NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities were launched in 2010 to support basic biological research at NIBB. They consist of three facilities that develop and provide state-of-the-art technologies aimed at increasing the understanding of biological functions through the application of functional genomics, bioimaging, and bioinformatics. The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among NIBB researchers and other academic institutions.

Functional Genomics Facility

Specially Appointed Associate Professor:
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
	BINO, Takahiro
Technical Assistant:	*ASAO, Hisayo*
	AKITA, Asaka
	MATSUMOTO, Miwako
Secretary:	*ICHIKAWA, Mariko*

The Functional Genomics Facility is a division of the NIBB Core Research Facilities organized jointly by NIBB and NIPS for the promotion of DNA and protein studies. The facility maintains a wide array of core research equipment, ranging from standard machinery (*e.g.* ultracentrifuges) to cutting edge tools (*e.g.* next generation DNA sequencers), which amount to 90 instruments in total. The facility is dedicated to fostering collaborations with researchers both at NIBB and other academic institutions worldwide through the provision of these tools as well as expertise. Our current focus is functional genomics. We also act as a bridge between experimental biology and bioinformatics through close consultation and training.

We recently conducted a large scale renovation of the Functional Genomics Facility building, and as part of this, the Visitors Lab and the Visitors Office were re-designed so that visiting scientists can work more effectively during their stay. In 2018, approximately 200 researchers came to use our facility and developed active collaborations, which consequently resulted in 20 co-authored papers published.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming modern biology thanks to ultra-high-throughput DNA sequencing. Utilizing HiSeq1500, NextSeq and MiSeq (Illumina), PacBio RS II and Sequel (PacificBio Sciences), and MinION and GridION (Oxford Nanopore Technologies), the Functional Genomics Facility is committed to joint research aimed at exploring new yet otherwise inaccessible fields in basic biology.

During 2018, we carried out 67 NGS projects in collaboration with researchers from academic institutions throughout the world. These projects cover a wide range of species (bacteria, animals, plants, and fungi) including both model and non-model organisms, and various other applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

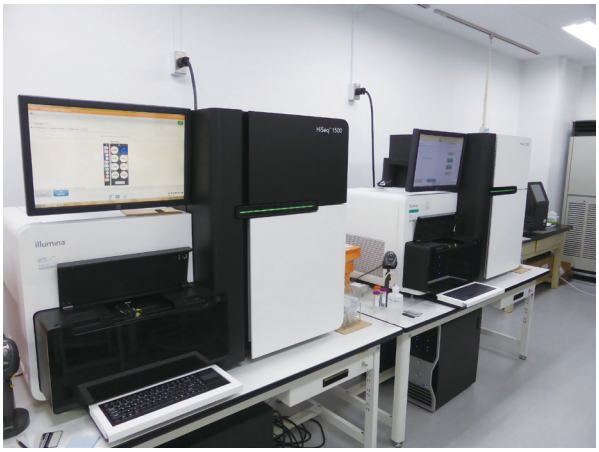

Figure 1. Next-generation sequencer

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2018, we analyzed approximately 1000 samples with mass spectrometers and protein sequencers.

- LC-MS (AB SCIEX TripleTOF 5600 system)
- LC-MS (Thermo Fisher SCIENTIFIC Orbtrap Elite)
- MALDI-TOF-MS (Bruker Daltonics REFLEX III)
- LC-Q-TOF MS (Waters Q-TOF Premier)
- Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

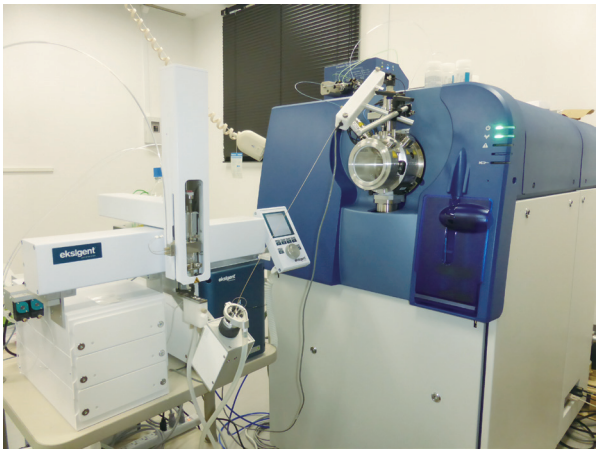

Figure 2. Triple TOF LC/MS/MS System

Other analytical instruments (excerpts)

