NIBB Core Research Facilities

Head
KOBAYASHI, Satoru

The NIBB Core Research Facilities were launched in 2010 to support basic biology research in NIBB. They consist of three facilities that are developing and providing state-of-the-art technologies to understand biological functions through functional genomics, bioimaging and bioinformatics.

The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among the researchers of NIBB and other academic institutions.

Functional Genomics Facility

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
	TANIGUCHI-SAIDA, Misako
Technical Assistant:	*ASAO, Hisayo*
Secretary:	*ICHIKAWA, Mariko*

The Functional Genomics Facility is a division of the NIBB Core Research Facilities and organized jointly by NIBB and NIPS for promoting DNA and protein studies. The facility maintains a wide array of core research equipment, from standard machinery like ultracentrifuges to cutting edge tools such as next generation DNA sequencers, which amount to 60 different kinds of instruments. The facility is dedicated to fostering collaborations with researchers both of NIBB and other academic institutions worldwide by providing these tools as well as expertise. Our current focus is supporting functional genomics works that utilize mass spectrometers and DNA sequencers. We also act as a bridge between experimental biology and bioinformatics.

Representative Instruments

Genomics

The advent of next-generation sequencing technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD 4 System (Applied Biosystems), the Functional Genomics Facility is committed to joint research aiming to exploring otherwise inaccessible new fields in basic biology. SOLiD 4 can output data up to 100 G/run.

During 2010 we started 14 next-generation sequencing projects in collaboration with NIBB laboratories as well as the researchers of other academic institutions. These projects cover a wide range of species (bacteria, animals, plants and human) including both model and non-model organisms, and various applications such as genomic re-sequencing, RNA-seq and ChIP-seq. A successful example is the mutant screening of *Arabidopsis*. We successfully identified causative mutations in EMS mutant screening by deep sequencing quickly at low cost, which is much more effective than conventional mapping-based cloning methods.

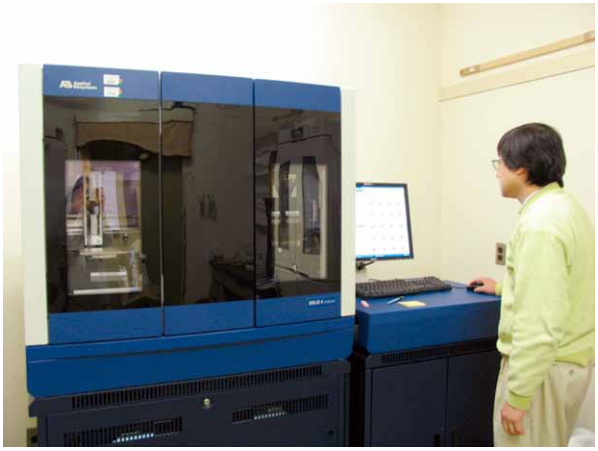

Figure 1. Next-generation sequencer SOLiD 4

Proteomics

Three different types of mass spectrometers and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2010, we analyzed approximately 400 samples by mass spectrometers and 80 samples by protein sequencers.

- GC/Mass Spectrometer (JEOL DX-300)
- 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)

Other analytical instruments

- Flow Cytometer (Coulter EPICS XL)
- Bio Imaging Analyzer (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser Capture Microdissection System (Arcturus XT)
- DNA Sequencer (ABI PRISM 310; ABI 3130xl)
- Real Time PCR (ABI 7500)
- Ultra Centrifuge (Beckman XL-80XP etc.)

Figure 2. LC/Q-TOF-MS

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

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

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SUZUKI, Miyuzu
Visiting Scientists: *GALLOT, Aurore*
SRINIVASAN, Dayalan

Symbiosis Genomics

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

Every creature on the earth exists among a network of various biological interactions. For example, many muticellular 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 indigestible by the host alone. In spite of 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 "Symbiosis Genomics", where we aim to understand the network of biological interactions at the molecular and genetic level. To this end, we take an advantage of state-of-the-art genomics such as next-generation sequencing technologies.

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

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, specialized cells for harboring the bacteria. The mutualism is so obligate that neither can reproduce independently. The newly released 464 Mb draft genome sequence of the pea aphid, *Acyrthosiphon pisum*, in consort with that of bacterial symbiont *Buchnera aphidicola* illustrates the remarkable interdependency between the two organisms. Genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. The 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 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.

Aphid research is entering the post-genome era. We analyzed the transcriptome of aphid bacteriocytes using RNA-seq technology featuring a next-generation DNA sequencer. We found thousands of genes over-represented in 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.

