antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids to obtain beneficial symbionts. Lineage-specific gene duplications have occurred in genes in a broad range of functional categories, which include signaling pathways, miRNA machinery, chromatin modification and mitosis. The importance of these duplications for symbiosis remains to be determined. We found several instances of lateral gene transfer from bacteria to the pea aphid genome. Some of them are highly expressed in bacteriocytes.

We recently discovered a novel class of genes in the pea aphid genome that encode small cysteine-rich proteins with secretion signals that are expressed exclusively in bacteriocytes of the pea aphid, and named these bacteriocyte-specific cysteine-rich proteins (BCR). The BCR mRNAs are first expressed at a developmental time point coincident with the incorporation of symbionts strictly in the cells that contribute to the bacteriocyte, and this bacteriocyte-specific expression is maintained throughout the aphid's life. Some BCRs showed an antibiotic activity. These results suggest that BCRs act within bacteriocytes to mediate the symbiosis with bacterial symbionts, which is reminiscent of the cysteine-rich secreted proteins of leguminous plants that also regulate endosymbionts. Employment of small cysteine-rich peptides may be a common tactic of host eukaryotes to manipulate bacterial symbionts.

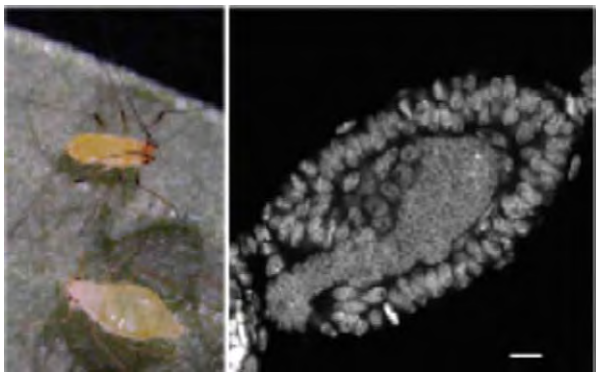

Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

Publication List:

〔Original papers〕

- Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., Baumann, A., and Blenau, W. (2015). Cockroach GABAB receptor subtypes: molecular characterization, pharmacological properties and tissue distribution. Neuropharmacol. *88*, 134–144.
- Bourguignon, T., Lo, N., Cameron, S.L., Sobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T., and Evans, T.A. (2015). The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. *32*, 406–421.
- Hojo, M.K., Ishii, K., Sakura, M., Yamaguchi, K., Shigenobu, S., and Ozaki, M. (2015). Antennal RNA-sequencing analysis reveals evolutionary aspects of chemosensory proteins in the carpenter ant, *Camponotus japonicus*. Sci. Rep. *5*, 13541.

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor:
KAMEI, Yasuhiro

Technical Staff: *KONDO, Maki*
TANIGUCHI-SAIDA, Misako
UCHIKAWA, Tamaki
Technical Assistant: *ICHIKAWA, Chiaki*
ISHIKAWA, Azusa

The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use "Light". The facility also provides technical support through management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers by Dr. Y. Kamei (refer to the Collaborative Research Group Research Enhancement Strategy Office section). Among its tools are advanced microscopes for biology and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism, capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve; allowing exposure to strong monochromatic light. The facility's microscopes, which are cutting edge devices such as confocal and multi-photon excitation microscopes, are used by both internal and external researchers as vital equipment for core and collaborative research projects.

Representative Instruments: Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum from 250 nm (ultraviolet) to 1,000 nm (infrared) onto its 10 m focal curve with an intensity of monochromatic light at each wavelength more than twice as much as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe *et al*., Photochem. Photobiol. *36*, 491-498, 1982). The spectrograph is dedicated to action spectroscopical studies of various light-controlled biological processes.

The NIBB Collaborative Research Program for the Use of the OLS supports about 10 projects every year conducted by

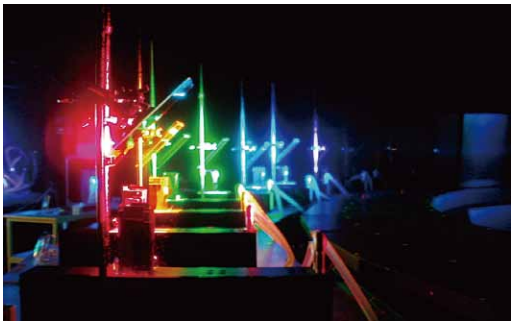

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

both visiting scientists, including foreign researchers, as well as those in NIBB.

Action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted.

Microscopes

This facility also has Bioimaging machines such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Olympus FV1000, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD camera), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced custom-made laser microscopes with special aims (Digital Scanned Light-sheet Microscope: DSLM and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began Collaborative Research Programs using these machines in 2010. In addition, transmission electron microscope service for plant biology has started from 2014.

The DSLM was developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissue by illuminating a specimen from the side with a light sheet (more information is given in Dr. Nonaka's section: Lab. for Spatiotemporal Regulations). Dr. Shigenori Nonaka conducted and supported 8 projects of the Collaborative Research Program for the Use of the DSLM. The IR-LEGO was developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST). This microscope can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (Kamei *et al*. Nat. Methods, 2009). Details are described in the next section. The IR-LEGO was also used for 10 Individual Collaborative Research projects, including applications for animals and plants.

