LABORATORY FOR SPATIOTEMPORAL REGULATIONS

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Our laboratory is currently pursuing two paths of scientific inquiry that are 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 biological analyses.

I. Initial step for left-right asymmetry

In mammalian development, the initial left-right asymmetry first appears as cilia-driven leftward fluid flow on the ventral surface of the node, and the flow provides the initial cue for asymmetric gene expressions that actually determine asymmetric morphogenesis. The mechanisms that convert the flow to asymmetric gene expression, i.e. the flow sensing mechanism, remains controversial, with several models being proposed, and involvement of Ca^{2+} being suggested.

We pursued this question by measuring Ca^{2+} dynamics in the node and found that the node cells cause apparently stochastic elevation of Ca^{2+}, and its spatiotemporal distribution is equal at the left and right sides but becomes more frequent at the left after late headfold stage, when the flow-induced commitment occurs. This asymmetry was disrupted in dynein mutant *iv/iv* and *pkd2*$^{-/-}$ mutants, in accordance to their left-right phenotypes.

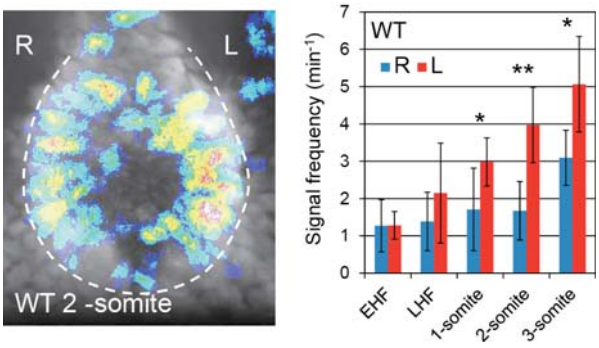

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

Based on these findings, we are now examining the validity of the two major proposed models of flow sensing, mechanosensing and chemosensing, and a third novel mechanism.

II. Development of light-sheet microscopy

Light-sheet microscopy has become popular over this decade for its advantages including low photodamage and fast image acquisition. We are now running two light-sheet microscopes, one commercial and the other self-made, maintaining them for both collaborations and for our own research interest, left-right asymmetry.

Over several years we have developed a fast light-sheet microscope named ezDSLM, in which the excitation light sheet and detection objective move instead of the specimen for z-scanning. We installed an electrically tunable lens (ETL) to achieve further speed and exclusion of mechanical vibrations that disturb continuous 4D imaging of floating or loosely held specimens.

Our light-sheet microscopes are open to other researchers via NIBB's Collaborative Research and MEXT's Advanced Bioimaging Support (ABiS) programs. Numerous collaborations are in progress including observation of moving *Amoeba proteus*, neuronal activity in *Drosophila* larvae, cell migration in zebrafish embryos, cleared mouse brains, and marine crustaceans, etc.

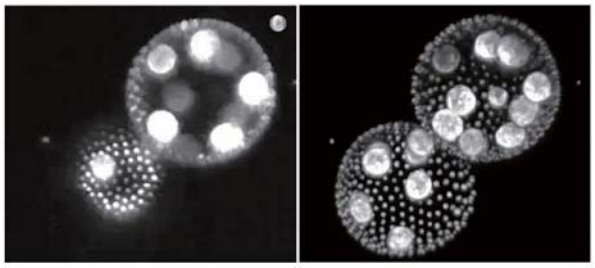

Figure 2. Images of floating volvox taken by ezDSLM with ETL. Left: Single optical section. Right: Maximum intensity projection.

Publication List:

〔Original papers〕

- Hashiura, T., Kimura, E., Fujisawa, S., Oikawa, S., Nonaka, S., Kurosaka, D., and Hitomi, J. (2017). Live imaging of primary ocular vasculature formation in zebrafish. PLoS ONE *12*, e0176456.
- Taniguchi, A., Kimura, Y., Mori, I., Nonaka, S., and Higashijima, S. (2017). Axially-confined in vivo single-cell labeling by primed conversion using blue and red lasers with conventional confocal microscopes. Dev. Growth Differ. *59*, 741-748.