

the symbiotic organ in comparison with the whole body. Many genes for amino acid metabolism are found to be over-represented as expected: the plant sap-eating insect depends on the bacterial symbionts to supply essential amino acids. In addition, many kinds of novel secretion proteins that are found only in aphid species are extremely enriched in the bacteriocytes. We also found that bacteriocytes express Distal-less (Dll), a homeodomain-containing transcription factor throughout the life cycle. Future study should focus on dissecting the genetic network of these components, which should allow us to understand the genetic basis on which symbiosis generates evolutionary novelty.



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

Spectrography and Bioimaging Facility



Specially Appointed Associate Professor
KAMEI, Yasuhiro

Technical Staff:

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Secretary:

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. 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.

Publication List

[Original papers]

- Chang, C.-C., Hsiao, Y.-M., Huang, T.Y., Cook, C.E., Shigenobu, S., and Chang, T.-H. (2013). Noncanonical expression of caudal during early embryogenesis in the pea aphid *Acyrtosiphon pisum*: maternal cad-driven posterior development is not conserved. *Insect Mol. Biol.* 22, 442–455.
- Hayashi, Y., Shigenobu, S., Watanabe, D., Toga, K., Saiki, R., Shimada, K., Bourguignon, T., Lo, N., Hojo, M., Maekawa, K., et al. (2013). Construction and characterization of normalized cDNA libraries by 454 pyrosequencing and estimation of DNA methylation levels in three distantly related termite species. *PLoS ONE* 8, e76678.
- Shibata, T.F., Maeda, T., Nikoh, N., Yamaguchi, K., Oshima, K., Hattori, M., Nishiyama, T., Hasebe, M., Fukatsu, T., Kikuchi, Y., et al. (2013). Complete genome sequence of *Burkholderia* sp. strain RPE64, bacterial symbiont of the bean bug *Riptortus pedestris*. *Genome Announc.* 1, e00441–13.
- Shigenobu, S., and Stern, D.L. (2013). Aphids evolved novel secreted proteins for symbiosis with bacterial endosymbiont. *Proc. Royal Soc. B Biol. Sci.* 280, 20121952.
- Suzuki, M.M., Yoshinari, A., Obara, M., Takuno, S., Shigenobu, S., Sasakura, Y., Kerr, A.R., Webb, S., Bird, A., and Nakayama, A. (2013). Identical sets of methylated and nonmethylated genes in *Ciona intestinalis* sperm and muscle cells. *Epigenetics Chromatin* 6, 38.

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).

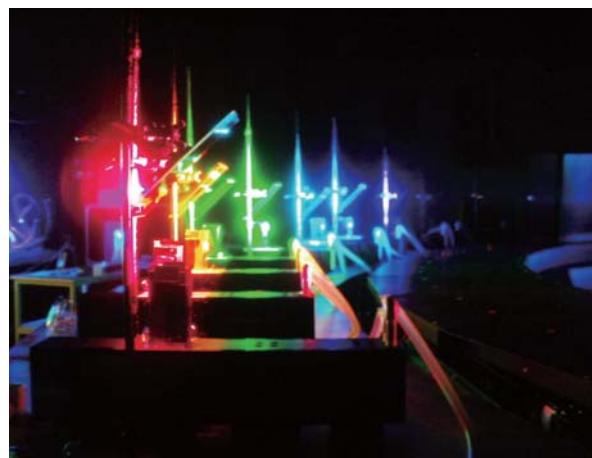


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

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 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 widefield microscopes (Olympus IX-81, BX-63 and KEYENCE BZ-8000), confocal microscopes (Olympus FV1000, Nikon A1R, Nikon A1Rsi, Carl Zeiss Duo 5 and Yokogawa CSU-X1), multi-photon microscopes (Olympus FV1000-MP, Leica 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.

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. Nonaka conducted and supported 7 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 higher plants.

Workshop and Symposium

In 2013, we held workshops (training courses) on IR-LEGO for vertebrates (frog and fish) and biological image processing. 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.

