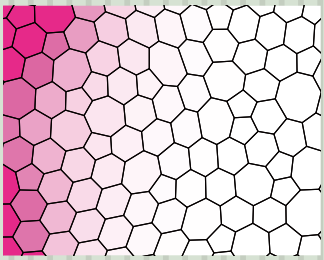
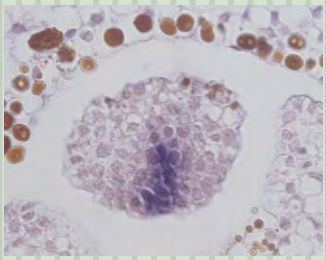

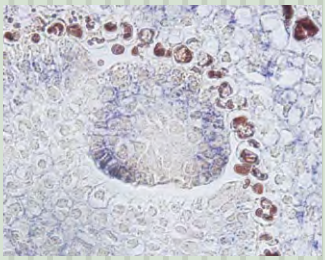
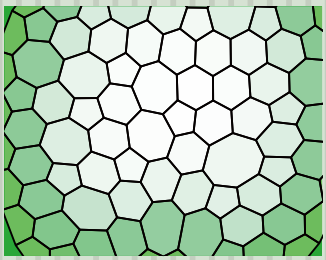

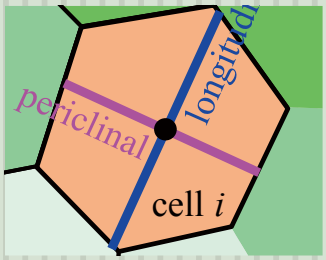

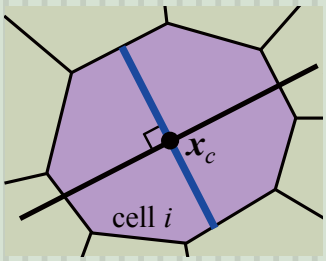

National Institute for Basic Biology

2015 ANNUAL REPORT

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Access to NIBB ..... Inside back cover

The cover items are related to a study dissecting the evolution of pitcher leaf development in *Sarracenia purpurea*, a carnivorous plant native to North America (Fukushima et al., Nat. Commun. 2015). A graduate student working at NIBB and his colleagues examined the process of pitcher leaf development by scanning electron microscopy, gene expression analyses, cell division pattern analyses, and a mathematical reconstruction of pitcher morphogenesis. They showed that a tissue-specific regulation of oriented cell division is the key factor for pitcher development. A computational modeling of leaf morphogenesis also supports these results. One of the most mysterious questions in evolutionary biology is the evolution of novel complex traits. This study shows that change at the cellular level can result in quite a big change in morphology. See page 40 of this report for details.

It is my great pleasure to introduce to you all the 2015 Annual Report of the National Institute for Basic Biology (NIBB), which outlines the high level research activities of the Institute and its effective function as a center for collaborative research in Japan over the last year. Given these important missions of the Institute, I recognize my strong responsibility to maintain the liberal atmosphere for research and discussion that NIBB has kept for many years, which I believe is a basis for these remarkable activities.

To improve the economic status of the country, the Japanese government continues to strengthen the demand that universities and inter-university research institute corporations, the latter of which includes NIBB, should reform themselves and draw actual profits from science. It is also necessary for the scientific community to achieve full compliance and establish integrity in research activities, to recover society's reliance on science. Under these circumstances NIBB must stride properly. We will maintain two directions steadily. One is that every person in NIBB should do his/her best in accomplishing good research in basic biology. Good science, even in a basic research field, will eventually benefit human beings. The history of science tells us this is true. The other is that NIBB must achieve high ethical standards for research that conform to the era of computer technology and data sharing through the internet. NIBB will work hard to be truly acknowledged as a remarkable institution by society.

Please find in this booklet a summary of the research, collaborative, educational, and international activities of NIBB in 2015. I would like to note that we welcomed several new colleagues in 2015, including one professor, one associate professor, one specially appointed associate professor, two assistant professors, and seven NIBB research fellows, while ten colleagues transferred to other institutes.

Finally I would like to congratulate Professor Emeritus Yoshinori Ohsumi for winning several outstanding international awards, and Adjunct Professor Takashi Yoshimura for winning the Van Meter Award in 2015 (Addendum: Prof. Ohsumi has been elected as a 2016 Nobel Laureate in Physiology or Medicine) . I would also like to congratulate our young colleagues for winning awards from academic societies as detailed on page 7.

To establish NIBB as an international leading institute in the field of basic biology, we always welcome your suggestions, comments and queries concerning our activities, in addition to your warm support.

Masayuki Yamamoto
Director General of NIBB
October 17, 2016

ORGANIZATION OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

The National Institute for Basic Biology (NIBB) was founded in 1977 on a hill overlooking the old town of Okazaki in Aichi prefecture as one of the Inter-University Research Institutes to promote and stimulate studies of biology. NIBB conducts first-rate research on site as well as in cooperation with national, public and private universities and research organizations. NIBB attempts to elucidate the general and fundamental mechanisms underlying various biological phenomena throughout a wide variety of biological fields, such as cell biology, developmental biology, neurobiology, evolutionary biology, environmental biology, and theoretical biology.

NIBB and four other national institutes, the National Astronomical Observatory of Japan (NAOJ), the National Institute for Fusion Science (NIFS), the National Institute for Physiological Sciences (NIPS), and the Institute for Molecular Science (IMS), constitute the National Institutes of Natural Sciences (NINS), which is one of the four Inter-University Research Institute Corporations. NIBB's activities are supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. The Okazaki Institute for Integrative Bioscience was established as a common facility for the three institutes (NIBB, NIPS, and IMS) of the Okazaki campus.

Buildings in the Myodaiji area (left) and Yamate area (right).

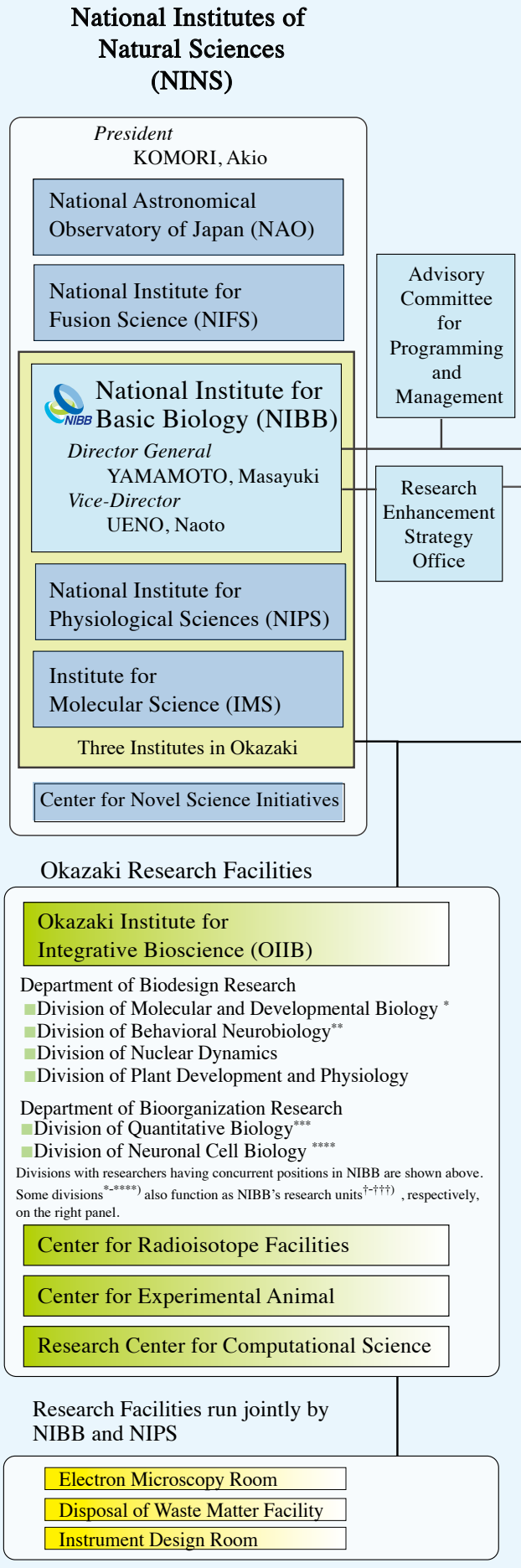
Policy, Decision Making, and Administration

