, M., Zhou, L., Komatsu, K., Shi, D., Fujimori, T., Labeau, J., Tyteca, D., Courtoy, P., Poumay, Y., Uemura, T., and Goffinet, A.M. (2010). Lack of cadherins Celsr2 and Celsr3 impairs ependymal cilliogenesis, leading to fatal hydrocephalus. Nature Neurosci. *13*, 700-707.
- Zheng, L., Ishii, Y., Tokunaga, A., Hamashima, T., Shen, J., Zhao, Q.L., Ishizawa, S., Fujimori, T., Nabeshima, Y., Mori, H., Kondo, T., and Sasahara, M. (2010). Neuroprotective effects of PDGF against oxidative stress and the signaling pathway involved. J. Neurosci. Res. *88*, 1273-1284.

〔Review Articles〕

- Fujimori, T. (2010). Preimplantation development of mouse: A view from cellular behavior. Develop. Growth Differ. *52*, 252-262.
- Niwa, H., and Fujimori, T. (2010). Stem cell systems in development of mammals. Develop. Growth Differ. *52*, 251.