Publication List on Cooperation

〔Original papers (Selected)〕

- Allorent, G., Tokutsu, R., Roach, T., Peers, G., Cardol, P., Girard-Bascou, J., Seigneurin-Berny, D., Petroutros, D., Kuntz, M., Breyton, C., Franck, F., Wollman, F.-A., Niyogi, K.K., Krieger-Liszka, A., Minagawa, J. and Finazzi, G. (2013). A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. *Plant Cell*. 25, 545-557.
- Ikehata, H., Higashi, S., Nakamura, S., Daigaku, Y., Furusawa, Y., Kamei, Y., Watanabe, M., Yamamoto, K., Hieda, K., Munakata, N., and Ono, T. (2013). Action spectrum analysis of UVR genotoxicity for skin:

the border wavelengths between UVA and UVB can bring serious mutation loads to skin. *J. Invest. Dermatol.* 133, 1850-1856.

- Shikata, T., Matsunaga, S., Iseki, M., Nishide, H., Higashi, S., Kamei, Y., Yamaguchi, M., Jenkinson, I.R., and Watanabe, M. (2013). Blue light regulates the rhythm of diurnal vertical migration in the raphidophyte red-tide alga *Chattonella antiqua*. *J. Plankton Res.* 35, 542-552.
- Okuyama, T., Isoe, Y., Hoki, M., Suehiro, Y., Yamagishi, G., Naruse, K., Kinoshita, M., Kamei, Y., Shimizu, A., Kubo, T., and Takeuchi, H. (2013). Controlled cre/loxP site-specific recombination in the developing brain in medaka fish, *Oryzias latipes*. *PLoS ONE*. 8, e66597.
- 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. *Nat. Commun.* 4, 1967.
- Sato-Numata, K., Numata, T., Okada, T., and Okada, Y. (2013). Acid-sensitive outwardly rectifying (ASOR) anion channels in human epithelial cells are highly sensitive to temperature and independent of CIC-3. *Pflugers Arch-Eur. J. Physiol.* 465, 1535-1543.
- Suzaki, T., Ito, M., and Kawaguchi, M. (2013). Induction of localized auxin response during spontaneous nodule development in *Lotus japonicus*. *Plant Signal Behav.* 8, e23359.
- Takahara, M., Magori, S., Soyano, T., Okamoto, S., Yoshida, C., Yano, K., Sato, S., Tabata, S., Yamaguchi, K., Shigenobu, S., Takeda, N., Suzuki, T., and Kawaguchi, M. (2013). Too much love, a novel Kelch repeat-containing F-box protein, functions in the long-distance regulation of the legume-Rhizobium symbiosis. *Plant Cell Physiol.* 54, 433-447.
- Yamagata, Y., Kaneko, K., Kase, D., Ishihara, H., Nairn, A.C., Obata, K., and Imoto, K. (2013). Regulation of ERK1/2 mitogen-activated protein kinase by NMDA-receptor- induced seizure activity in cortical slices. *Brain Res.* 24, 1-10.
- Kimura, E., Deguchi, T., Kamei, Y., Shoji, W., Yuba, S., and Hitomi, J. (2013). Application of infrared laser to the zebrafish vascular system: gene induction, tracing, and ablation of single endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 33, 1264-1270.
- Hira, R., Ohkubo, F., Tanaka, Y.R., Masamizu, Y., Augustine, J.G., Kasai, H., and Matsuzaki, M. (2013). In vivo optogenetic tracing of functional corticocortical connections between motor forelimb areas. *Front. Neural Circuits.* 7, 55.
- Shimada, A., Kawanishi, T., Kaneko, T., Yoshihara, H., Yano, T., Inohaya, K., Kinoshita, M., Kamei, Y., Tamura, K., and Takeda, H. (2013). Trunk exoskeleton in teleosts is mesodermal in origin. *Nat. Commun.* 4, 1639.
- Kobayashi, K., Kamei, Y., Kinoshita, M., Czerny, T., and Tanaka, M. (2013). A heat-inducible Cre/LoxP gene induction system in medaka. *Genesis*. 51, 59-67.
- Moritoh, S., Komatsu, Y., Yamamori, T., and Koizumi, A. (2013). Diversity of retinal ganglion cells identified by transient GFP transfection in organotypic tissue culture of adult marmoset monkey retina. *PLoS ONE*. 8, e54667.
- Suzaki, T., Kim, C.S., Takeda, N., Szczyglowski, K., and Kawaguchi, M. (2013). TRICOT encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in *Lotus japonicus*. *Development* 140, 353-361.