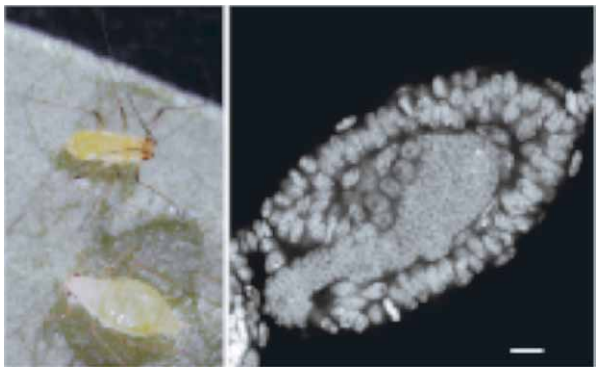

Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo in which symbionts are infecting (Right). Scale bar = 20um.

Publication List

〔Original papers〕

- Huang, T.-Y, Cook, C.E., Davis, G.K., Shigenobu, S., Chen, R. P.-Y., and Chang, C.-C. (2010). Anterior development in the parthenogenetic and viviparous form of the pea aphid, *Acyrthosiphon pisum*: hunchback and orthodenticle expression. Insect Mol. Biol. *19*, 75-85.
- Legeai, F., Shigenobu, S., Gauthier, J., Colbourne, J., Rispe, C., Collin, O., Richards, R., Wilson, A., and Tagu, D. (2010). AphidBase: A centralized bioinformatic resource for annotation of the pea aphid genome. Insect Mol. Biol. *19*, 5-12.
- Nakabachi, A., Shigenobu, S., and Miyagishima, S. (2010). Chitinase-like proteins encoded in the genome of the pea aphid, *Acyrthosiphon pisum*. Insect Mol. Biol. *19*, 175-185.
- Niwa, R., Namiki, T., Ito, K., Shimada-Niwa, Y., Kiuchi, M., Kawaoka, S., Kayukawa, T., Banno, Y., Fujimoto, Y., Shigenobu, S., Kobayashi, S., Shimada, T., Katsuma, S., and Shinoda, T. (2010). *Non-molting glossy/shroud* encodes a short-chain dehydrogenase/reductase that functions in the "Black Box" of the ecdysteroid biosynthesis pathway. Development *137*, 1991-1999.
- Price, D.R.G., Tibbles, K., Shigenobu, S., Smertenko, A., Russel, C.W., Douglas, A.E., Fitches, E., Gatehouse, A.M.R., and Gatehouse, J.A. (2010). Sugar transporters of the major facilitator superfamiliy in aphids; from gene prediction to fucntional characterization. Insect Mol. Biol. *19*, 97-112.
- Shigenobu, S., Bickel, R.D., Brisson, J.A., Butts, T., Chang, C., Christiaens, O., Davis, G.K., Duncan, E.J., Ferrier, D.E.K., Iga, M., Janssen, R., Lin, G., Lu, H., McGregor, A.P., Miura, T., Smagghe, G. Smith, J.M., van der Zee, M., Velarde, R., Wilson, M.J., Dearden, P.K., and Stern, D.L. (2010). Comprehensive survey of developmental genes in the pea aphid, *Acyrthosiphon pisum*: frequent lineage-specific duplications and losses of developmental genes. Insect Mol. Biol. *19*, 47-62.
- Shigenobu, S., Richards, S., Cree, A.G., Morioka, M., Fukatsu, T., Kudo, T., Miyagishima, S., Gibbs, R.A., Stern, D.L., and Nakabachi, A. (2010). A full-length cDNA resource for the pea aphid, *Acyrthosiphon pisum*. Insect Mol. Biol. *19*, 23-32.
- The International Aphid Genomics Consortium. Genome Sequence of the Pea Aphid *Acyrthosiphon pisum*. PLoS Biol. *8*, e1000313.

Spectrography and Bioimaging Facility

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Staff: *HIGASHI, Sho-ichi*
TANIGUCHI-SAIDA, Misako
Technical Assistants: *ENDOU, Seiichiro*
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 confocal microscopes and the Okazaki Large Spectrograph. 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 two-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 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 and Nikon A1R) and other custom-made laser microscopes (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 last April.

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 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 5 Individual Collaborative Research projects, including applications for higher plants and small fish.

Publication List on Collaboration

〔Original papers〕

- Arimoto-Kobayashi, S., Sano, K., Machida, M., Kaji, K., and Yakushi, K. (2010). UVA activation of N-dialkylnitrosamines releasing nitric oxide, producing strand breaks as well as oxidative damages in DNA, and inducing mutations in the Ames test. Mut. Res. *691*, 47-54.
- Hayashi, Y., Kobira, H., Yamaguchi, T., Shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y., and Kitano, T. (2010). High temperature causes masculinization of genetically female medaka by elevation of cortisol level. Mol. Reprod. Dev. *77*, 679-686.