The Director General oversees the operation of NIBB, assisted by the Advisory Committee for Programming and Management. The Advisory Committee, comprised of eleven professors within NIBB and ten leading biologists outside NIBB, advises the Director General on important matters such as planning joint research programs as well as on the scientific activities of NIBB. The Advisory Committee makes recommendations on the appointments of new Directors General, faculty appointments, NIBB's annual budget and future prospects.

The Research Enhancement Strategy Office, aimed at supporting researchers in order to improve NIBB's abilities as a collaborative research institution, was restructured in 2013 from the former Strategic Planning Department, Office of Public Relations, and Office of International Cooperation. The Office is made up of five groups (p. 85) and its activities are mainly carried out by URAs (University Research Administrators) in close collaboration with the NINS's Research Enhancement Promotion Headquarters.

Administration of NIBB is undertaken by the Okazaki Administration Office under the direct auspices of the Administration Office of NINS.

Organization



National Institute for Basic Biology (NIBB)

As of April 1, 2016

Research Units

- **Cell Biology**
  - Division of Cellular Dynamics
  - Division of Quantitative Biology†††
  - Laboratory of Cell Responses
  - Laboratory of Neuronal Cell Biology††††
  - Laboratory of Stem Cell Biology
- **Developmental Biology**
  - Division of Morphogenesis
  - Division of Molecular and Developmental Biology †
  - Division of Embryology
  - Division of Germ Cell Biology
  - Laboratory of Molecular Genetics for Reproduction
- **Neurobiology**
  - Division of Molecular Neurobiology
  - Division of Behavioral Neurobiology††
  - Division of Brain Circuits
  - Laboratory of Neurophysiology
- **Evolutionary Biology and Biodiversity**
  - Division of Evolutionary Biology
  - Division of Symbiotic Systems
  - Division of Evolutionary Developmental Biology
  - Laboratory of Morphodiversity
  - Laboratory of Bioresources
  - Laboratory of Biological Diversity
- **Environmental Biology**
  - Division of Environmental Photobiology
  - Division of Seasonal Biology (Adjunct)
- **Theoretical Biology**
  - Laboratory of Genome Informatics
- **Imaging Science**
  - Laboratory for Spatiotemporal Regulations

Research Support Facilities

- **NIBB Core Research Facilities**
  - Functional Genomics Facility
  - Spectrography and Bioimaging Facility
  - Data Integration and Analysis Facility
- **NIBB BioResource Center**
  - Model Animal Research Facility
  - Model Plant Research Facility
  - Cell Biology Research Facility
- **NIBB Center of the Interuniversity Bio-Backup Project (IBBP Center)**
- **NIBB Center for Model Organism Development**

Research Support Section

- **Technical Division**

- Evaluation and Information Group
- Public Relations Group
- International Cooperation Group
- Collaborative Research Group
- Gender Equality Promotion Group

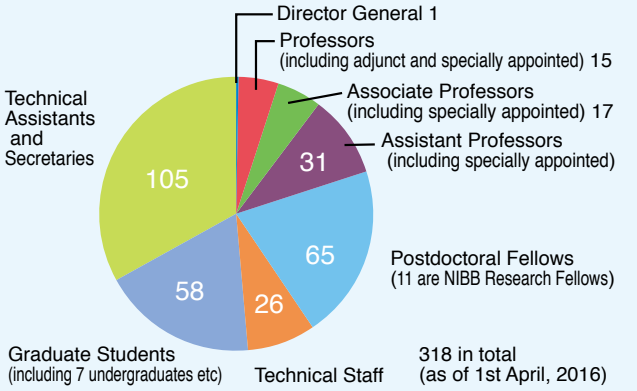
- **Okazaki Administration Office**

Research and Research Support

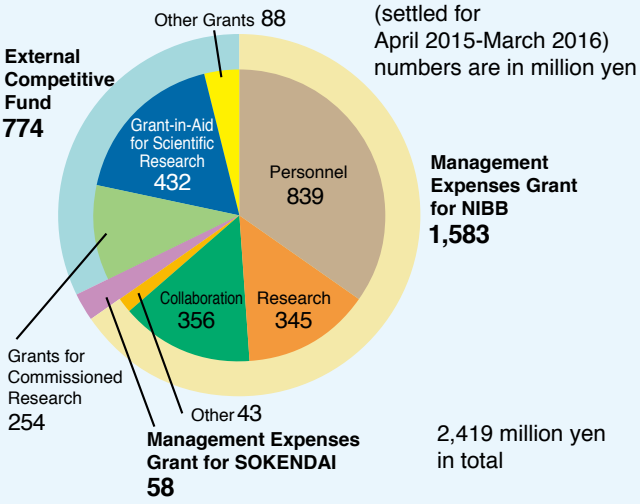
NIBB's research programs are conducted in research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research Facilities and NIBB BioResource Center were launched in 2010 to support research in NIBB and also to act as an intellectual hub to promote collaboration among the researchers of NIBB and other institutions. The NIBB Center of the Inter-University Bio-Backup Project (IBBP Center) was founded in 2012 to prevent loss of invaluable biological resources. Projects for the development of bio-resource preservation technology are solicited by the IBBP center from 2013. The NIBB Center for Model Organism Development was founded in 2013 to promote development of new model organisms and research using them. The Technical Division manages the activities of the technical staff and helps to promote the activities of each research unit and to maintain the common resources of both the research support units of NIBB and the research facilities of the Okazaki campus. Center for Radioisotope Facilities are one of the latter and run by the technical staff of NIBB.

The Okazaki Institute for Integrative Bioscience was founded to conduct interdisciplinary molecular research on various biological phenomena and is shared by all three institutes in Okazaki. Some of the divisions also function as NIBB divisions.

