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.

A complete understanding of early events during embryogenesis in mammals, as compared to other animals, has been relatively delayed. This is mainly due to difficulties in the approaches to the developing embryos 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. Cell differentiation in pre-implantation development

During the 4 days after fertilization, the mouse embryo proceeds in its development within the zona-pellucida, an extra-embryonic membrane surrounding the embryo proper, and reaches the blastocyst stage. In the mouse blastocyst, two types of cells, namely cells of the inner cell mass (ICM) and cells of the trophectoderm (TE) can be distinguished. This is the initial cell differentiation during mouse development. Oct4 (Oct3/4) and Cdx2 are differentially expressed in the ICM and TE respectively, and it is suggested that Oct4 functions as a factor to maintain pluripotency of ICM cells. The onset of the specification of these two cell types has not been defined. The initial stage when Oct4 and Cdx2 are differentially expressed is known to be early in the morula stage. The cells located at the outside express Cdx2, suggesting that specification has occurred in these outside cells. It is hypothesized that the cells located inside the morula mainly contribute to the ICM and cells facing the embryonic surface will differentiate into TE cells. However, this inside-outside hypothesis is based on the analysis of separated blastomeres, and continuous information from whole embryos was very limited. We therefore analyzed division orders of cells and expression profiles of differentiation markers relating to the position of cells within embryos to reveal the different characteristics of each blastomere in the embryo.

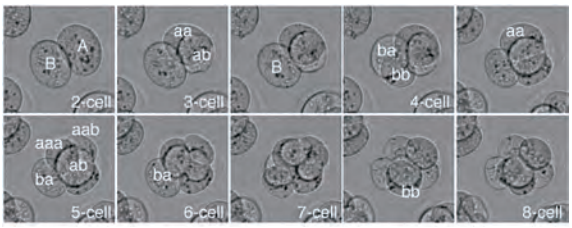

Figure 1. Analysis of division patterns until the 8-cell stage. Blastomere A divided earlier than blastomere B to give rise to the 3-cell stage. The four blastomeres aa, ab, ba, bb divided to reach 5, 6, 7, and 8-cell stages, respectively.

The dividing patterns of blastomeres until the late 8-cell stage were analyzed using bright field time-lapse recording images. The time point of each blastomere division was recorded, and the division patterns were analyzed. All six possible types of division pattern were observed. Of all cases, the type where all the daughter cells of the early dividing cell precede in the next pattern was most common. When the period of the 3-cell stage was examined, there was correlation with the appearance of different patterns and the length of the stage, which showed a wide variety lasting up to 220min. The most common pattern was seen in embryos with a longer 3-cell stage period, and variant patterns occurred more often during short 3-cell stage periods. This suggests that the division order might be altered when the divisions of blastomeres occurs synchronously. We did not observe any relationship between the division order and future cell fates.

We analyzed the positioning of cells within each embryo using optical section images of phalloidin and Hoechst staining obtained by confocal microscopy. We defined an outer cell as a cell facing the embryonic surface of the

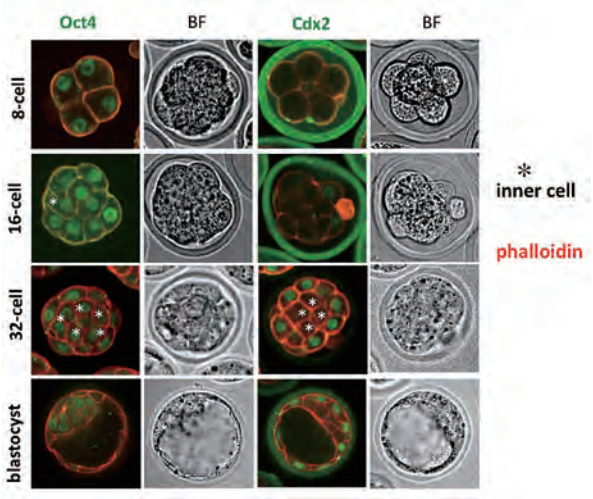

Figure 2. Localization of Oct 4 and Cdx2 protein during preimplantation mouse development. Cell shape was visualized by phalloidin staining.

embryo and an inner cell as a cell not facing the surface of the embryo but surrounded by other cells. At the 8-cell stage, even after compaction, almost all the cells were outer cells. At the 16-cell stage, inner cells were observed. The average number of inner cells was 1.7. All the embryos contained inner cells at the 32-cell stage. Thus, the geographical differences between the inner and outer cells can be initiated later than the 8-cell stage. The Oct4 proteins staining signals were observed in all embryos both in inner and outer cells until the 32-cell stage. The Cdx2 staining signals were different from that of Oct4. Cdx2 was initially observed in a few cells of the 8-cell stage, and the numbers of Cdx2-positive cells increased as development progressed. Cdx2 signals were observed in most of the outer cells at the 32-cell stage. Thus Cdx2 and Oct4 were co-expressed in most outer cells at the 32-cell stage. These results suggest that the differentiation of the two cell types might correlate with the position of the cells within the embryo.