- Cell sorter (SONY SH800)
- Bioimaging analyzer (Fujifilm LAS 3000 mini; GE FLA9000)

- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500)
- Ultracentrifuge (Beckman XL-80XP *etc*.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1)

Publication List on Cooperation:

〔**Original papers**〕

- Ando, T., Matsuda, T., Goto, K., Hara, K., Ito, A., Hirata, J., Yatomi, J., Kajitani, R., Okuno, M., Yamaguchi, K., Kobayashi, M., Takano, T., Minakuchi, Y., Seki, M., Suzuki, Y., Yano, K., Itoh, T., Shigenobu, S., Toyoda, A., and Niimi, T. (2018). Repeated inversions within a *pannier* intron drive diversification of intraspecific colour patterns of ladybird beetles. Nat. Commun. *9*, 3843.
- Fallon, T.R., Lower, S.E., Chang, C.-H., Bessho-Uehara, M., Martin, G.J., Bewick, A.J., Behringer, M., Debat, H.J., Wong, I., Day, J.C., Suvorov, A., Silva, C.J., Stanger-Hall, K.F., Hall, D.W., Schmitz, R.J., Nelson, D.R., Lewis, S., Shigenobu, S., Bybee, S.M., Larracuente, A.M., Oba, Y., and Weng, J.-K. (2018). Firefly genomes illuminate parallel origins of bioluminescence in beetles. eLife *7*, e36495.
- Ishishita, S., Takahashi, M., Yamaguchi, K., Kinoshita, K., Nakano, M., Nunome, M., Kitahara, S., Tatsumoto, S., Go, Y., Shigenobu, S., and Matsuda, Y. (2018). Nonsense mutation in *PMEL* is associated with yellowish plumage colour phenotype in Japanese quail. Sci. Rep. *8*, 16732.
- Kinjo, Y., Bourguignon, T., Tong, K.J., Kuwahara, H., Lim, S.J., Yoon, K.B., Shigenobu, S., Park, Y.C., Nalepa, C.A., Hongoh, Y., Ohkuma, M., Lo, N., and Tokuda, G. (2018). Parallel and gradual genome erosion in the *Blattabacterium* endosymbionts of *Mastotermes darwiniensis* and *Cryptocercus* wood roaches. Genome Biol. Evol. *10*, 1622–1630.
- Kobayashi, Y., Maeda, T., Yamaguchi, K., Kameoka, H., Tanaka, S., Ezawa, T., Shigenobu, S., and Kawaguchi, M. (2018). The genome of *Rhizophagus clarus* HR1 reveals a common genetic basis for auxotrophy among arbuscular mycorrhizal fungi. BMC Genomics *19*, 465.
- Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T., and Hasebe, M. (2018). *Physcomitrella* MADS-box genes regulate water supply and sperm movement for fertilization. Nat. Plants *4*, 36–45.
- Maeda, T., Kobayashi, Y., Kameoka, H., Okuma, N., Takeda, N., Yamaguchi, K., Bino, T., Shigenobu, S., and Kawaguchi, M. (2018). Evidence of non-tandemly repeated rDNAs and their intragenomic heterogeneity in *Rhizophagus irregularis*. Commun. Biol. *1*, 87.
- Masuoka, Y., Yaguchi, H., Toga, K., Shigenobu, S., and Maekawa, K. (2018). TGFβ signaling related genes are involved in hormonal mediation during termite soldier differentiation. PLoS Genet. *14*, e1007338.
- Miura, C., Yamaguchi, K., Miyahara, R., Yamamoto, T., Fuji, M., Yagame, T., Imaizumi-Anraku, H., Yamato, M., Shigenobu, S., and Kaminaka, H. (2018). The mycoheterotrophic symbiosis between orchids and mycorrhizal fungi possesses major components shared with mutualistic plant-mycorrhizal symbioses. Mol. Plant. Microbe. Interact. *31*, 1032–1047.
- Nikoh, N., Tsuchida, T., Maeda, T., Yamaguchi, K., Shigenobu, S., Koga, R., and Fukatsu, T. (2018). Genomic insight into symbiosis-induced insect color change by a facultative bacterial endosymbiont, "*Candidatus* Rickettsiella viridis." MBio *9*, e00890-18.
- Ohde, T., Morita, S., Shigenobu, S., Morita, J., Mizutani, T., Gotoh, H., Zinna, R.A., Nakata, M., Ito, Y., Wada, K., Kitano, Y., Yuzaki, K., Toga, K., Mase, M., Kadota, K., Rushe, J., Lavine, L.C., Emlen, D.J., and Niimi, T. (2018). Rhinoceros beetle horn development reveals deep parallels with dung beetles. PLoS Genet. *14*, e1007651.
- Ravinet, M., Yoshida, K., Shigenobu, S., Toyoda, A., Fujiyama, A., and Kitano, J. (2018). The genomic landscape at a late stage of stickleback speciation: High genomic divergence interspersed by small localized regions of introgression. PLoS Genet. *14*, e1007358.
- Sato, K., Kadota, Y., Gan, P., Bino, T., Uehara, T., Yamaguchi, K., Ichihashi, Y., Maki, N., Iwahori, H., Suzuki, T., Shigenobu, S., and Shirasu, K. (2018). High-quality genome sequence of the root-knot nematode *Meloidogyne arenaria* genotype A2-O. Genome Announc. *6*, e00519-18.
- Suzuki, M., Hayashi, T., Inoue, T., Agata, K., Hirayama, M., Suzuki, M., Shigenobu, S., Takeuchi, T., Yamamoto, T., and Suzuki, K.-I.T. (2018). Cas9 ribonucleoprotein complex allows direct and rapid analysis of coding and noncoding regions of target genes in *Pleurodeles waltl* development and regeneration. Dev. Biol. *443*, 127–136.
- Xu, C., Li, Q., Efimova, O., He, L., Tatsumoto, S., Stepanova, V., Oishi, T., Udono, T., Yamaguchi, K., Shigenobu, S., Kakita, A., Nawa, H., Khaitovich, P., and Go, Y. (2018). Human-specific features of spatial gene expression and regulation in eight brain regions. Genome Res. *28*, 1097–1110.
- Yaguchi, H., Shigenobu, S., Hayashi, Y., Miyazaki, S., Toga, K., Masuoka, Y., and Maekawa, K. (2018). A lipocalin protein, Neural Lazarillo, is key to social interactions that promote termite soldier differentiation. Proc. Biol. Sci. *285*, 20180707.
- Yamaoka, S., Nishihama, R., Yoshitake, Y., Ishida, S., Inoue, K., Saito, M., Okahashi, K., Bao, H., Nishida, H., Yamaguchi, K., Shigenobu, S., Ishizaki, K., Yamato, K.T., and Kohchi, T. (2018). Generative cell specification requires transcription factors evolutionarily conserved in land plants. Curr. Biol. *28*, 479–486.e5.