Members in NIBB



Financial Configuration of NIBB



Members of the Advisory Committee for Programming and Management (as of April, 2016)

Non-NIBB members	HAKOSHIMA, Toshio ##	Professor, Nara Institute of Science and Technology
	HIGASHIYAMA, Tetsuya	Professor, Nagoya University
	KOHSHIMA, Shiro	Professor, Kyoto University
	KONDO, Shigeru	Professor, Osaka University
	KUME, Shoen	Professor, Tokyo Institute of Technology
	KURUMIZAKA, Hitoshi	Professor, Waseda University
	NISHITANI, Kazuhiko	Professor, Tohoku University
	NOSE, Akinao	Professor, The University of Tokyo
	OHTA, Kunihiro	Professor, The University of Tokyo
	TSUKITA, Sachiko	Professor, Osaka University
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	HIGASHIJIMA, Shin-ichi*	Professor, National Institute for Basic Biology
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	MINAGAWA, Jun	Professor, National Institute for Basic Biology
	NIIMI, Teruyuki*	Professor, National Institute for Basic Biology
	NODA, Masaharu	Professor, National Institute for Basic Biology
	TAKADA, Shinji #	Professor, Okazaki Institute for Integrative Bioscience
	UENO, Naoto	Professor, National Institute for Basic Biology
	YOSHIDA, Shosei	Professor, National Institute for Basic Biology
	YOSHIMURA, Takashi	Adjunct Professor, National Institute for Basic Biology

* new member from April 2016
\# Chairperson
Vice-Chair

GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

The National Institute for Basic Biology (NIBB) sets five goals for its activities in pursuing the progress of biology. We contribute to the world-wide community of biologists through our efforts to accomplish these goals. This chapter briefly explains four of these goals. The last goal, the promotion of academic research, is accomplished through our research activities, which are introduced throughout this brochure.

Promotion of Collaborative Research

Collaborative Research Support

Research activities in collaboration with NIBB's divisions/laboratories using NIBB's facilities are solicited from external researchers. "Individual collaborative research projects" are the basic form of collaboration support which provide external researchers with travel and lodging expenses to visit NIBB's laboratories for collaborative research. For the use of NIBB's unique and excellent research instruments, such as the large spectrograph, the DSLM, and next generation DNA sequencers experimental projects are solicited and reviewed to provide machine time and travel expenses. "Priority collaborative research projects" are carried out as group research by internal and external researchers to develop pioneering research fields. "Collaborative research projects for model organism/technology development" and "Collaborative research projects for bioresource preservation technology development" are for developing and establishing new model organisms and new research technology. For these projects, research expenses in addition to travel expenses are provided. Bioimage processing and analysis collaborative research projects were started in 2015 and are for development and application of image analysis methods and travel expenses are supported.

year	2012	2013	2014	2015
Priority collaborative research projects	5	2	1	2
Collaborative research projects for model organisms/ technology development	3	4	2	3
Individual collaborative research projects	89	89	87	88
NIBB workshops	6	4	3	6
Collaborative experiments using the large spectrograph	14	15	12	10
Collaborative experiments using the DSLM	5	9	10	11
Bioimage processing and analysis collaborative research projects				14
Collaborative experiments using the next generation DNA sequencer	47	41	37	46
Facility Use (Training Course Facility)	2	1	0	1
Collaborative research projects for bioresource preservation technology development		9	10	9
total	171	174	162	190

Collaborative Research Projects by Year

NIBB Core Research Facilities

The NIBB Core Research Facilities support research in NIBB and also act as an intellectual hub to promote collaboration between NIBB and other academic institutions. They consist of three facilities that are developing and providing state-of-the-art technologies through functional genomics, bioimaging and bioinformatics (p. 71).

The Functional Genomics Facility maintains a ide array of core research equipment, including next generation DNA sequencers. The facility is dedicated to fostering NIBB's collaborative research by providing these tools as well as expertise. The current focus is supporting functional genomics works that utilize mass spectrometers and DNA sequencers, holding training courses as one of these undertakings (p. 90). The Spectrography and Bioimaging Facility manages research tools, such as confocal microscopes, DSLM and the large spectrograph, and provides technical support and scientific advice to researchers. These two facilities hold specially appointed associate professors, an expert in each field, with a mission to manage each facility as well as conducting his own academic research. The Data Integration and Analysis Facility supports analysis of large-scale biological data, such as genomic sequence data, gene expression data, and imaging data. For this purpose, the facility maintains high-performance computers with large-capacity storage systems.

Bio-Resources

The National BioResource Project (NBRP) is a national project for the systematic accumulation, storage, and supply of nationally recognized bio-resources (experimental animals and plants, cells, DNA, and other genetic resources), which are widely used as materials in life science research. To promote this national project, NIBB has been appointed as a center for research on Medaka (*Oryzia latipes*) whose usefulness as a vertebrate model was first shown by Japanese researchers. The usability of Medaka as a research material in biology has drawn increasing attention since its full genome sequence recently became available. NIBB is also a sub-center for the NBRP's work with Japanese morning glory. The NIBB BioResource Center has equipment, facilities, and staff to maintain Medaka and Japanese morning glory safely, efficiently, and appropriately. The center also maintains other model organisms, such as mice, zebrafish, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, and provides technical support and advice for the appropriate use of these organisms (p. 77).

Strains of Japanese morning glory maintained in the center

NIBB Center of the Inter-University Bio-Backup Project (IBBP Center)

To prevent damage to important biological resources by natural disasters, NIBB established the IBBP Center in 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources under cutting-edge research (p.81).

International Cooperation and Outreach

Collaborative Programs with Overseas Institutes

NIBB takes a leading role in the collaborative research programs between the European Molecular Biology Laboratory (EMBL) and the National Institutes of Natural Sciences (NINS) and promotes personal and technological exchange through joint meetings, exchange between researchers and graduate students, and the introduction of experimental equipment.

NIBB formed an agreement with the Temasek Life Sciences Laboratory (TLL) of Singapore and the Max Planck Institute for Plant Breeding Research (MPIPZ) to promote joint research projects, collaborative symposia, training courses and student exchange programs. The 5th NIBB-MPIPZ-TLL Joint Symposium " Horizons in Plant Biology " was held in November, 2014 at NIBB.

NIBB Conference

The NIBB Conferences are international conferences on hot topics in biology organized by NIBB's professors. Since the first conference in 1977 (the year of NIBB's foundation), NIBB Conferences have provided researchers in basic biology with valuable opportunities for international exchange. The 63rd conference " Environment to Bioresponse " was held in November, 2015 (p.88).

International Practical Course

With the cooperation of researchers from Japan and abroad the NIBB international practical course is given at a laboratory specifically prepared for its use. The 8th course "Experimental Techniques Using Medaka and *Xenopus* - The Merits of Using Both -" was held jointly with TLL and the National University of Singapore (NUS) in September, 2014 at NIBB. Graduate students and young researchers from various areas including Taiwan, Hong Kong, India, Indonesia, Bangladesh, Germany, the United States, and Japan, were provided with training in state-of-the-art research techniques. International conferences and courses are managed by the International Cooperation Group of the Research Enhancement Strategy Office.

Outreach

NIBB's outreach activities aim to present cutting edge research results to the public via mass media through press releases or directly through the internet, such as web pages, Facebook, and Twitter. Our triannual open campus event was held in 2013 at which we welcomed more than 1,300 local citizens. NIBB also cooperates in the education of undergraduate and younger students through lectures and workshops. Outreach activities are mostly managed by the Public Relations Group of the Research Enhancement Strategy Office.

Development of New Fields of Biology

Bioimaging

NIBB aims to maximize the application of modern light microscopes and biophotonic probes for real time visualization of biological phenomena and to develop new imaging techniques. As part of our collaborative work with EMBL, NIBB introduced a DSLM, which is effective for the three-dimensional observation of living organisms, and has developed an improved model using two-photon optics (p. 68). The application of the adaptive optics to microscopy is under way in collaboration with the National Astronomical Observatory of Japan. The Advisory Committee on Bioimaging, comprised of leading researchers in the bioimaging field in Japan, is organized to formulate advice on NIBB's imaging research. The Bioimaging Forum provides an opportunity for researchers and company engineers to frankly discuss practical difficulties and needs regarding imaging. The 9th Forum "Imaging of Physical Properties" was held in January 2015 (p. 90). A training course in bioimage analysis was also held in 2015 (p. 91).