● Research activity by Y. Kamei

Specially Appointed Associate Professor:
KAMEI, Yasuhiro
NIBB Research Fellow: **HATTORI, Masayuki**

My research group promotes two advanced 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 of telescopes on the earth may be disturbed by fluctuation in the atmosphere, AO technology can cancel this disturbance. On the other hand, living materials have particular refractive indexes, therefore, some organelles act as a disturbance of the ideal optical path for microscope observation just like the atmosphere does for telescopes. AO technology also can recover 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 as a collaboration with Dr. Tamada in NIBB and Dr. Hayano in the National Astronomical Observatory of Japan (NAOJ) and got high-resolution bright field and fluorescent images of living cells. Our result indicated that improvement of optical resolution was restricted in a small

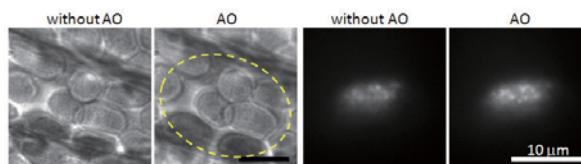


Figure 1. Effects of adaptive optics (AO) to the wide-field microscope images (bright field and fluorescence) of plant cells.

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

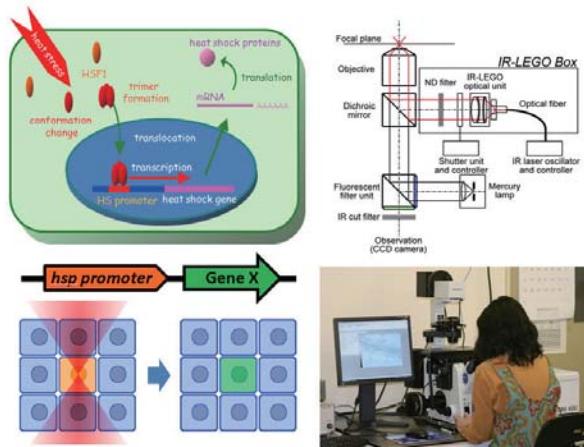


Figure 2. Schematic illustration of local gene induction system and an infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

(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 *C. elegans*, medaka, zebrafish, *Xenopus* and *Arabidopsis*, to induce the heat shock response at a desired timing.

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, 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 the species

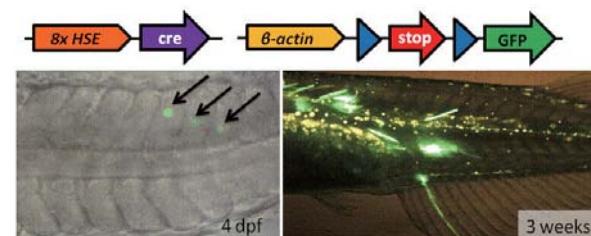


Figure 3. Cre-loxP mediated long-term GFP marking in a living medaka individual for lineage tracing.

as expected.

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. By Applying IR-LEGO to a mutant and its rescue transgenic strain; using hsp-cre with a rescue gene which is sandwiched by loxP sequences, we will achieve single-cell knockout experiments in living organisms, and reveal fine interaction between the cells. We are now testing this system using medaka. We have already constructed a medaka TILLING library and a screening system for reverse genetic mutant screening, furthermore we have confirmed a system operation of a cre-loxP system in medaka using IR-LEGO (Figure 3).