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

- Matsunami, M., Nozawa, M., Suzuki, R., Toga, K., Masuoka, Y., Yamaguchi, K., Maekawa, K., Shigenobu, S., and Miura, T. Caste-specific microRNA expression in termites: insights into soldier differentiation. Insect Mol. Biol. 2018 Oct 16.
- Yoshida, K., Ishikawa, A., Toyoda, A., Shigenobu, S., Fujiyama, A., and Kitano, J. Functional divergence of a heterochromatin-binding protein during stickleback speciation. Mol. Ecol. 2018 Aug 17.

● ***Research activity by S. Shigenobu***

Specially Appointed Associate Professor:	*SHIGENOBU, Shuji*
NIBB Research Fellow:	*OGAWA, Kota**
SOKENDAI Graduate Student:	*YORIMOTO, Shunta*
Visiting Scientist:	*CHUNG, Chen-yo*
	OGAWA, Kota
Technical Assistant:	*SUZUKI, Miyuzu*

Symbiogenomics

"Nothing, it seems, exists except as part of a network of interactions." (Gilbert & Epel, 2008)

Every creature on Earth exists among a network of various biological interactions. For example, many multicellular organisms, including humans, harbor symbiotic bacteria in their bodies. Some of them provide their hosts with essential nutrients deficient in the host's diet and others digest foods that are indigestible by the host alone. Despite numerous examples of symbioses and its intriguing outcomes, the genetic and molecular basis underlying these interactions remains elusive. The goal of our group is to establish a new interdisciplinary science known as "Symbiogenomics", where we aim to understand the network of biological interactions at the molecular and genetic level. To this end, we take advantage of state-of-the-art genomics, such as next-generation sequencing technologies.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2018. The former title is indicated by an asterisk (*).

Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, which are specialized cells for harboring said bacteria. This mutualism is so obligate that neither can reproduce independently. The genome sequence of the pea aphid, *Acyrthosiphon pisum*, in consort with that of bacterial symbiont *Buchnera aphidicola* illustrates the remarkable interdependency between these two organisms. The genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. Genome analysis revealed that the pea aphid has undergone characteristic gene losses and duplications. The IMB antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids in obtaining beneficial symbionts. Lineage-specific gene duplications have occurred in genes over 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 the 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 coinciding 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. Furthermore, some BCRs showed 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 = 20 um.

Publication List:

〔Original papers〕

- Nikoh, N., Tsuchida, T., Maeda, T., Yamaguchi, K., Shigenobu, S., Koga, R., and Fukatsu, T. (2018). Genomic insight into symbiosis-induced insect color change by a facultative bacterial endosymbiont, "*Candidatus* Rickettsiella viridis." MBio *9*, e00890-18.
- Suzuki, M., Hayashi, T., Inoue, T., Agata, K., Hirayama, M., Suzuki, M., Shigenobu, S., Takeuchi, T., Yamamoto, T., and Suzuki, K.-I.T. (2018). Cas9 ribonucleoprotein complex allows direct and rapid analysis of coding and noncoding regions of target genes in *Pleurodeles waltl* development and regeneration. Dev. Biol. *443*, 127–136.

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor:
KAMEI, Yasuhiro

Technical Staff:	*KONDO, Maki*
	TANIGUCHI-SAIDA, Misako
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, under the guidance of Dr. Kamei, also provides technical support through the management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers (refer to the Collaborative Research Group Research Enhancement Strategy Office section for more information). Among the equipment available are advanced biological microscopes, and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism and is capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve, thus allowing exposure to strong monochromatic light. The facility's microscopes, which include cutting edge devices such as confocal and multi-photon excitation microscopes, are an indispensable part of core and collaborative projects conducted by both internal and external researchers.

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.

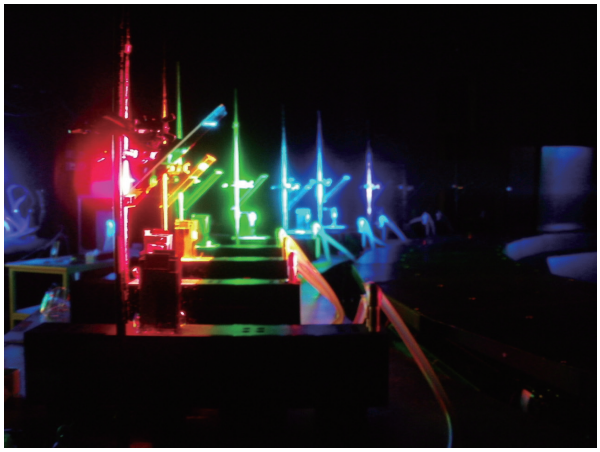

Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously to samples stored in cooling chambers.

In addition to the other action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted since it's establishment, 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.

Microscopes

This facility also provides bioimaging machinery, such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Leica TCS-SP8, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD/ CMOS cameras), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced laser microscopes boasting specialized, cutting edge technology (Light-sheet Microscope and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO), which can be utilized by researchers within NIBB, as well as collaborative guest researchers. We began two new types of Collaborative Research Programs from 2016. One is a new category of the NIBB Collaborative Research for Integrative Bioimaging program using machinery and bioimage processing/analysis techniques, and the other is the Advanced Bioimaging Support Program (ABiS) which operates under the framework of the Grant-in-aid for Scientific Research on Innovative Areas.

The light-sheet microscope 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 tissues by illuminating specimens 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 about 10 Collaborative Research Program projects for Integrative Bioimaging. The IR-LEGO, developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST), can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (details are provided in the next section). The IR-LEGO was also used for about 10 Collaborative Research projects, including applications for animals and plants.

Workshop, symposium and training course

In 2018 we held the 5th biological image processing training course with Drs. Kagayaki Kato, Shigenori Nonaka, Takashi Murata and Hiroshi Koyama (p. 104). 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 12th NIBB Bioimaging Forum focused on artificial intelligence (AI) for imaging field (p. 103). In addition, we held training courses focused on medaka, *Xenopus* and a new emerging model animal, Iberian ribbed newt, for domestic and also international participants (p. 101).

