LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

Technical Staff: *KAJIURA-KOBAYASHI, Hiroko*
Postdoctoral Fellow: *ICHIKAWA, Takehiko*
TAKAO, Daisuke
MARUYAMA, Atsushi
Technical Assistant: *SHINTANI, Atsuko*
ISHIBASHI, Tomoko

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, while several models have been proposed, and involvement of Ca^{2+} has been 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.

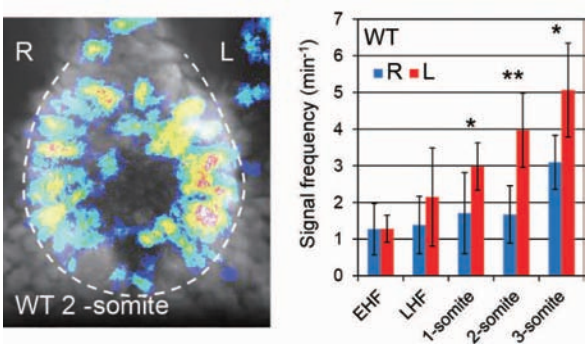

Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

II. Development of light-sheet microscopy

A remarkable characteristic of light-sheet microscopy is high efficiency of light detection against illumination power. This feature results in several advantages in live imaging: detection of weak signals, low phototxicity and bleaching, and fast image acquisition.

We took advantage of light-sheet microscopy by applying long-term observation of intact mouse embryos at gastrulating stages for tracking cell movements, raman imaging of living medaka, and ultra-fast 4D data acquisition of freely moving *Amoeba proteus*.

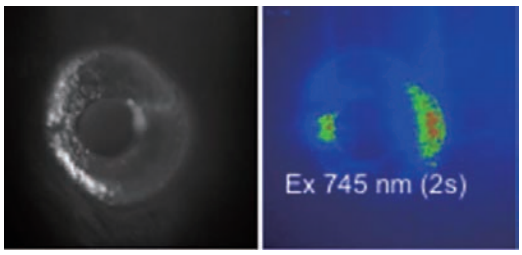

Figure 2. Raman signal of medaka Left: Dark-field image of a living Quintet medaka eye taken by oblique illumination. Right: Raman image of the same area.

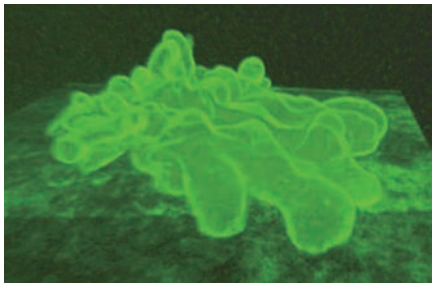

Figure 3. 3D image of Amoeba Proteus reconstructed from 100 optical sections taken in 0.5 sec.

Publication List

〔Original papers〕

- Morita, H., Kajiura-Kobayashi, H., Takagi, C., Yamamoto, T.S., Nonaka, S., and Ueno, N. (2012). Cell movements of the deep layer of non-neural ectoderm underlie complete neural tube closure in Xenopus. Development *139*, 1417-1426.
- Oshima, Y., Sato, H., Kajiura-Kobayashi, H., Kimura, T., Naruse, K., and Nonaka, S. (2012). Light sheet-excited spontaneous Raman imaging of a living fish by optical sectioning in a wide field Raman microscope. Optics Express *20*, 16195-16204.
- Takao, D., Taniguchi, A., Takeda, T., Sonobe, S., and Nonaka, S. (2012). High-speed imaging of amoeboid movements using light-sheet microscopy. PLoS ONE *7*, e50846.
- Yoshiba, S., Shiratori, H., Kuo, I.Y., Kawasumi, A., Shinohara, K., Nonaka, S., Asai, Y., Sasaki, G., Belo, J.A., Sasaki, H., et al. (2012). Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. Science *338*, 226-231.