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

Specially Appointed Associate Professor
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
Technical Assistant:	*ASAO, Hisayo*
	WAKAZUKI, Sachiko
	MATSUMOTO, Miwako
	FUJITA, Miyako
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 instrument. 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 (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD5500xl (Applied Biosystems), HiSeq2500, HiSeq1500 (Illumina), and MiSeq (Illumina) the Functional Genomics Facility is committed to joint research aiming to explore otherwise inaccessible new fields in basic biology.

During 2013 we carried out 41 NGS 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 humans) including both model and non-model organisms, and various 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 2013, we analyzed approximately 300 samples with mass spectrometers and 50 samples with 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

- Cell sorter (SONY SH800)
- Bioimaging 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.)

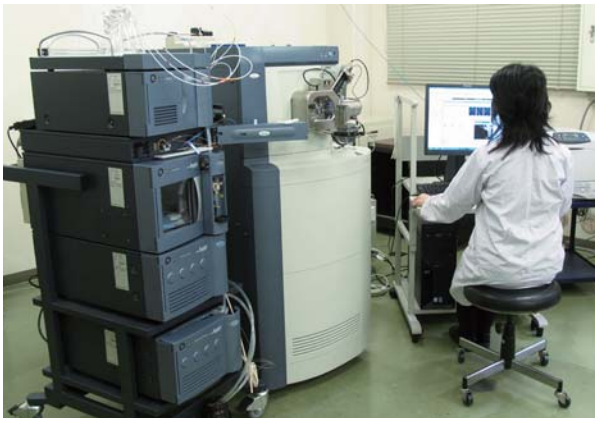

Figure 2. LC-Q-TOF mass spectrometer

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2013, we provided two two-day training courses on next-generation sequence data analyses and transcriptome analysis. These courses are designed to introduce the basic knowledge and skills of bioinformatics analysis to biologists who are not familiar with bioinformatics.

Figure 3. NIBB Genome Informatics Training Course

Publication List on Cooperation

〔Original papers〕

- Arimura, T., Onoue, K., Takahashi-Tanaka, Y., Ishikawa, T., Kuwahara, M., Setou, M., Shigenobu, S., Yamaguchi, K., Bertrand, A.T., Machida, N., *et al*. (2013). Nuclear accumulation of androgen receptor in gender difference of dilated cardiomyopathy due to lamin A/C mutations. Cardiovasc. Res. *99*, 382–394.
- Ishikawa, T., Okada, T., Ishikawa-Fujiwara, T., Todo, T., Kamei, Y., Shigenobu, S., Tanaka, M., Saito, T.L., Yoshimura, J., Morishita, S., *et al*. (2013). ATF6α/β-mediated adjustment of ER chaperone levels is essential for development of the notochord in medaka fish. Mol. Biol. Cell *24*, 1387–1395.
- Okamoto, S., Shinohara, H., Mori, T., Matsubayashi, Y., and Kawaguchi, M. (2013). Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nat. Commun. *4*, 2191.
- Tabata, R., Kamiya, T., Shigenobu, S., Yamaguchi, K., Yamada, M., Hasebe, M., Fujiwara, T., and Sawa, S. (2013). Identification of an EMS-induced causal mutation in a gene required for boron-mediated root development by low-coverage genome re-sequencing in Arabidopsis. Plant Signal. Behav. *8*, e22534.
- Takahara, M., Magori, S., Soyano, T., Okamoto, S., Yoshida, C., Yano, K., Sato, S., Tabata, S., Yamaguchi, K., Shigenobu, S., *et al*. (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.
- Tokuda, G., Elbourne, L.D.H., Kinjo, Y., Saitoh, S., Sabree, Z., Hojo, M., Yamada, A., Hayashi, Y., Shigenobu, S., Bandi, C., *et al*. (2013). Maintenance of essential amino acid synthesis pathways in the *Blattabacterium cuenoti* symbiont of a wood-feeding cockroach. Biol. Lett. *9*, 20121153.
- Wang, Z., Pascual-Anaya, J., Zadissa, A., Li, W., Niimura, Y., Huang, Z., Li, C., White, S., Xiong, Z., Fang, D., *et al*. (2013). The draft genomes of soft-shell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. Nat. Genet. *45*, 701–706.

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

- Uehara, M., Wang, S., Kamiya, T., Shigenobu, S., Yamaguchi, K., Fujiwara, T., Naito, S., and Takano, J. (2014). Identification and characterization of an Arabidopsis mutant with altered localization of NIP5;1, a plasma membrane boric acid channel, reveals the requirement for D-galactose in endomembrane organization. Plant Cell Physiol. 2013 Dec. 15.

● *Research activity by S. Shigenobu*

Specially Appointed Associate Professor: *SHIGENOBU, Shuji*
NIBB Research Fellow: *MAEDA, Taro*
Technical Assistant: *SUZUKI, Miyuzu*
Visiting Graduate Student: *OGAWA, Kota*

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 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 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 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 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.

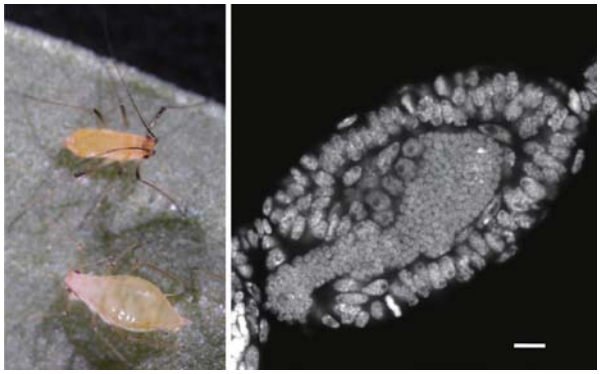

Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

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 *Acyrthosiphon 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.

Spectrography and Bioimaging Facility

Specially Appointed Associate Professor
KAMEI, Yasuhiro

Technical Staff: *HIGASHI, Sho-ichi*
TANIGUCHI-SAIDA, Misako
UCHIKAWA, Tamaki
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).

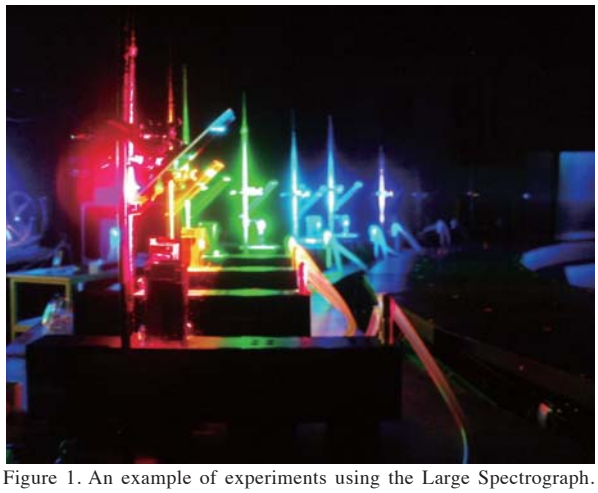

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.