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.

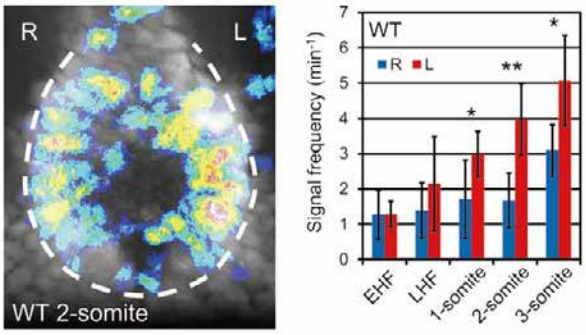

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

During the analysis we generated transgenic lines carrying ultrasensitive Ca^{2+} sensor proteins. We demonstrated these lines are useful for visualizing Ca^{2+} dynamics of adult tissues, while they did not work in the embryonic tissues.

II. Development of light-sheet microscopy

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

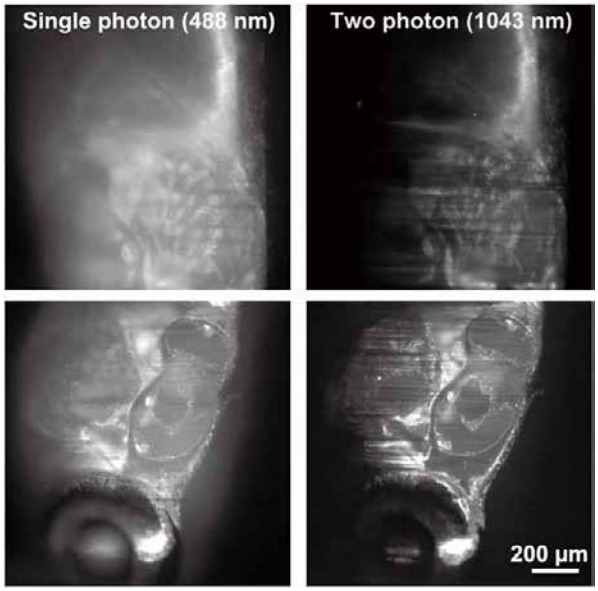

Figure 2. Two-photon light-sheet microscopy. Left: Fluorescent images of a medaka taken by a conventional (one-photon) light-sheet microscope. Right: Images of the same area taken by our two-photon light-sheet microscope.

Publication List

〔Original papers〕

- Ichikawa, T., Nakazato, K., Keller, P.J., Kajiura-Kobayashi, H., Stelzer, E.H., Mochizuki, A., and Nonaka, S. (2014). Live imaging and quantitative analysis of gastrulation in mouse embryos using light-sheet microscopy and 3D tracking tools. Nature Protoc. *9*, 575-585.
- Maruyama, A., Oshima, Y., Kajiura-Kobayashi, H., Nonaka, S., Imamura, T., and Naruse, K. (2014). Wide field intravital imaging by two-photon-excitation digital-scanned light-sheet microscopy (2p-DSLM) with a high-pulse energy laser, Biomed. Opt. Express *5*, 3311-3325.
- Oshima, Y., Imamura, T., Shintani, A., Kajiura-Kobayashi, H., Hibi, T., Nagai, T., Nonaka, S., and Nemoto, T. (2014). Ultrasensitive imaging of Ca^{2+} dynamics in pancreatic acinar cells of yellow cameleon-nano transgenic mice. Int. J. Mol. Sci. *15*, 19971-19986.