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〕

- Gusev, O., Suetsugu, Y., Cornette, R., Kawashima, T., Logacheva, M.D., Kondrashov, A.S., Penin, A.A., Hatanaka, R., Kikuta, S., Shimura, S., Kanamori, H., Katayose, Y., Matsumoto, T., Shagimardanova, E., Alexeev, D., Govorun, V., Wisecaver, J., Mikheyev, A., Koyanagi, R., Fujie, M., Nishiyama, T., Shigenobu, S., Shibata, T.F., Golygina, V., Hasebe, M., Okuda, T., Satoh, N., and Kikawada, T. (2014). Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. Nature Commun. 5, 4784.
- Kaiwa, N., Hosokawa, T., Nikoh, N., Tanahashi, M., Moriyama, M., Meng, X.-Y., Maeda, T., Yamaguchi, K., Shigenobu, S., Ito, M., and Fukatsu, T. (2014). Symbiont-supplemented maternal investment underpinning host's ecological adaptation. Curr. Biol. 24, 2465-2470.
- Kodama, Y., Suzuki, H., Dohra, H., Sugii, M., Kitazume, T., Yamaguchi, K., Shigenobu, S., and Fujishima, M. (2014). Comparison of gene expression of Paramecium bursaria with and without Chlorella variabilis symbionts. BMC Genomics 15, 183.
- Takeshita, K., Shibata, T.F., Nikoh, N., Nishiyama, T., Hasebe, M., Fukatsu, T., Shigenobu, S., and Kikuchi, Y. (2014). Whole-genome sequence of Burkholderia sp. strain RPE67, a bacterial gut symbiont of the bean bug Riptortus pedestris. Genome Announc. 2, e00556-14.

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

- Bourguignon, T., Lo, N., Cameron, S.L., Sobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T., and Evans, T.A. The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. 2014 Nov 10.

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).

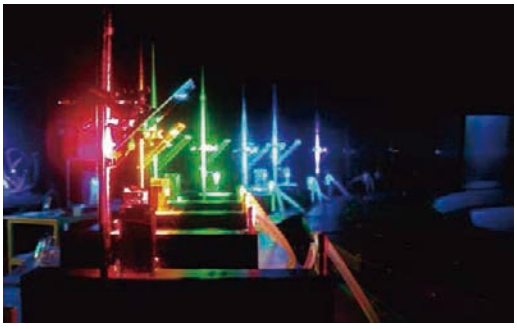

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

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, FV1200-MPs, 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. 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. 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 2014, we held the 8th International training courses on frog and fish (refer to international practical course) and the 2nd 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 8th NIBB Bioimaging Forum focused on establishment of a network among Imaging Centers in Japan. In addition, we held a symposium focused on new microscope methods using adaptive optics, "Subaru microscope", with the National Astronomical Observatory of Japan (NAOJ).

Publication List of Collaborative Research

〔Original papers (Selected)〕

- Fang, X., Ide, N., Higashi, S., Kamei, Y., Toyooka, T., Ibuki, Y., Kawai, K., Kasai, H., Okamoto, K., Arimoto-Kobayashi, S., and Negishi, T. (2014). Somatic cell mutaions caused by 365 nm LED-UVA double-strand breaks through oxidative damage. Photochem. Photobiol. Sci. *13*, 1338-1346.
- Goto-Yamada, S., Mano, S., Nakamori, C., Kondo, M., Yamawaki, R., Kato, A., and Nishimura, M. (2014). Chaperone and protease functions of LON protease 2 modulate the peroxisomal transition and degradation with autophagy. Plant Cell Physiol. *55*, 482-496.
- Hayashi, S., Ochi, H., Ogino, H., Kawasumi, A., Kamei, Y., Tamura, K., and Yokoyama, H. (2014). Transcriptional regulators in the Hippo1 signaling pathway control organ growth in Xenopus tadpole tail regeneration. Dev. Biol. *396*, 31-41.
- Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y.I., Yamamoto, S., Yabe, T., Takada, S., Kinoshita, M., Kuroiwa, A., and Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. Proc. Natl. Accad. Sci. USA *111*, 7343-7348.
- Masamizu, Y., Tanaka, Y.R., Tanaka, Y.H., Hira, R., Ohkubo, F., Kitamura, K., Isomura, Y., Okada, T., and Matsuzaki, M. (2014). Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task. Nature Neurosci. *17*, 987-994.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Wakamatsu, Y., Kelsh, R.N., Hibi, M., and Hashimoto, H. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genetics *10*, e1004246.
- Ogino, Y., Hirakawa, I., Inohaya, K., Sumiya, E., Miyagawa, S., Denslow, N., Yamada, G., Tatarazako, N., and Iguchi, T. (2014). Bmp7 and Lef1 are the downstream effectors of androgen signaling in androgen-induced sex characteristics development in medaka. Endocrinology *155*, 449-462.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.
- Tamada, Y., Murata, T., Hattori, M., Oya, S., Hayano, Y., Kamei, Y., and Hasebe, M. (2014). Optical property analyses of plant cells for adaptive optics microscopy. Int. Optomechatroni. *8*, 89-99.

