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

● Shigenobu, S., and Stern, D. Aphids evolved novel secreted proteins for symbiosis with bacterial endosymbiont. Proc. Royal Soc. B: Biol. Sci. 2012 Nov 21.

Spectrography and Bioimaging Facility

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Staff: *HIGASHI, Sho-ichi*
TANIGUCHI-SAIDA, Misako
Technical Assistant: *ICHIKAWA, Chiaki*
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.

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.

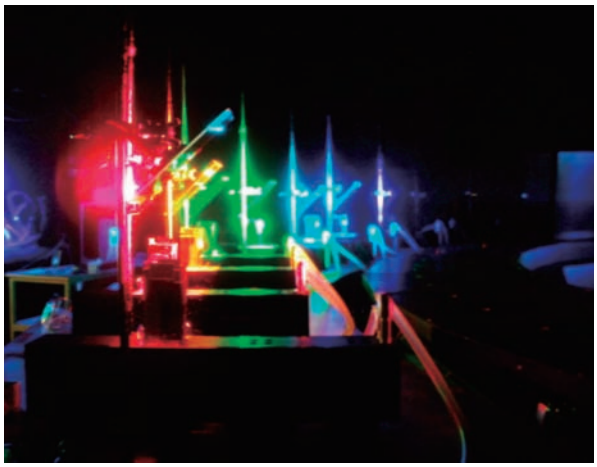

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

The 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, Leica TCS SP2, Nikon A1R, Nikon A1Rsi, Carl Zeiss Duo 5 and Yokogawa CSU-X1) and other advanced custom-made laser microscopes for 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 since 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 from the side of a specimen with a light sheet (more information is described in Dr. Nonaka's section: Lab. for Spatiotemporal Regulations). Dr. Nonaka conducted and supported about 7 projects of the Collaborative Research Program for the Use of the DSLM. On the other, 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 about 10 Individual Collaborative Research projects, including applications for animals and higher plant.

Workshop and Symposium

In 2012, we held workshops (training course) on IR-LEGO for plants (*Arabidopsis*) and fish (medaka) in Japan and Singapore (as a joint workshop by NIBB, the National University of Singapore, and Temasek Lifesciences Laboratory) respectively. 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. In 2012, we held the 6th and 7th forums which focused on all imaging sciences, from astronomy to biology, and optogenetics and adaptive optics, respectively.

Publication List on Cooperation

〔Original papers〕

- Moritoh, S., Eun, C-H., Ono, A., Asao, H., Okano, Y., Yamaguchi, K., Shimatani, Z., Koizumi, A., and Terada, R. (2012). Targeted disruption of an orthologue of DOMAINS REARRANGED METHYLASE 2, OsDRM2, impairs the growth of rice plants by abnormal DNA methylation. Plant J. *71*, 85-98.
- Satoh, C., Kimura, Y., and Higashijima, S. (2012). Generation of multiple classes of V0 neurons in zebrafish spinal cord: Progenitor heterogeneity and temporal control of neuronal diversity. J. Neurosci. *32*, 1771-1783.
- Suzaki, T., Yano, K., Ito, M, Umehara, Y., Suganuma, N., and Kawaguchi, M. (2012). Positive and negative regulation of cortical cell division during root nodule development in Lotus japonicus is accompanied by auxin response. Development *139*, 3997-4006.
- Takeda, N., Maekawa, T., and Hayashi, M. (2012). Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates Rhizobial and Mycorrhizai responses in Lotus japonicus. Plant Cell *24*, 810-822.
- Watakabe, A., Kato, S., Kobayashi, K., Takaji, M., Nakagami, Y., Sadakane, O., Ohtsuka, M., Hioki, H., Kaneko, T., Okuno, H., Kawashima, T., Bito, H., Kitamura, Y., and Yamamori, T. (2012). Visualization of Cortical Projection Neurons with Retrograde TET-Off Lentiviral Vector. PLoS ONE *7*, e46157.

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

- Suzaki, T., Kim, C.S., Takeda, N., Szczyglowski, K., and Kawaguchi, M. TRICOT encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in Lotus japonicus. Development 2012 Dec. 18.

● *Research activity by Y. Kamei*

Associate Professor (Specially appointed)
KAMEI, Yasuhiro
Technical Assistant: *KANIE, Yuta*

To investigate a gene function in each cell we have to express the gene in the cell *in vivo*, and ideally the expression must be limited only to the single cell. Tissue or cell specific promoters were used to reveal gene functions, however promoter-driven gene expression was governed by cell fate or environment, therefore we could not control the timing of gene expression. To achieve timing-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 heating.