Publication List

[Original papers]

- Ikehata, H., Higashi, S., Nakamura, S., Daigaku, Y., Furusawa, Y., Kamei, Y., Watanabe, M., Yamamoto, K., Hieda, K., Munakata, N., and Ono, T. (2013). Action spectrum analysis of UVR genotoxicity for skin: the border wavelengths between UVA and UVB can bring serious mutation loads to skin. *J. Invest. Dermatol.* **133**, 1850-1856.
- Ishikawa, T., Okada, T., Ishikawa-Fujiwara, T., Todo, T., Kamei, Y., Shigenobu, S., Tanaka, M., Saito, T.L., Yoshimura, J., Morishita, S., Toyoda, A., Sakaki, Y., Taniguchi, Y., Takeda, S., and Mori, K. (2013). ATF6a/b-dedicated adjustment of ER chaperone levels is essential for development of the notochord in medaka fish. *Mol. Biol. Cell* **24**, 1387-1395.
- Kimura, E., Deguchi, T., Kamei, Y., Shoji, W., Yuba, S., and Hitomi, J. (2013). Application of infrared laser to the zebrafish vascular system: gene induction, tracing, and ablation of single endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **33**, 1264-1270.
- Kobayashi, K., Kamei, Y., Kinoshita, M., Czerny, T., and Tanaka, M. (2013). A heat-inducible Cre/LoxP gene induction system in medaka. *Genesis* **51**, 59-67.
- Okuyama, T., Isoe, Y., Hoki, M., Suehiro, Y., Yamagishi, G., Naruse, K., Kinoshita, M., Kamei, Y., Shimizu, A., Kubo, T., and Takeuchi H. (2013). Controlled Cre/loxP site-specific recombination in the developing brain in medaka fish, *Oryzias latipes*. *PLoS ONE* **8**, e66597.
- Shikata, T., Matsunaga, S., Iseki, M., Nishide, H., Higashi, S-I., Kamei, Y., Yamaguchi, M., Jenkinson, I.R., and Watanabe, M. (2013). Blue light regulates the rhythm of diurnal vertical migration in the raphidophyte red-tide alga *Chattonella antiqua*. *J. Plankton Res.* **35**, 542-552.
- Shimada, A., Kawanishi, T., Kaneko, T., Yoshihara, H., Yano, T., Inohaya, K., Kinoshita, M., Kamei, Y., Tamura, K., and Takeda, H. (2013). Trunk exoskeleton in teleosts is mesodermal in origin. *Nature Commun.* **4**, 1639.

Data Integration and Analysis Facility

Assistant Professor:
Technical Staff:

UCHIYAMA, Ikuo
MIWA, Tomoki
NISHIDE, Hiroyo
NAKAMURA, Takanori
OKA, Naomi

Technical Assistant:



Figure 1. Biological Information Analysis System

cluster system (DELL PowerEdge M1000e+M610; 32 nodes/256 cores, 768GB memory) and a large-capacity storage system (DELL Equallogic; 35TB SAS, 26TB SATA, 750GB SSD). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be processed efficiently. Some personal computers and color/monochrome printers are also available. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for institute members. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

The facility also provides network communication services. Most of the PCs in each laboratory, as well as all of the above-mentioned service machines, are connected by a local area network, which is linked to the high performance backbone network ORION connecting the three research institutes in Okazaki. Many local services, including sequence analysis services, file sharing services, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (<http://www.nibb.ac.jp/>).

Research activity by I. Uchiyama

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p. ##).

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence analysis, expression data analysis, and imaging data analysis. For this purpose, the facility maintains high-performance computers with large-capacity storage systems. On the basis of this system, the facility supports development of data analysis pipelines, database construction and setting up websites to distribute the data worldwide. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the network system in the institute and computer/network consultation for institute members.

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a shared memory parallel computer (DELL PowerEdge R905; 4 nodes/16 cores, 256GB memory), a high-performance