Okazaki Biology Conferences

NIBB holds Okazaki Biology Conferences (OBC) that, with the endorsement of the Union of Japanese Societies for Biological Science, aim to explore new research fields in biology and support the formation of international communities in these fields. Dozens of top-level researchers from Japan and abroad spend nearly one week together for intensive discussions seeking strategies for addressing future critical issues in biology. Past Conferences have promoted the formation of international researcher communities.

Cultivation of Future Researchers

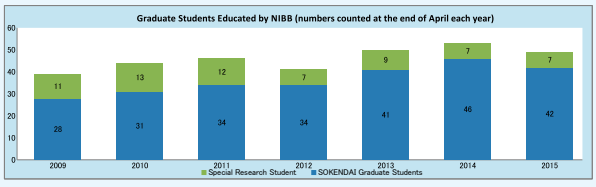
NIBB constitutes the Department of Basic Biology in the School of Life Science of the SOKENDAI (Graduate University for Advanced Studies). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree.

Graduate students enrolled in other universities and institutions can apply to be special research students eligible to conduct research under the supervision of NIBB professors.

In both cases above, graduate students can receive financial support from NIBB based on the research assistant (RA) system from the beginning of the five-year course.

Due to the international collaboration with EMBL, graduate students are encouraged to attend PhD student symposia held at EMBL and provided an opportunity to give oral and poster presentations, at least once during their master's and doctoral program (p. 89).

Students from Japan and abroad can also come to NIBB through our Internship Program. Internships give students an excellent way to build international connections while experiencing hands on research in a world class research institute (p. 92).



Graduate students educated by NIBB

Personnel changes in 2015*

Newly assigned in NIBB

Name	Position	Research Unit	Date
TSUBOUCHI, Tomomi	Associate Professor	Laboratory of Stem Cell Biology	February 1
KAWADE, Kensuke	Specially Appointed Associate Professor	Laboratory of Plant Development and Physiology (BIONEXT Project)	March 1
YASUGI, Masaki	NIBB Research Fellow	Laboratory of Neurophysiology	April 1
HIRA, Seiji	NIBB Research Fellow	Division of Germ Cell Biology	April 1
AIHARA, Yusuke	NIBB Research Fellow	Division of Environmental Photobiology	April 1
TOYOTA, Kenji	NIBB Research Fellow	Division of Molecular Environmental Endocrinology	April 1
NISHIMURA, Toshiya	NIBB Research Fellow	Laboratory of Molecular Genetics for Reproduction	April 1
OGAWA, Kota	NIBB Research Fellow	Functional Genomics Facility	April 1
NIIMI, Teruyuki	Professor	Division of Evolutionary Developmental Biology	June 1
NAKAGAWA, Toshinori	Assistant Professor	Division of Germ Cell Biology	August 1
KAMIKAWA, Yasunao	NIBB Research Fellow	Laboratory of Stem Cell Biology	August 1
OHDE, Takahiro	Assistant Professor	Division of Evolutionary Developmental Biology	September 1

Newly affiliated in other universities and institutes

Name	New Affiliation	Position	Date
YAMAMORI, Tetsuo	RIKEN, Brain Science Institute	Team Leader	April 1
KOBAYASHI, Satoru	University of Tsukuba	Professor	April 1
WATAKABE, Akiya	RIKEN, Brain Science Institute	Researcher	April 1
HAYASHI, Yoshiki	University of Tsukuba	Assistant Professor	April 1
SATO, Masanao	Keio University	Project Research Associate	April 1
SADAKANE, Osamu	RIKEN, Brain Science Institute	Researcher	April 1
HARA, Kenshiro	Tohoku University	Associate Professor	April 1
MIYAKAWA, Hitoshi	Utsunomiya University	Associate Professor	June 1
SUZAKI, Takuya	University of Tsukuba	Associate Professor	August 16
TANAKA, Daisuke	National Institute of Agrobiological Sciences	Researcher	October 1

* Changes in professors, associate and assistant professors, and NIBB research fellows are shown.

Awardees in 2015

Name	Position	Award
OHSUMI, Yoshinori	Professor Emeritus	Canada Gairdner International Award, The 31st International Prize for Biology, The 20th Keio Medical Science Prize, Bunkakorosya (Person of Cultural Merits)
YOSHIMURA, Takashi	Adjunct Professor	2015 Van Meter Award, The Japan Society for Comparative Endocrinology's Incentive Award
NAKAMURA, Yoshiaki	Postdoctoral Fellow	The Society for Reproduction and Development's Young Investigator Award
TOYOTA, Kenji	NIBB Research Fellow	Inoue Research Award for Young Scientist, The Japan Society of Endocrine Disrupters Research's Poster Award, The Japan Society for Comparative Endocrinology's Best Presentation Award

LABORATORY OF CELL RESPONSES

Director General
YAMAMOTO, Masayuki

Specially Appointed Associate Professor
YAMASHITA, Akira

Postdoctoral Fellow: *OTSUBO, Yoko*
SHICHINO, Yuichi

Secretary: *SAKAGAMI, Mari*

Cells sense the environment around them, for example the amount of nutrients and hormones, as well as the temperature and pressure, and decide what kind of activities to undertake using this information. Germ cells, which produce sperm and eggs, begin halving their number of chromosomes during a special kind of cell division called meiosis, in response to the ambient conditions. In our laboratory we use the fission yeast *Schizosaccharomyces pombe*, the simplest organism that performs meiosis, to research the mechanism by which cells switch from mitosis, the kind of cell division that divides cells equally to create two identical cells, to meiosis, which is essential for bringing forth genetically diverse progeny.

I. Signaling pathways that regulate the onset of sexual differentiation

We have been trying to elucidate how fission yeast cells switch their mode of cell cycle from mitotic to meiotic. We focus on a highly conserved kinase, namely Target of rapamycin (TOR) kinase, which plays key roles in the recognition of nutrition and the onset of sexual differentiation in fission yeast. TOR kinase forms two types of complexes, namely TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential to suppress sexual differentiation in the presence of nitrogen. TORC2 contains Tor1 and, in contrast to TORC1, is required for onset of sexual differentiation under nitrogen starvation (Figure 1).

Temperature-sensitive *tor2* mutants initiate sexual differentiation on rich medium at the restrictive temperature. To gain insights into the TORC1 signaling pathway, we have isolated mutants that initiate sexual differentiation ectopically under nutrient-rich conditions. We are currently characterizing these mutants.

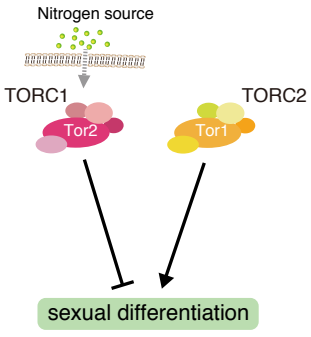


Figure 1. The two TOR complex pathways in *S. pombe*. TORC1, containing Tor2, and TORC2, containing Tor1, regulate sexual differentiation oppositely. TORC1 suppresses sexual differentiation in the presence of ample nitrogen.

II. The molecular mechanisms that establish the meiosis-specific transcription profile

Expression of hundreds of genes are upregulated during meiosis. We have shown that specific control of the stability of meiotic transcripts, which is orchestrated by the interplay between RNA-binding proteins and a long non-coding RNA, contributes to the meiosis-specific gene expression in fission yeast. Understanding precise mechanisms of this control will shed light on the regulation of timely gene expression during meiosis.