We have started to prepare transgenic mice where fluorescent protein expressions are driven by the gene regulatory elements of genes involved in cell differentiation. These will be used for live imaging of gene expression during cell differentiation.

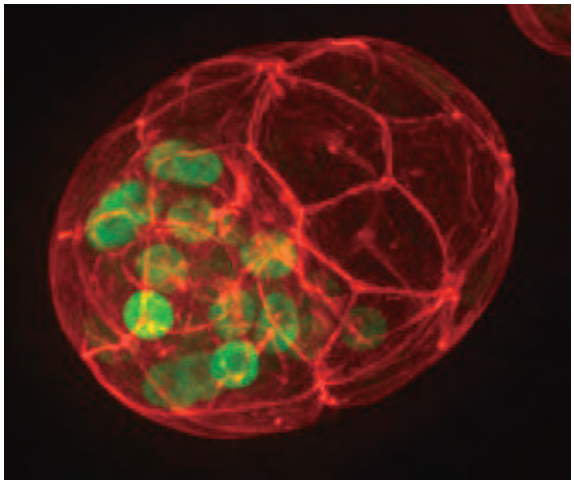

Figure 3. Localization of Nanog protein at mid blastocyst stage. This protein is one of the candidates applied for live cell imaging.

II. Planar cell polarity in mouse oviduct

Planar Cell Polarity (PCP) is the asymmetric organization within the 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 cell surface beat back and forth along the axis from the ovary to the uterus, and this beating must be directionally controlled to transport eggs to the uterus. Thus, 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 observed the movement of oviduct epithelial cilia by high-speed CCD camera, and confirmed that the cilia generally beat along the axis from ovary to uterus. To understand how these movements of cilia can make a flow of fluid to transport follicles from the ovary to the uterus, we

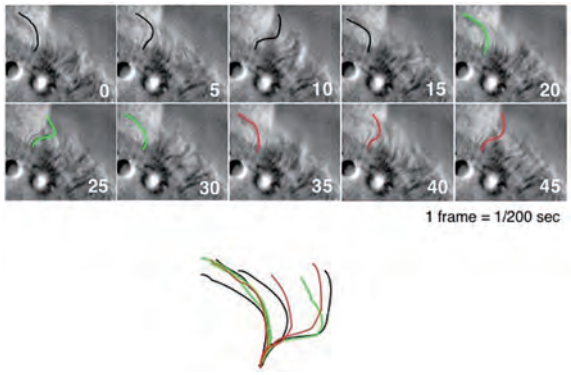

Figure 4. Observation of movements of the multiple cilia of the oviduct epithelial cells. The numbers shown in bottom indicate frame numbers of the movie. The shape of a cilium is traced on the snap shot images taken by high speed CCD camera. The movements of the cilium are represented on the bottom.

carefully analyzed the movements of cilium. There was a wide variety of frequency in the beating of cilia. These cilia did not necessarily makea coordinated synchronous beating. The effective stroke (forward movement along the ovary-uterus axis) of cilia is 2-3 folds faster than the recovery stroke (backward movement). This differential speed of each stroke might provide a force to generate the functional flow. We are studying how these ciliated cells generate coordinated and functional flow in the oviduct. To analyze the beating in more detail, we are planning to develop a tool to automatically analyze cilia beat frequency at multiple points simultaneously. We are also preparing experiments to uncover molecular mechanisms underlying PCP formation in the oviducts, mainly by focusing on the involvement of classical PCP genes and their products.

Publication List

〔Original papers〕

- Fujimori, T., Kurotaki, Y., Komatsu, K., and Nabeshima, Y. (2009). Morphological organization of the mouse preimplantation embryo. Reprod Sci. *16*, 171-177.
- Yuri, S., Fujimura, S., Nimura, K., Takeda, N., Toyooka, Y., Fujimura, Y., Aburatani, H., Ura, K., Koseki, H., Niwa, H., Nishinakamura, R. (2009). Sall4 is essential for stabilization, but not for pluripotency, of embryonic stem cells by repressing aberrant trophectoderm gene expression. Stem Cells *27*, 796-805.