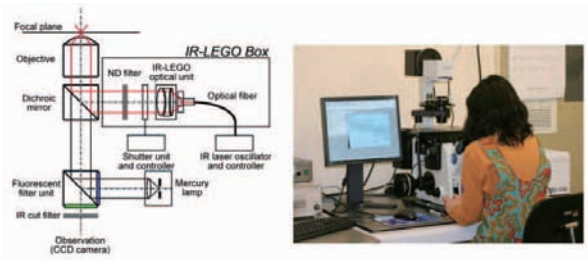

Figure 1. An infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

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 1). The IR-LEGO microscope can irradiate an IR laser to a single cell *in vivo* such as *C. elegans*, medaka 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; however, there was no way to measure temperature in a microenvironment under microscopic observation. To achieve this we employed green fluorescent protein (GFP) as a thermometer. Since fluorescent matter has the common property of temperature dependent decrease of emission intensity, we can estimate temperature shift by emission intensity change. GFP expressing E. coli was used to measure temperature as a micro thermometer. Using this probe, we evaluated heating properties of IR-LEGO such as speed of temperature rise and 3-dimensional distribution of temperature during IR irradiation. In a model tissue which contained GFP expressing bacteria in polyacrylamide gel, temperature rose rapidly with IR irradiation and kept a constant level dependant on IR laser power (Figure 2 left). On the other hand, the heated area was limited to a small volume about as large as a typical cell (Figure 2 right).

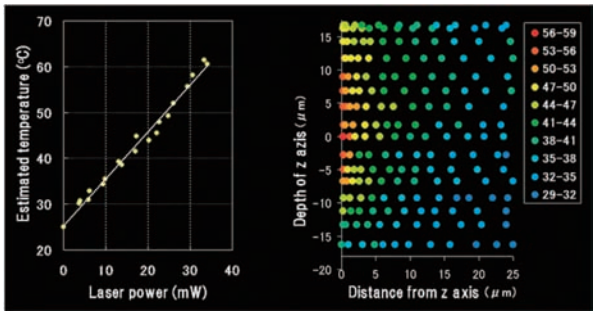

Figure 2. Heating profiles (laser power dependency of focus temperature and 3-D temperature map) of IR irradiation.

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 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 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

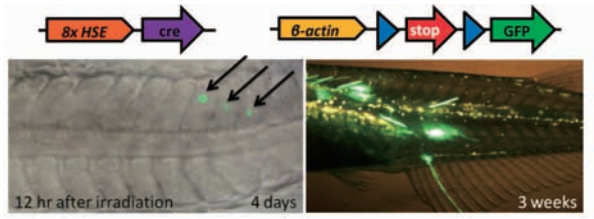

Figure 3. Cre-loxP mediated long-term GFP marking in a living medaka individual for linage tracing.

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〕

- Ansai, S., Ochiai, H., Kanie, Y., Kamei, Y., Gou, Y., Kitano, T., Yamamoto, T., and Kinoshita, M. (2012). Targeted disruption of exogenous EGFP gene in medaka using zinc-finger nucleases. Dev. Growth Differ. *54*, 546-556.
- Kitano, T., Hayashi, Y., Shiraishi, E., and Kamei, Y. (2012). Estrogen rescues masculinization of genetically female medaka by exposure to cortisol or high temperature. Mol. Reprod. Dev. *79*, 719-726.
- Masuyama, H., Yamada, M. Kamei, Y., Fujiwara-Ishikawa, T., Todo, T., Nagahama, Y., and Matsuda, M. (2012). Dmrt1 mutation causes a male-to-female sex reversal after the sex determination by Dmy in the medaka. Chromosome Res. *20*, 163-176.
- Yasuda, T., Oda, S., Li, Z., Kimori, Y., Kamei, Y., Ishikawa, T., Todo, T., and Mitani, H. (2012). Gamma-ray irradiation promotes premature meiosis of spontaneously differentiating testis-ova in the testis of p53-deficient medaka (Oryzias latipes). Cell Death Dis. *3*, e395

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

- Kobayashi, K., Kamei, Y., Kinoshita, M., Czerny, T., and Tanaka, M. A heat-inducible cre/loxP gene induction system in medaka. Genesis 2012 Nov 3.

Data Integration and Analysis Facility

Assistant Professor:	*UCHIYAMA, Ikuo*
Technical Staff:	*MIWA, Tomoki*
	NISHIDE, Hiroyo
	NAKAMURA, Takanori
Technical Assistant:	*YAMAMOTO, Kumi*
	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. 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 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

Figure 1. Biological Information Analysis System

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. 65).