A YTH-family RNA-binding protein Mmi1 plays a crucial role in the selective elimination system of meiosis-specific transcripts during the mitotic cell cycle. Mmi1 recognizes a region termed DSR (Determinant of Selective Removal) in meiotic transcripts, which is enriched with repeats of hexanucleotide motifs. Meiotic transcripts bound to Mmi1 are degraded by the RNA-degradation nuclear exosome machinery. Mmi1 also induces formation of facultative heterochromatin at a subset of its target genes.

During meiosis, a meiosis-specific nuclear body, called Mei2 dot, blocks the Mmi1-mediated elimination system. The Mei2 dot is composed of the RNA-binding protein Mei2 and a long non-coding RNA species termed meiRNA. Mei2 physically interacts with meiRNA and forms the dot structure at the chromosomal *sme2* locus, which encodes meiRNA. The Mei2 dot lures Mmi1 through numerous copies of the DSR motif on meiRNA and inhibits its function, so that meiotic transcripts harboring DSR are stably expressed (Figure 2).

We have shown, in collaboration with a group at the University of Cambridge, that a conserved multifunctional protein complex Ccr4/Not is recruited by Mmi1 to its target transcripts and plays an essential role for heterochromatin formation in the Mmi1-dependent pathway (Cotobal *et al.*, 2015).

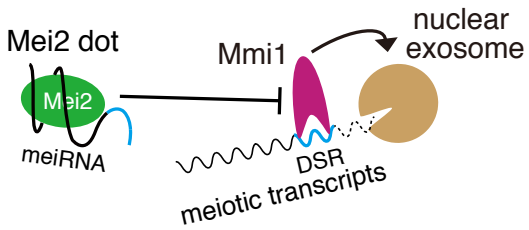


Figure 2. Selective elimination of meiosis-specific transcripts by the Mmi1/DSR system. Mmi1 binds to DSR in meiotic transcripts and induces their degradation by the nuclear exosome during the mitotic cell cycle. In meiotic cells, the Mei2 dot, composed of Mei2 and meiRNA, sequesters and inhibits Mmi1, so that DSR-harboring meiotic transcripts escape from Mmi1-mediated selective elimination.

III. Regulation of nuclear oscillation driven by cytoplasmic dynein during meiotic prophase

During meiotic prophase in fission yeast, the nucleus migrates back and forth between two poles of the cell. This oscillatory nuclear movement is called 'horse-tail' movement due to its characteristic shape and motion (Figure 3). Horse-tail nuclear movement enhances pairing of homologous chromosomes and facilitates meiotic recombination. Horse-tail movement is driven by cytoplasmic dynein, which forms a huge minus-end-directed microtubule motor complex. Cytoplasmic dynein that is anchored to the cell cortex generates a pulling force on the microtubule emanating from the leading edge of the nucleus. This dynein-mediated pulling is the major contributor to horse-tail movement. Cortical anchoring of dynein is crucial for the generation of horse-tail movement.

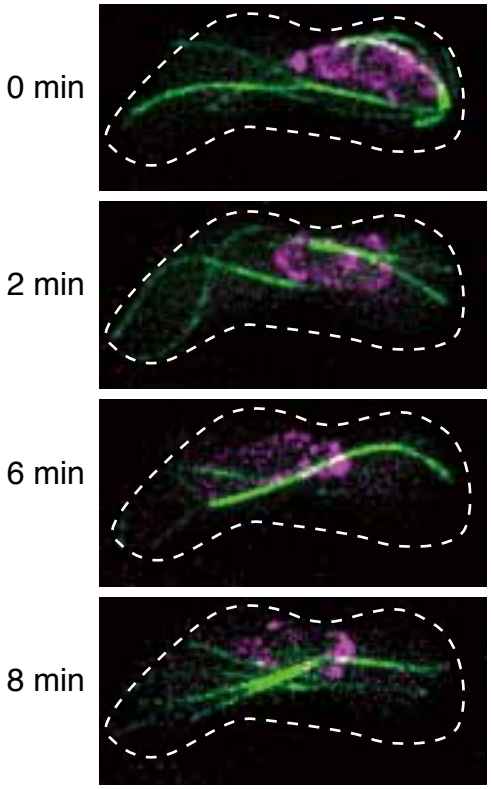


Figure 3. Horse-tail nuclear movement during meiotic prophase in *S. pombe*. Time-lapse images of nuclear membrane (Cut11, magenta) and microtubules (Atb2, green) in the wild-type strain. The cellular contour is shown by the dotted line.

We identified three subunits of dynactin, a protein complex that is required for most dynein-mediated cellular activities (Fujita *et al*., 2015). The three subunits, namely Arp1, Mug5 and Jnm1, transiently colocalized with dynein at the cell cortex and were essential for the cortical anchoring of dynein. We also found that another dynein-related cortical factor, Num1, cooperates with dynactin to establish dynein anchoring at the cell cortex (Figure 4).

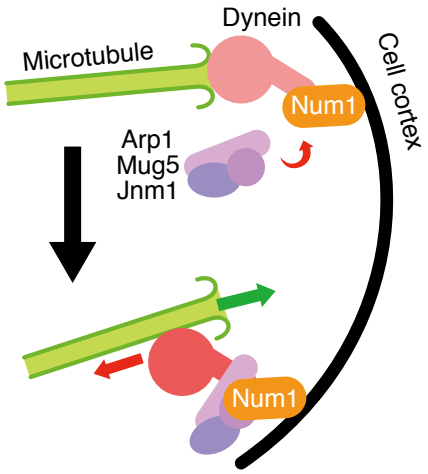


Figure 4. Cortical anchoring of cytoplasmic dynein in *S. pombe*. Dynein is captured by Num1 at the cell cortex. Dynactin subunits, Arp1, Mug5 and Jnm1 assemble with dynein at the cell cortex and activate dynein. Dynactin and Num1 cooperate to establish dynein anchoring and enable dynein to generate microtubule-pulling force.

Publication List:

〔Original papers〕

- Cotobal, C., Rodríguez-López, M., Duncan, C., Hasan, A., Yamashita, A., Yamamoto, M., Bähler, J., and Mata, J. (2015). Role of Ccr4-Not complex in heterochromatin formation at meiotic genes and subtelomeres in fission yeast. Epigenet. Chromatin *8*, 28.
- Fujita, I., Yamashita, A., and Yamamoto, M. (2015). Dynactin and Num1 cooperate to establish the cortical anchoring of cytoplasmic dynein in *S. pombe*. J. Cell Sci. *128*, 1555-1567.

LABORATORY OF NEURONAL CELL BIOLOGY

Associate Professor
SHIINA, Nobuyuki

Assistant Professor: *NAKAYAMA, Kei*
SOKENDAI Graduate Student: *OHASHI, Rie*
KATAYAMA, Kaori
Technical Assistant: *MATSUDA, Chisato*

The transport of specific mRNAs and local control of translation in neuronal dendrites represent an important gene expression system that provides dendritic protein synthesis at just the right time and place. It is believed that this system controls the location at which neurites will connect to each other, thereby forming neural networks. Our main interest is to understand the mechanisms and roles of mRNA transport and local translation in neuronal dendrites.

