NIBB Core Research Facilities

Head
YOSHIDA, Shosei

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
	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 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.

During 2014, the Functional Genomics Facility was largely renovated. For example, the Visitors Lab and the Visitors Office were newly designed to promote collaboration projects.

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 (Life technologies), HiSeq2500, HiSeq1500, and MiSeq (Illumina), the Functional Genomics Facility is committed to joint research aiming to explore otherwise inaccessible new fields in basic biology.

During 2014 we carried out 37 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 sequencers

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2014, we analyzed approximately 200 samples with mass spectrometers and 50 samples with 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)

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. A mass spectrometry system

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2014, we provided two three-day training courses on RNA-seq data 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 of Collaborative Research

〔Original papers〕

- Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., Baumann, A., and Blenau, W. (2014). Cockroach GABAB receptor subtypes: Molecular characterization, pharmacological properties and tissue distribution. Neuropharmacology. 88, 134-144.
- Furuta, Y., Namba-Fukuyo, H., Shibata, T.F., Nishiyama, T., Shigenobu, S., Suzuki, Y., Sugano, S., Hasebe, M., and Kobayashi, I. (2014). Methylome diversification through changes in DNA methyltransferase sequence specificity. PLoS Genet. 10, e1004272.
- Ishida, T., Tabata, R., Yamada, M., Aida, M., Mitsumasu, K., Fujiwara, M., Yamaguchi, K., Shigenobu, S., Higuchi, M., Tsuji, H., Shimamoto, K., Hasebe, M., Fukuda, H., and Sawa, S. (2014). Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. EMBO Rep. 5, 1202-1209.
- 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.
- Matsui, H., Takahashi, T., Murayama, S.Y., Uchiyama, I., Yamaguchi, K., Shigenobu, S., Matsumoto, T., Kawakubo, M., Horiuchi, K., Ota, H., Osaki, T., Kamiya, S., Smet, A., Flahou, B., Ducatelle, R., Haesebrouck, F., Takahashi, S., Nakamura, S., and Nakamura, M. (2014). Development of new PCR primers by comparative genomics for the detection of Helicobacter suis in gastric biopsy specimens. Helicobacter 19, 260–271.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, Sdgc, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development 141, 3363-3369.
- 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. 55, 704-714.
- Yoshida, K., Makino, T., Yamaguchi, K., Shigenobu, S., Hasebe, M., Kawata, M., Kume, M., Mori, S., Peichel, C.L., Toyoda, A., Fujiyama, A., and Kitano, J. (2014). Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. PLoS Genet. 10, e1004223.

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

Specially Appointed Associate Professor:	*SHIGENOBU, Shuji*
NIBB Research Fellow:	*MAEDA, Taro*
Postdoctoral ellow:	*HOJO, Masaru*
	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 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 "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.

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.

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).

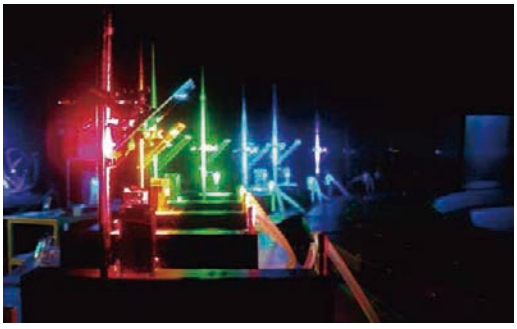

Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.