LABORATORY FOR SPATIOTEMPORAL REGULATIONS

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Our laboratory is currently pursuing two paths of scientific inquiry of interest to developmental biology. One goal is to clarify the mechanism of left-right (L-R) determination. The other is to develop imaging technologies for developmental biology.

I. Initial step for left-right asymmetry

The first L-R asymmetry in mammalian development arises on the embryonic surface. A gastrulating mouse embryo has a shallow hollow on its ventral surface, called 'the node,' with hundreds of cilia moving in a clockwise rotational manner (Figure 1; Nonaka et al., 1998). The sum of the vortical motions of the cilia, however, generates a leftward flow of the surrounding fluid rather than a vortex. The cilia can generate L-R asymmetry *de novo*, i.e. without pre-existing left-right asymmetry, by their posteriorly tilted rotation axis (Nonaka et al., 2005).

The leftward flow, called nodal flow, determines subsequent L-R development. This principle has been confirmed by our experiments, which demonstrated that embryos raised with an artificial rightward flow develop reversed L-R asymmetry (Nonaka et al., 2002).

While it is clear that nodal flow conveys asymmetric information along the L-R axis, the nature of the information remains unclear. We are now working to clarify how the direction of nodal flow is converted to the subsequent step, asymmetric gene expression.

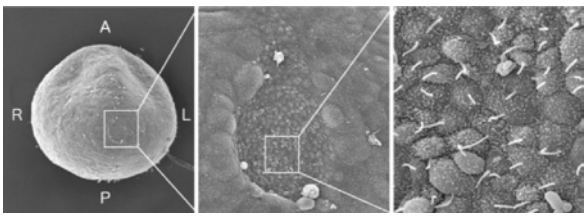

Figure 1. Left: ventral view of a 7.5-day mouse embryo. Middle: the node. Right: node cilia.

II. Imaging technologies

Long-term live imaging of large specimens, such as embryos, is very useful in developmental biology but technically challenging, mainly because of phototoxicity and the limitations of deep imaging. Light-sheet microscopy

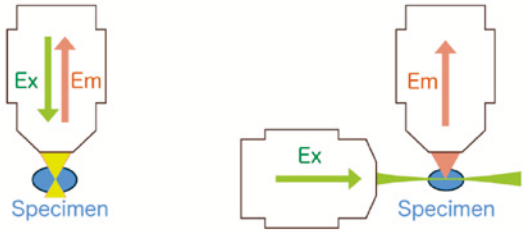

Figure 2. Principle of light-sheet microscopy. Left: light path of conventional fluorescent microscopes using single objective lens for both illumination (Ex) and detection (Em). Right: light-sheet microscopes including DSLM, where illumination light is limited to the focal plane of the detection objective.

including Digital Scanned Light-sheet Microscope (DSLM, Figure 2) is extremely suitable for this purpose, and we have applied it to analyze cell movements in intact mouse embryos at gastrulating stages (Figure 3). We have been also developing another light-sheet microscope for wider application of living samples: our embed-free and fast image acquisition system enables 4D data acquisition of freely moving *Amoeba proteus*.

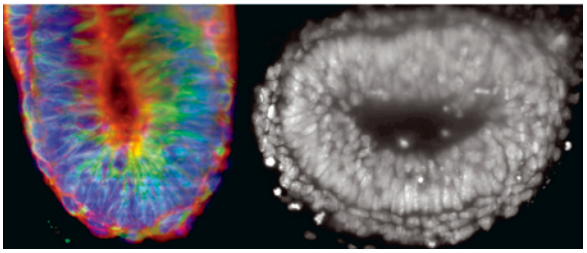

Figure 3. 6.5-day mouse embryos visualized by DSLM. Left: an optical transverse section of a fixed embryo. Right: cross section of a living one expressing GFP in the neurons.

In addition to our own research projects, we support researchers who are interested in using our DSLM and two-photon microscopes. Several collaborations including live imaging of developing brains, hair bulbs, salivary glands and renal tubules are in progress.

Publication List

〔Original paper〕

● Kishimoto., N., Alfaro-Cervello, C., Shimizu, K., Asakawa, K., Urasaki, A., Nonaka, S., Kawakami, K., Garcia-Verdugo, JM., and Sawamoto, K. (2011). Migration of neuronal precursors from the telencephalic ventricular zone into the olfactory bulb in adult zebrafish. Journal of Comparative Neurology *519*, 3549-3565.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).