Specific mRNAs are recruited into "RNA granules" and transported to dendrites. RNA granules are macromolecular complexes composed mainly of mRNAs, ribosomes and RNA-binding proteins, and mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1). We are researching RNA granule factors regulating mRNA transport and local translation, their target mRNAs, and the mechanisms of localized protein synthesis using mice in order to better understand their relation to the formation of synapses and neural networks, memory, learning, and behavior.

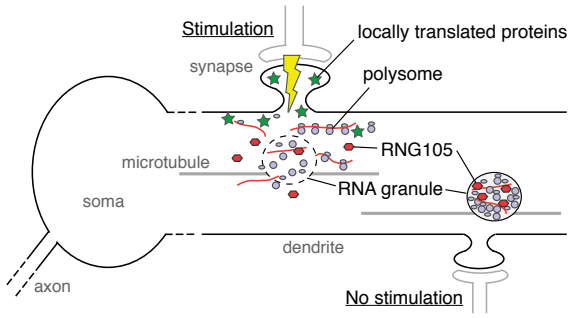


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Comprehensive behavioral analysis of RNG105 heterozygous mice: Implications in autism spectrum disorder (ASD)

We previously identified RNA granule protein 105 (RNG105)/caprin1, an RNA-binding protein, as a component of RNA granules. RNG105 is responsible for mRNA transport to dendrites, which is required for the encoded proteins to be translated and function in dendrites for proper networking of neurons. RNG105 knockout mice exhibit reduced dendritic synapse formation and reduced dendritic arborization, which results in poor development of neuronal networks. The knockout neonates die soon after birth due to respiratory failure that is associated with defects in fetal brainstem development (Shiina *et al*., J. Neurosci. *30,* 12816-12830, 2010).

A recent study aiming to detect genetic variants in ASD by whole-genome sequencing reported that a heterozygous *de novo* nonsense mutation in the *Rng105/Caprin1* gene was found in a patient with Asperger's syndrome, a form of ASD. The patient's intelligence quotient (IQ) was above average, but adaptive behavior and sociability were delayed. The report suggested that *Rng105/Caprin1* is a candidate risk gene for ASD, but it remained unclear as to whether there was a causal relation between RNG105 deficiency and ASD.

To investigate the influence of RNG105 deficiency on mouse behavior, we subjected RNG105 heterozygous ($Rng105^{+/-}$) mice to a comprehensive behavioral test battery. One of the marked changes in $Rng105^{+/-}$ mice was sociality. $Rng105^{+/-}$ mice showed reduced social interaction with familiar mice in a home cage test and reduced social interest/preference for novel mice over familiar mice in a three-chambered social approach test (Figure 2). The latter change can be characterized as indistinguishable responses of $Rng105^{+/-}$ mice to familiarity and novelty, as the $Rng105^{+/-}$ mice also showed reduced interest/preference for novel objects and places over familiar objects and places, respectively. In several maze tasks, $Rng105^{+/-}$ mice showed normal acquisition of memory, and in some of the tasks, they showed higher performance compared to wild-type mice. However, they showed relative difficulty in reversal learning,

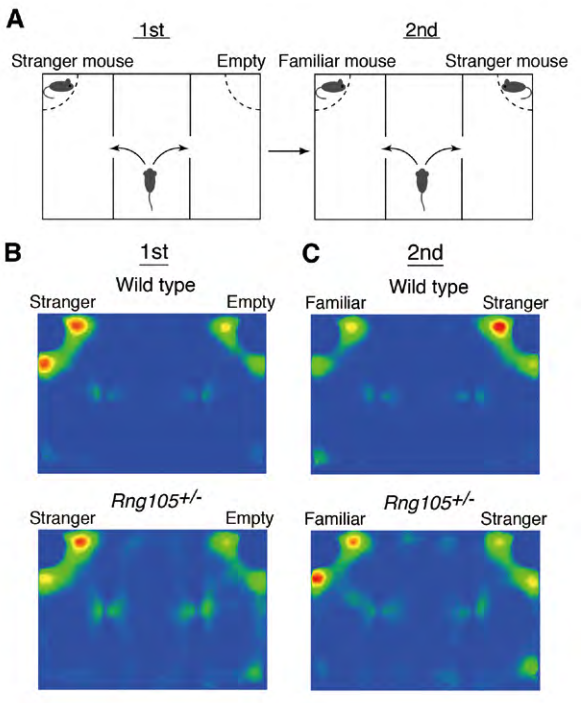


Figure 2. Three-chambered social approach test. (A) Schematic diagram of the test. In the first session, one of the cages (dotted lines) contains a stranger mouse. A test mouse is allowed to freely explore the three chambers. In the second session, a familiar mouse and a stranger mouse are put in each cage. (B and C) Heat maps showing the average traces of wild-type mice (top panels) and $Rng105^{+/-}$ mice (bottom panels) in the first session (B) and the second session (C). Social interaction with a stranger mouse is normal, but there is a lack of interest/preference for a stranger mouse over a familiar mouse in $Rng105^{+/-}$ mice.

in which the target was changed from the initial position. These results suggest that social interaction, responses to novel situations, and flexibility to changes were reduced in *Rng105*$^{+/-}$ mice.

Furthermore, RNG105-deficient neurons showed a reduction in AMPA glutamate receptor (AMPAR) cell surface distribution in dendrites (Figure 3), which has been reported in other ASD-like mutant mice and is thought to be related with the neuropathology of ASD. The behavioral test battery, together with the analysis of AMPAR distribution, suggest that an RNG105 deficiency leads to ASD-like behavior. In particular, *Rng105*$^{+/-}$ mice performed well in specific tasks, suggesting that the phenotype of *Rng105*$^{+/-}$ mice was related to Asperger's syndrome-like behavior.

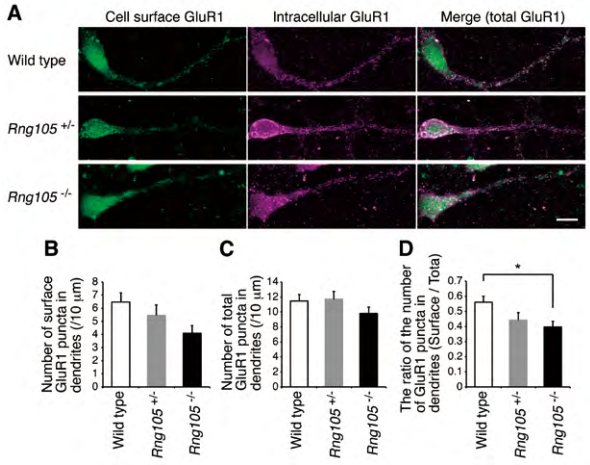


Figure 3. The cell surface distribution of AMPAR subunit GluR1 is reduced in dendrites of RNG105-deficient neurons. (A) Immunostaining for GluR1 in cultured neurons (9 DIV) from cerebral cortexes of E17.5 wild-type, *Rng105*$^{+/-}$ and *Rng105*$^{-/-}$ mice. Scale bar, 10 μm. (B and C) The number of surface (B) and total (C) GluR1 puncta in dendrites. (D) The ratio of surface/total number of GluR1 puncta in dendrites. *p<0.05, one-way ANOVA followed by Turkey-Kramer test.

II. Learning and memory deficits in RNG105 conditional knockout (cKO) mice

Although learning and memory were normal in mice with a moderate deficiency of RNG105 (*Rng105*$^{+/-}$), learning and memory were markedly impaired in mice with severe deficiencies of RNG105: RNG105 conditional knockout (cKO) in the brain after birth led to reduced contextual and spatial memories.