Workshop and Symposium

In 2015 we held courses on both medaka basic training, which focused on basic techniques for medaka research including imaging, and the 3nd biological image processing training course. We also have been holding a "Bioimaging Forum" every year which discusses Bioimaging from various directions such as microscopy, new photo-technology, and computer science. This year we held the 9th NIBB Bioimaging Forum focused on physical properties on biology including heat (temperature) and force. In addition, we held symposiums focused on new emerging model animals and amphibians.

Publication List on Cooperation

〔Original papers (Selected)〕

- Kawasumi-Kita, A., Hayashi, T., Kobayashi, T., Nagayama, C., Hayashi, S., Kamei, Y., Morishita, Y., Takeuchi, T., Tamura, K., and Yokoyama, H. (2015). Application of local gene induction by infrared laser-mediated microscope and temperature stimulator to amphibian regeneration study. Dev. Growth Differ. *57*, 601-613.
- Kuboyama, K., Fujikawa, A., Suzuki, R., and Noda, M. (2015). Inactivation of protein tyrosine phosphatase receptor type Z by pleiotrophin promotes remyelination through activation of differentiation of oligodendrocyte precursor cells. J Neurosci. *35*, 12162-12171.
- Nakashima, M., Suzuki, M., Saida, M., Kamei, Y., Hossain, M.B., and Tokumoto, T. (2015). Cell-based assay of nongenomic actions of progestins revealed inhibitory G protein coupling to membrane progestin receptor α (mPRα). Steroids *100*, 21–26.
- Oikawa, K., Matsunaga, S., Shoji Mano, S., Kondo, M., Yamada, K., Hayashi, M., Kagawa, T., Kadota, A., Sakamoto, W., Higashi, S., Watanabe, M., Mitsui, T., Shigemasa, A., Iino, T., Hosokawa, Y., and Nishimura, M. (2015). Physical interaction between peroxisomes and chloroplasts elucidated by in situ laser analysis. Nat. Plants *1*, 15035.
- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2015). Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in Lotus japonicus. Plant Physiol. *167*, 545-557.

〔Original paper (E-publication ahead of print)〕

- Kagawa, N., Honda, A., Zenno, A., Omoto, R., Imanaka, S., Takehana, Y., and Naruse, K. Arginine vasotocin neuronal development and its projection in the adult brain of the medaka. Neurosci. Let. 2015 Dec. 29.

● *Research activity by Y. Kamei*

Specially Appointed Associate Professor: *KAMEI, Yasuhiro*
NIBB Research Fellow: *HATTORI, Masayuki*
Technical Assistant: *CHISADA, Eriko*

Our research group promotes two cutting-edge microscope projects; "observation" and "manipulation" using optical and biological technologies. The aim of our "observation project" is deep-seeing in living organisms using adaptive optics (AO) which were well-developed in the field of astronomy as a key technology of large telescopes such as the Subaru telescope in Hawaii. Although observation using telescopes on the earth may be disturbed by fluctuations in the atmosphere, AO technology can cancel this disturbance. On the other hand, living materials have particular refractive indexes, therefore, some organelles act as disturbances of the ideal optical path for microscope observation just like the atmosphere does for telescopes. AO technology can also compensate for this disturbance by sensing and correcting wave fronts using a wave front sensor and deformable mirror. Hence, we developed a custom-made wide-field microscope equipped with an AO system for observation of living organisms in collaboration with Dr. Tamada in NIBB and Dr. Hayano in the National Astronomical Observatory of Japan (NAOJ) and got high-resolution bright field and

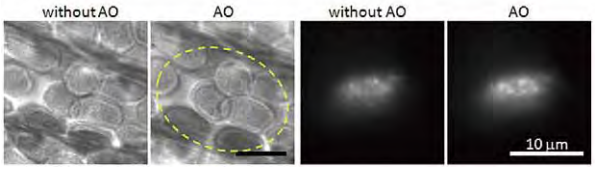

Figure 1. Effects of adaptive optics (AO) to wide-field microscope images (bright field and fluorescence of plant cells).

fluorescent images of living cells. Our results indicated that improvement of optical resolution was restricted to a small area which is called the “isoplanatic patch” (Figure 1).

Second, the aim of our “manipulation project” is to control gene expression *in vivo*. Gene function analysis must be evaluated at the cell level *in vivo*. To achieve spatiotemporal-controlled gene expression we employed one of the stress responses, the heat shock response. The heat shock promoter is the transcription regulation region of heat shock proteins and all organisms have this mechanism. Positioning the target gene downstream of the promoter, we can induce the target gene expression by local heating.