Publication List on Cooperation

〔Original papers (Selected)〕

- Hamada, T., Yako, M., Minegishi, M., Sato, M., Kamei, Y., Yanagawa, Y., Toyooka, K., Watanabe, Y., and Hara-Nishimura, I (2018). Stress granule formation is induced by a threshold temperature rather than a temperature difference in Arabidopsis. J. Cell Sci. *131*, jcs216051.
- Hasugata, R., Hayashi, S., Kawasumi-Kita, A., Sakamoto, J., Kamei, Y., and Yokoyama, H. (2018). Infrared laser-mediated single-cell-level gene induction in the regenerating tail of *Xenopus laevis* tadpoles. Cold Spring Harb. Protoc. *2018(12)*:pdb.prot101014.
- Kamijo, M., Kawamura, M. and Fukamachi, S. (2018). Loss of red opsin genes relaxes sexual isolation between skin-colour variants of medaka. Behav. Processes *150*, 25-28.
- Koshimizu, S., Kofuji, R., Sasaki-Sekimoto, Y., Kikkawa, M., Shimojima, M., Ohta, H., Shigenobu, S., Kabeya, Y., Hiwatashi, Y., Tamada, Y., Murata, T., and Hasebe, M. (2018). Physcomitrella MADS-box genes regulate water supply and sperm movement for fertilization. Nature Plants *4*, 36-45.
- Mano, S., Nishihama, R., Ishida, S., Hikino, K., Kondo, M., Nishimura, M., Yamato, K. T., Kohchi, T., and Nakagawa, T. (2018). Novel gateway binary vectors for rapid tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha*. PLoS ONE *13*, e0204964.
- Matsuo, M., Yoriko Ando, Y., Kamei, Y., and Fukamachi, S. (2018). A semi-automatic and quantitative method to evaluate behavioral photosensitivity in animals based on the optomotor response (OMR). Biology Open *7*, bio033175.
- Nagao,Y., Takada, H., Miyadai, M., Adachi, T., Seki, R., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Hibi, M., Kelsh, R.N., and Hashimoto, H. (2018). Distinct interactions of Sox5 and Sox10 in fate specification of pigment cells in medaka and zebrafish. PLoS Genet. *14*, e1007260.
- Nonomura, K., Lukacs, V., Sweet, D.T., Goddard, L.M., Kanie, A., Whitwam, T., Ranade, S.S., Fujimori, T., Kahn, M.L., and Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proc. Natl. Acad. Sci. USA *115*, 12817-12822.
- Tominaga, J., Nakahara, Y., Horikawa, D., Tanaka, A., Kondo, M., Kamei, Y., Takami, T., Sakamoto, W., Unno, K., Sakamoto, A., and Shimada, H. (2018). Overexpression of the protein disulfide isomerase AtCYO1 in chloroplasts slows darkinduced senescence in Arabidopsis. Plant Biol. *18*, 80.

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

- Amemiya, S., Hibino, T., Minokawa, T., Naruse, K., Kamei, Y., Uemura, I., Kiyomoto, M., Hisanaga, S., and Kuraishi, R. Development of the coelomic cavities in larvae of the living isocrinid sea lily *Metacrinus rotundus*. Acta Zool. 2018 Sep 23.

● *Research activity by Y. Kamei*

Specially Appointed Associate Professor: *KAMEI, Yasuhiro*
NIBB Research Fellow: *SAKAMOTO, Joe*
CREST Researcher: *KAMIKAWA, Yuko*
Technical Assistant: *NAKAGAWA, Mami*
TAMADA, Tomoko

Our research group promotes two cutting-edge microscope projects: “observation” and “manipulation” using optical and biological technologies.

The aim of our “observation project” is seeing deep into 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 Earth based telescopes may be disturbed by fluctuations in the atmosphere, AO technology can mitigate this disturbance. However, living materials have particular refractive indexes, so some organelles may hinder the ideal optical path for microscope observation, similar to the situation with the atmosphere and 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 the observation of living organisms in collaboration with Dr. Tamada of NIBB and Dr. Hayano of the National Astronomical Observatory of Japan (NAOJ) and subsequently acquired high-resolution bright field and fluorescent images of living cells. Our results indicated that improvements in optical resolution were restricted to a small area which is called the “isoplanatic patch”.