● *Research activity by Y. Kamei*

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

To investigate a gene function in each cell we have to express the gene in the cell *in vivo*, 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.

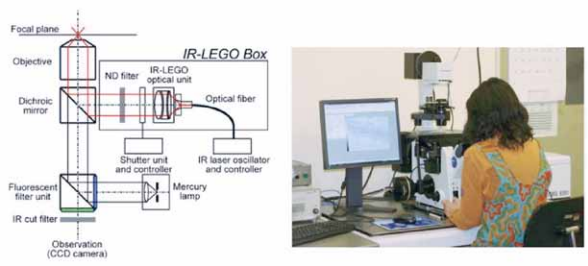

Figure 1. Infrared laser evoked gene operator (IR-LEGO) microscope system at NIBB

In 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 time course 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). On the other hand, the heated area was limited to a small volume about as large as a typical cell.

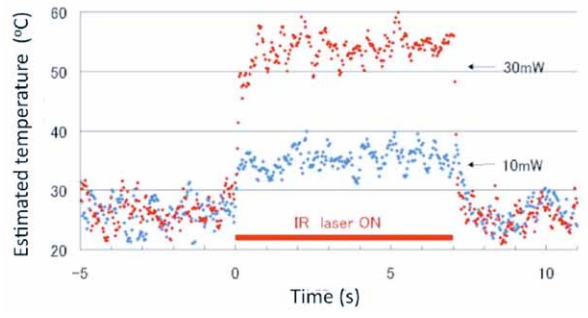

Figure 2. Time course of temperature at IR-laser focus
IR laser was irradiated to EGFP expressing bacterium by IR-LEGO microscope. Fluorescent intensity change was used to estimate temperature shift. Temperature was raised by laser irradiation and kept at a constant level during the irradiation.

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 fishes, medaka and zebrafish, 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 (Figure 3).

Studies for 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. Applying to a mutant and its rescue transgenic strain; hsp-cre and rescue gene which is sandwiched by loxP sequence, we will achieve single-cell knockout experiments in living organism, 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 started establishment of a cre-loxP system in medaka.

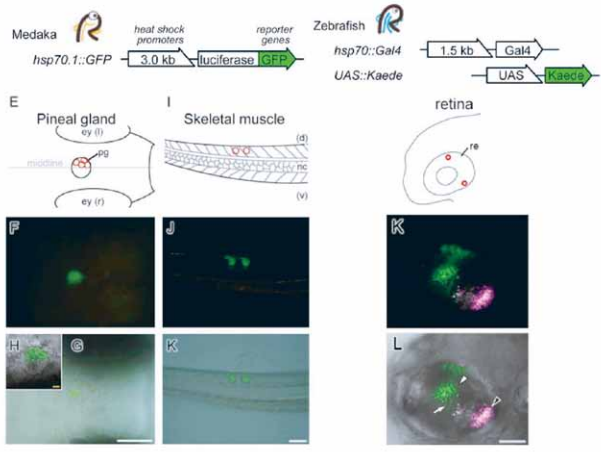

Figure 3. Local gene expression in fish by IR-LEGO
GFPs were expressed in various tissue in medaka and zebrafish. IR-LEGO can induce local gene expression by heat shock response in many organisms.

Publication List

〔Original papers〕

- Hayashi, Y., Kobira, H., Yamaguchi, T., Shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y., and Kitano, T. (2010). High temperature causes masculinization of genetically female medaka by elevation of cortisol level. Mol. Reprod. Dev. *77*, 679-686.
- Ishikawa, T.#, Kamei Y.#, Otozai, S., Kim, J., Sato, A., Kuwahara, Y., Tanaka, M., Deguchi, T., Inohara, H., Tsujimura, T., and Todo, T. (2010). High-resolution melting curve analysis for rapid detection of mutations in a Medaka TILLING library. BMC Mol. Biol. *11*, 70. (#: equally contribution)
- Oda, S., Mikami, S., Urushihara, Y., Murata, Y., Kamei, Y., Deguchi, T., Kitano, T., Fujimori, K.E., Yuba, S., Todo, T., and Mitani, H. (2010). Identification of a functional Medaka heat shock promoter and characterization of its ability to induce in vitro and in vivo exogenous gene expression in Medaka. Zool. Sci. *27*, 410-415.

Data Integration and Analysis Facility

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

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

The 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 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.

Figure 1. Biological Information Analysis System

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.71).