Infrared (IR) beams can heat water molecules, which are the main constituent of cells, hence, we can heat a single cell by irradiating IR to a target cell using a microscope. We have developed a microscope, IR laser evoked gene operator (IR-LEGO), specialized for this purpose (Figure 2). The IR-LEGO microscope can irradiate an IR laser to a single cell *in vivo* such as in *C. elegans*, *Drosophila*, medaka, zebrafish, *Xenopus* and *Arabidopsis*, to induce the heat shock response at a desired timing. In 2015, additionally, we confirmed the system was effective in the moss *Marchantia polymorpha* and in the newt *Pleurodeles waltl*.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating. We evaluated time course and spatial heating profiles, and the results presented that temperature of the target area rose rapidly and kept a constant level dependant on IR laser power, additionally, the heated area was adequately as small as a typical cell size.

With this in mind, we tried to induce gene expression in various species. At first, we reported an IR-LEGO experiment in living *C. elegans*. Target gene expression in a target cell could be induced with only 1 s-IR irradiation. Whereas the optimal power range which can induce gene induction without cell damage was limited. Excess laser power resulted in cell death or cessation of cell division. We confirmed that an optimal irradiation, e.g. 11 mW for 1 s, induced physiological gene expression in the target cell and subsequent cell division or morphogenesis underwent normal development. Next, we tried the experiment in other animals, such as, medaka, zebrafish and *Xenopus*, and the higher plant, *Arabidopsis*, since all organisms have a heat shock response system. We succeeded in local gene induction in all the species as expected.

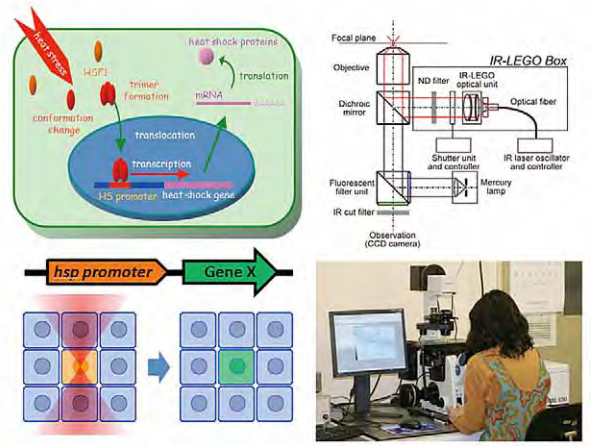

Figure 2. Schematic illustration of local gene induction system and an infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

Studies of cell fates, cell-cell interaction, or analysis of non-cell autonomous phenomena require a fine control system of gene expression in experiments. IR-LEGO will be a powerful tool for these studies in combination with molecular biological techniques, such as the cre-loxP system. Dr. Shimada in the University of Tokyo wanted to confirm the cell lineage of exo-skeletal tissue such as the scales of medaka fish. She questioned the traditional belief concerning the origin of the exo-skeleton of the body-trunk using transplantation studies. We then started a collaboration to establish a local permanent labeling system in medaka and to make clear the origin of exo-skeletal cells. The system was well working (Figure 3), and the fate tracking results indicated that exo-skeletal tissues were mesodermal in origin, not from neural crest cells, as previously believed (Shimada et al, Nat. Commun, 2013).

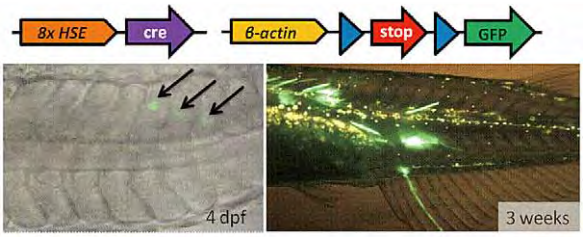

Figure 3. Examples of Cre-loxP mediated long-term GFP marking using IR-LEGO in living medaka individuals for cell linage tracing.

Publication List:

〔Original papers〕

- Kawasumi-Kita, A., Hayashi, T., Kobayashi, T., Nagayama, C., Hayashi, S., Kamei, Y., Morishita, Y., Takeuchi, T., Tamura, K., and Yokoyama, H. (2015). Application of local gene induction by infrared laser-mediated microscope and temperature stimulator to amphibian regeneration study. Dev. Growth Differ. *57*, 601-613.
- Nakashima, M., Suzuki, M., Saida, M., Kamei, Y., Hossain, B., and Tokumoto, T. (2015). Cell-based assay of nongenomic actions of progestins revealed inhibitory G protein coupling to membrane progestin receptor α (mPRα). Steroids *100*, 21-26.
- Yokoi, S., Okuyama, T., Kamei, Y., Naruse, K., Taniguchi, Y., Ansai, S., Kinoshita, M., Young, L. J., Takemori, N., Kubo, T., and Takeuchi, H. (2015). An essential role of the arginine vasotocin system in mate-guarding behaviors in triadic relationships of medaka fish (*Oryzias latipes*). PLoS Genetics *11*, e1005009.

〔Original paper (E-publication ahead of print)〕

- Nishihama, R., Ishida, S., Urawa, H., Kamei, Y., and Kohchi, T. Conditional gene expression/deletion systems for Marchantia polymorpha using its own heat-shock promoter and the cre/loxP-mediated site-specific recombination. Plant Cell Physiol. 2015 Jul. 6.