Because memory formation is highly correlated with increases in the size of spine heads (postsynapses) on neurons, we measured the size of spines in the hippocampus of RNG105 cKO mice. The size of the spine heads was significantly smaller in RNG105 cKO mice than in wild-type mice, which may underlie the impaired memory formation in RNG105 cKO mice.

RNG105/caprin1 has one paralog, RNG140/caprin2, which has RNA-binding domains highly conserved with RNG105. RNG105 and RNG140 are localized to different kinds of RNA granules and their timing of expression is also different: RNG105 is highly expressed in embryos, but RNG140 is highly expressed in adults (Shiina and Tokunaga, J. Biol. Chem. *285,* 24260-24269, 2010). To investigate the role of RNG140 in higher brain functions in adult mice, we have started to generate RNG140 knockout mice with the CRISPR/Cas9 system.

III. RNA granule dynamics regulated by PRMT1

Regulation of RNA granule assembly and disassembly is emerging as a key mechanism of translational control, defects of which are linked with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). We conducted proteomic analyses to identify proteins associated with RNG105, and identified PRMT1 as a factor which disassembles RNG105-containing RNA granules.

PRMT1 is a major arginine methyltransferase that methylates arginine residues in the arginine and glycine-rich (RG-rich) domain. We found that the RG-rich domain of RNG105 was bound and methylated by PRMT1. A methyltransferase activity-deficient mutant PRMT1 also bound to RNG105, but had reduced ability to methylate RNG105 and disassemble RNA granules. These results suggested that not only the association with RNG105, but also the methylation activity of PRMT1 is required to disassemble RNA granules. Because various RNA granule components, including an ALS and FTLD risk factor FUS, have the RG-rich domain, it will be a future challenge to investigate whether methylation of these RNA granule factors is implicated in the regulation of RNA granule dynamics and neurodegenerative disease.

Publication List:

〔Original paper〕

- Tsuboi, D., Kuroda, K., Tanaka, M., Namba, T., Iizuka, Y., Taya, S., Shinoda, T., Hikita, T., Muraoka, S., Iizuka, M., Nimura, A., Mizoguchi, A., Shiina, N., Sokabe, M., Okano, H., Mikoshiba, K., and Kaibuchi, K. (2015). Disrupted-in-schizophrenia 1 regulates transport of ITPR1 mRNA for synaptic plasticity. Nat. Neurosci. *18*, 698-707.

LABORATORY OF STEM CELL BIOLOGY

Associate Professor
TSUBOUCHI, Tomomi

NIBB Research Fellow: *KAMIKAWA, Yasunao*
Technical Assistant: *YASUI, Naomi*

DNA is constantly damaged from both endogenous and exogenous sources. One of the most important challenges for all living organisms is to prevent genome instability that can lead to malfunction of a cell. Our group is interested in the strategies through which cells protect themselves from alterations in the genome. To date, much information has been gained from various model organisms and tissue culture cells, and we are beginning to learn that the choice of genome-maintenance strategies taken by a cell depends on the cell type, cell cycle- and developmental stages. In the Laboratory of Stem Cell Biology, we are currently focusing our attention on the genome maintenance mechanisms of the embryonic stem cells, and their roles during differentiation and reprogramming processes.

I. Self-renewal of Embryonic Stem Cells and Their Genome-Maintenance Mechanisms

Embryonic stem (ES) cells are derived from the blastocyst stage of embryonic development, and are capable of differentiating into all cell types that compose our body (i.e., ES cells are "pluripotent"). Pluripotent cells exist only transiently and are lost as development proceeds. On the other hand, ES cells are capable of proliferating indefinitely when given an appropriate culturing condition. Curiously, ES cells proliferate with truncated gap phases while S (DNA replication) and M (mitosis) phases are as lengthy as other cell types. ES cells also appear to lack some of the mechanisms that ensure genome integrity (i.e., checkpoint mechanisms), the significance of which remains a mystery.

To date, studies on cell cycle regulation in ES cells have not been straightforward compared to that of other cell types, as many commonly used cell-synchronization protocols are ineffective for ES cells. We have established a protocol to synchronize ES cells using centrifugal elutriation (Tsubouchi et al., Cell, 2013), which allowed us to investigate specific stages of the ES cell cycle. We are currently aiming to address why and how ES cells maintain such unique cell cycle regulation and how that is interlinked with pluripotency by carrying out side-by-side analyses between ES cells and differentiated populations.

II. Genome Instability during Nuclear Reprogramming

In order to gain a deeper understanding of the relationship between the choice of genome maintenance mechanisms and pluripotency, we are investigating the behavior of factors involved in genome maintenance mechanisms during nuclear reprogramming towards pluripotency.

Specifically, we take advantage of the cell-to-cell fusion approach, in which a target cell is fused to a pluripotent stem cell to induce pluripotency within a target nucleus (Figure 1). The cell fusion system is a simple, versatile way to induce reprogramming towards another lineage, not limited to pluripotency. Using this system, the first sign of reprogramming can be detected from within a few hours to one day after fusion, allowing us to monitor the initial events of reprogramming after induction.

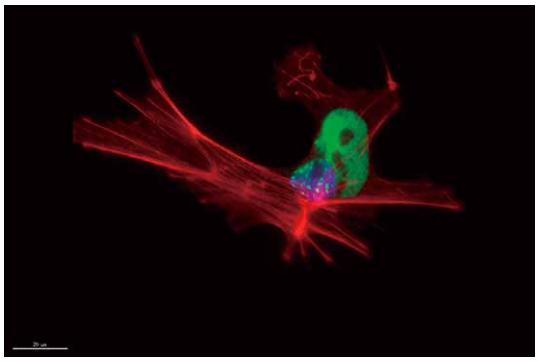


Figure 1. Reprogramming induced through cellular fusion: a human lymphoblastoid nucleus (blue, with green spots) is induced to undergo nuclear reprogramming towards pluripotency upon fusion with mouse ES cells (green).

Using this system, we previously found that DNA synthesis is an important event for successful reprogramming (Tsubouchi et al., Cell, 2013). Recent reports indicate that reprogramming may cause genetic instabilities, some of which are thought to arise as DNA replication errors. We are currently investigating how such genetic instability may arise, and how it is linked to reprogramming-specific events.

III. Future Perspective

While the fundamental mechanisms that maintain genome integrity have been widely studied using various models, the danger a cell might face when altering their cellular identity (through differentiation, reprogramming etc.) is unknown. Recent studies of cancer genome sequencing repeatedly identified mutations in the factors that govern cellular identities, leading us to hypothesize that cells may experience genome instability when their identity is unstable. Our goal is to uncover the nature of such genetic instability and to gain a comprehensive understanding of the mechanisms that maintain genome integrity.

Publication List:

〔Original paper〕

● Leung, W.-K., Humphryes, N., Afshar, N., Argunhan, B., Terentyev, Y., Tsubouchi, T., and Tsubouchi, H. (2015). The synaptonemal complex is assembled by a polySUMOylation-driven feedback mechanism in yeast. J. Cell Biol. *211*, 785-793.

LABORATORY OF CELL SOCIOLOGY †

Assistant Professor
HAMADA, Yoshio

Technical Assistant: *GONDA, Naoko*

Mammals have evolved placentae that facilitate the transport of nutrients and oxygen into the fetus. Primates and rodents have an ancestral type of placenta in which maternal blood is not contained within endothelial cell lined vessels but rather is in direct contact with epithelial cells of the fetal placenta (so called hemochorial placentation) that are derived from the trophoblast cell lineage (Figure 1). How maternal blood vascular circuits develop in the hemochorial placenta without immunological rejection has been a longstanding mystery in biology.