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 as such, 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), that has been specialized for this purpose (Figure 1). 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 a heat shock

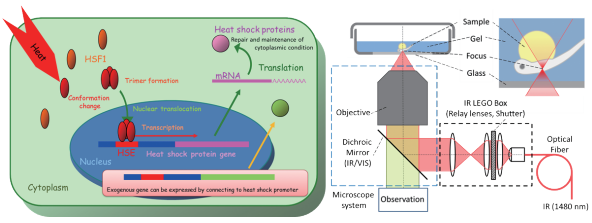

Figure 1. Schematic illustration of heat shock response of cells (left) and an infrared laser-evoked gene operator (IR-LEGO) microscope system.

response at the desired time. In 2015, we also confirmed the system was effective in the moss *Marchantia polymorpha* and in the newt *Pleurodeles waltl*.

Optimal heating induces a 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 showed that the temperature of the target area rose rapidly and kept a constant level dependent on IR laser power. Furthermore, the heated area was as small as the size of a typical cell.

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, while 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. We then tried this 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 of these species as was expected. Moreover, this system can be combined to the cre/loxP recombination technique for long-term gene expression (Figure 2).

As mentioned above, excess irradiation resulted in cell damage, so we utilized the system to ablate target cells with strong pulsed irradiation. In collaboration with National Taiwan University, we used the system for a neuronal regeneration study in zebrafish and revealed that a kind of neuronal precursor cell played an important role within the neuron regeneration step the in the zebrafish spinal cord (Zeng *et al*. Biol. Cell 2016). In addition, the IR-LEGO system can be utilized for biothermology, a new field that examines temperature or heat in biological systems, because spatiotemporal micrometer order local heating is difficult to perform without this system. We are currently trying to estimate the thermal properties of cells and biomaterials

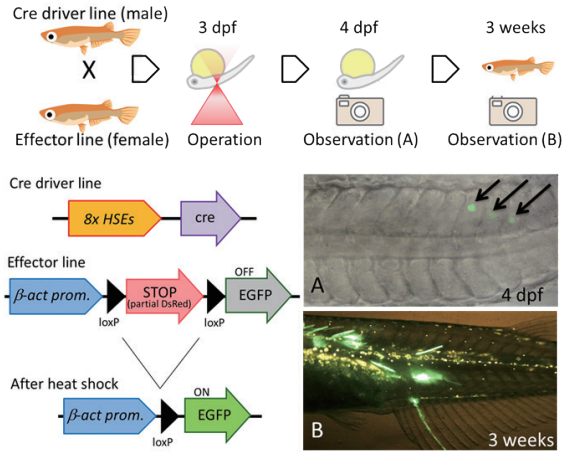

Figure 2. Long-term gene expression system with cre/loxP recombination system and an example of a practical experiment in medaka ranging embryos to adults.

in vivo using a newly developed thermo-probe (Nakano *et al*. PLoS One 2017) and the IR-laser heating microscope system. We held the 3rd Biothermology Workshop 2018 in Okazaki to build up the new basic biology field with other scientific fields, (such as chemistry and physics) and mathematics in parallel with the above mentioned research.

Publication List:

〔Original papers〕

- Hamada, T., Yako, M., Minegishi, M., Sato, M., Kamei, Y., Yanagawa, Y., Toyooka, K., Watanabe, Y., and Hara-Nishimura, I. (2018). Stress granule formation is induced by a threshold temperature rather than a temperature difference in Arabidopsis. J. Cell Sci. jcs216051.
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DATA INTEGRATION AND ANALYSIS FACILITY

Assistant Professor: *UCHIYAMA, Ikuo*
Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
SUGIURA, Hiroki
Technical Assistant: *OKA, Naomi*

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence, expression data, 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 the development of data analysis pipelines, construction of databases, and website setup to distribute data worldwide as well as providing basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the institute's network systems and provides 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 (HPE Apollo r2800, 20 nodes/800 cores, 192 GB memory/node), a shared memory parallel computer (HPE ProLiant DL560, 72 cores, 3TB memory; HP ProLiant DL980 G7, 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700X, 1.52PB+880TB), 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 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. We have especially provided support in 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 ORION network connecting the three research institutes in Okazaki. Many local services, including sequence analysis, file sharing, 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/en).

Figure 1. Biological Information Analysis System

Research activity conducted by Ikuo Uchiyama

Assistant Professor Ikuo 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.