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 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 mechanism that converts the flow to the 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.

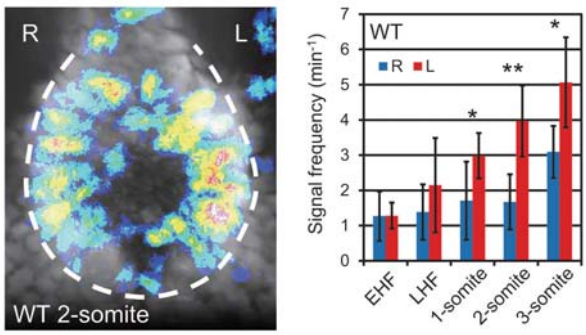

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

II. Development of light-sheet microscopy

Light-sheet microscopy has many advantages for live imaging including low photobleaching and phototoxicity, high penetration of depth, and fast imaging acquisition. This method has also peculiar disadvantages, however.

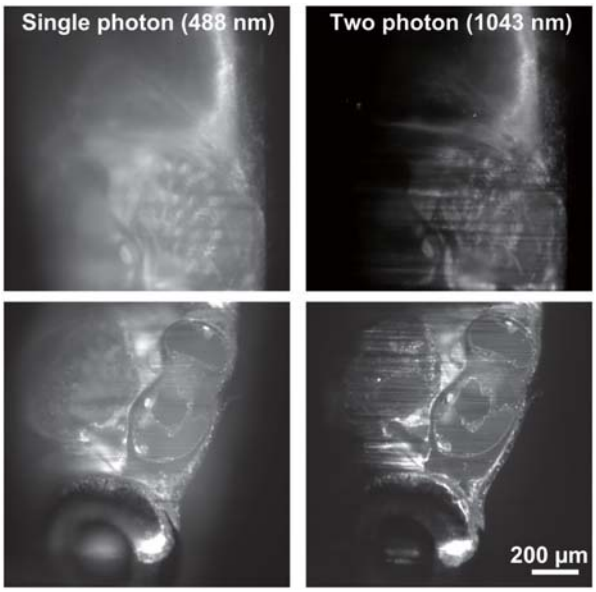

Figure 2. Two-photon light-sheet microscopy. Left: Fluorescent images of a medaka taken by a conventional (one-photon) light-sheet microscope. Right: Images of the same area taken by our two-photon light-sheet microscope.

Specifically scattering of excitation light within the specimen and illumination of areas besides the focal plane, and deterioration of contrast. A solution to these problems is combining light-sheet microscopy with two-photon excitation (TPE), but this results in a narrow field of view, because generation of TPE images requires very high photon density, i.e. focusing with a high numerical aperture (NA) lens.

We utilized a new fiber laser with high peak power to overcome this problem, and enabled observation of larger specimens using a hybrid TPE light-sheet microscope.

Publication List

〔Original papers〕

- Ichikawa, T., Nakazato, K., Keller, P.J., Kajiura-Kobayashi, H., Stelzer, E.H., Mochizuki, A., and Nonaka, S. (2013). Live imaging of whole mouse embryos during gastrulation: migration analyses of epiblast and mesodermal cells. PLoS ONE *8*, e64506.
- Murata, T., Sano, T., Sasabe, M., Nonaka, S., Higashiyama, T., Hasezawa, S., Machida, Y., and Hasebe, M. (2013). Mechanism of microtubule array expansion in the cytokinetic phragmoplast. Nature Commun. *4*, 1967.
- Takao, D., Nemoto, T., Abe, T., Kiyonari, H., Kajiura-Kobayashi, H., Shiratori, H., and Nonaka, S. (2013). Asymmetric distribution of dynamic calcium signals in the node of mouse embryo during left-right axis formation. Develop. Biol. *376*, 23-30.

〔Review article〕

- Nonaka, S. (2013). Visualization of mouse nodal cilia and nodal flow. Methods Enzymol. *525*, 149-157.