● *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 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).

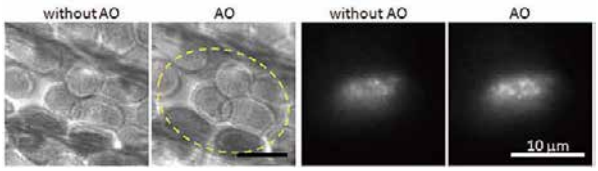

Figure 1. Effects of adaptive optics (AO) to the wide-field microscope images (bright field and fluorescence of plant cells).

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 *C. elegans*, *Drosophila*, 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, 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.

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). This year, our group and other collaborators were applying the same system to amphibians, *Xenopus* and salamanders, for regeneration studies.

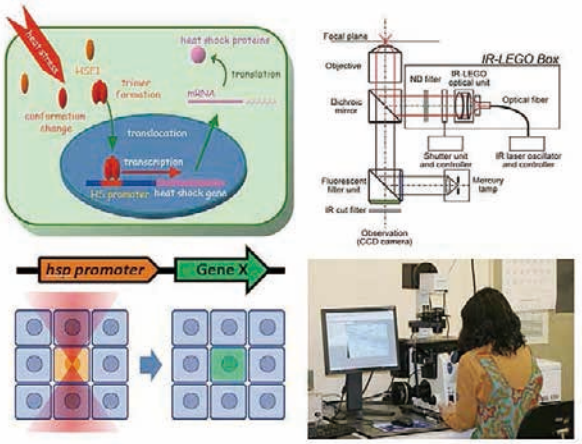

Figure 2. Schematic illustration of local gene induction system and an infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

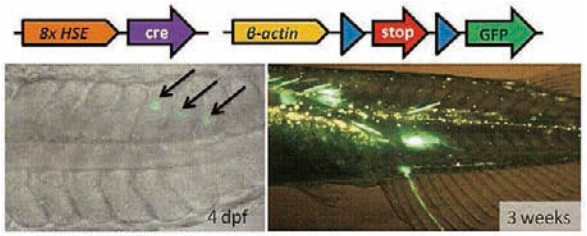

Figure 3. Examples of Cre-loxP mediated long-term GFP marking using IR-LEGO in living medaka individuals for cell linage tracing.

Publication List

〔Original papers〕

- Fang, X., Ide, N., Higashi, S., Kamei, Y., Toyooka, T., Ibuki, Y., Kawai, K., Kasai, H., Okamoto, K., Arimoto- Kobayashi, S., and Negishi, T. (2014). Somatic cell mutations caused by 365 nm LED-UVA double-strand breaks through oxidative damage. Photochem. Photobiol. Sci. *13*, 1338-1346.
- Hayashi, S., Ochi, H., Ogino, H., Kawasumi, A., Kamei, Y., Tamura, K., and Yokoyama, H. (2014). Transcriptional regulators in the Hippo1 signaling pathway control organ growth in Xenopus tadpole tail regeneration. Dev. Biol. *396*, 31-41.

- Murozumi, N., Nakashima, R., Hirai, T., Kamei, Y., Ishikawa-Fujiwara, T., Todo, T., and Kitano, T. (2014). Loss of follicle-stimulating hormone receptor function causes masculinization and suppression of ovarian development in genetically female medaka. Endocrinology *155*, 3136-3145.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Wakamatsu, Y., Kelsh, R.N., Hibi, M., and Hashimoto, H. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genetics *10*, e1004246.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.
- Otozai, S., Ishikawa-Fujiwara, T., Oda, S., Kamei, Y., Ryo, H., Sato, A., Nomura, T., Mitani, H., Tsujimura, T., Inohara, H., and Todo T. (2014). p53-Dependent suppression of genome instability in germ cells. Mutat. Res. Fundam. Mol. Mech. Mutagen. *760*, 24-32.
- Tamada, Y., Murata, T., Hattori, M., Oyac, S., Hayano, Y., Kamei, Y., and Hasebe, M. (2014). Optical property analyses of plant cells for adaptive optics microscopy. Int. J. Optomechatroni. *8*, 89-99.

Data Integration and Analysis Facility

Assistant Professor:	*UCHIYAMA, Ikuo*
Technical Staff:	*MIWA, Tomoki*
	NISHIDE, Hiroyo
	NAKAMURA, Takanori
Technical Assistant:	*OKA, Naomi*

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 as well as providing users' basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the network systems 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 high-performance cluster system (SGI Rackable server C2112-4RP; 40 nodes/800 cores, 96GB memory/node), a shared memory parallel computer (HP ProLiant DL980 G7; 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700; 480TB), and a large capacity storage system (DELL PowerEdge R620; 720TB). 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 and collaborative researchers. Especially,

Figure 1. Biological Information Analysis System

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. 70).