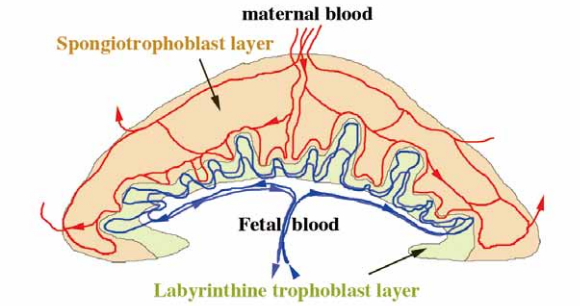


Figure 1. Fetal and maternal blood flow in the mouse placenta. Feto-maternal exchanges take place in the labyrinthine layer (green). Polyploid trophoblasts are observed in the spongiotrophoblast layer (orange) where *Notch2* is expressed.

The *Notch2* null mutant dies at mid-gestation due to impaired maternal circuit formation. Two mechanisms for the formation of spaces for the maternal circuit have been speculated. One is by polyploid Trophoblast Giant Cells (TGCs) that retain the ability to form cavities, analogous to endothelial cells. Notch signaling mutations reduce the population or cause dysfunction of TGCs. The other is by deletion of trophoblasts, which we have proposed. Notch mutation would lead the trophoblasts to resist the deletion.

We carried out a survey of authentic cell death, apoptosis and necrosis, related to the formation of the maternal blood circuit, but failed to find meaningful signals. Therefore, we suspected that an unknown type of cell death or deletion might be involved. Because histological studies of developing mouse placentae show that formation of open spaces begins at E10.5 and become bigger at later stages in the spongiotrophoblast layer (Figure 2), it was rational to speculate that cell death or deletion occurs in the spongiotrophoblast layer. Study of the nuclear size of spongiotrophoblast cells shows a constant and substantial population of polyploid trophoblast cells throughout development.

How is the population of polyploid trophoblast cells kept constant? Considering that diploid spongiotrophoblast cells differentiate into polyploidy during normal development, the supply from diploid trophoblast cells and deletion could maintain a constant population. Now, our working hypothesis

†: This laboratory was closed on 31 March, 2016.

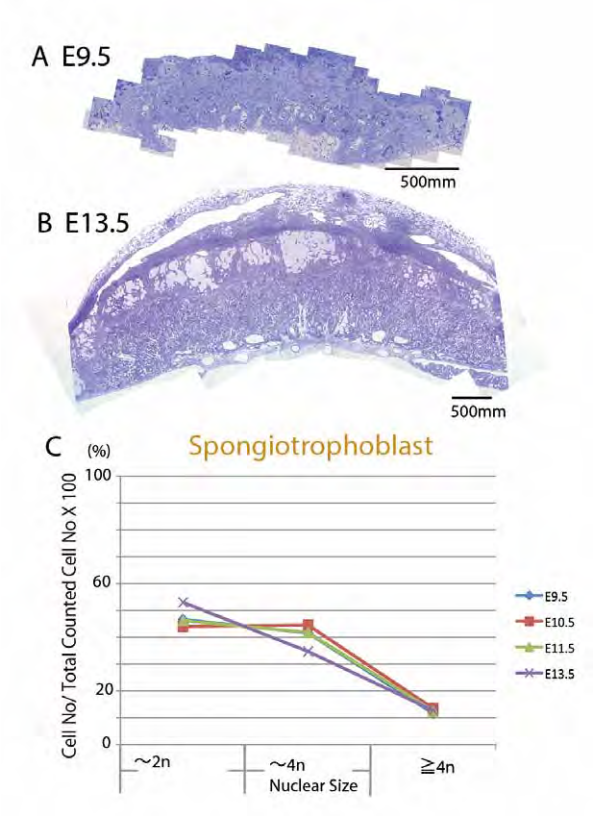


Figure 2. Drastic morphological change and unchanged population of polyploid trophoblast cells. Open spaces are barely seen at E9.5 (A) and become large enough to contribute to half of the spongitotrophoblast layer at E13.5 (B). In contrast to the morphological changes, the proportion of diploid, replicating dipoloid and polyploidy is unchanged throughout E9.5-E13.5 (C).

became testable. Polyploid trophoblasts should be deleted and *Notch2* mutation should cause them to resist the deletion.

Although tetraploid embryo derived trophoblasts are able to support ES cell derived embryos to grow to term, they are deleted from the placenta by E11.5 if they co-exist with diploid trophoblasts (Figure 3). Contrastingly, *Notch2* null mutant tetraploid embryo derived trophoblasts resist the deletion and even dominate diploid trophoblasts. From these results we conclude that maternal blood spaces throughout the trophoblast are created by polyploid trophoblast cell deletion.

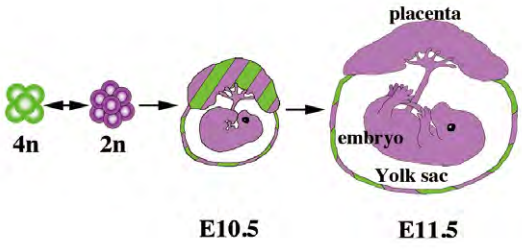
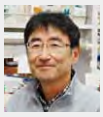


Figure 3. Fate of tetraploid trophoblast cells in chimera embryos. A tetraploid embryo was aggregated with a diploid embryo *in vitro* and transplanted onto a pseudo-pregnant female's uterus. While the 4n embryo contributes to the placenta and yolk sac, it does not contribute to the embryo proper by E10.5, 4n embryo derived trophoblasts disappear by E11.5.

DIVISION OF MORPHOGENESIS

Professor
UENO, Naoto



Associate Professor
KINOSHITA, Noriyuki

Assistant Professor:	*TAKAHASHI, Hiroki*
	SUZUKI, Makoto
Technical Staff:	*TAKAGI, Chiyo*
NIBB Research Fellow:	*YAMAGUCHI, Takeshi*
Postdoctoral Fellow:	*NEGISHI, Takefumi*
	SUZUKI, Miho
	NINOMIYA, Hiromasa
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	MIYAGI, Asuka
	YASUE, Naoko
	WATANABE, Mika
Secretary:	*MIYAKE, Satoko*
	TSUGE, Toyoko

The complex morphogenesis of organisms is achieved by dynamic rearrangements of tissues during embryogenesis, in which change in cellular morphology as well as orchestrated cell movements are involved. For cells to know how they should change their shape and where they should move, information called “cell polarity” is essential. How then is the cell polarity established within cells? Is it intrinsically formed within the cells or triggered by extracellular cues? Furthermore, little is known as to how coordinated and complex cell movements are controlled in time and space. We attempt to understand the mechanisms underlying these events using several model animals, including frogs, fish, mice and ascidians, taking physical parameters such as force in consideration, in addition to conventional molecular and cellular biology.

I. A novel signaling pathway for head formation

The head is formed in the most anterior part of the neural tube which is the anlage of the brain and spinal cord. How is the head region selected in its particular region of the tube? Patterning of the anterior-posterior axis is controlled by secreted molecules known as growth factors whose effects are mediated by their downstream mediators which facilitate differentiation of the brain and eyes. It was previously shown that the inhibition of the Wnt/β-catenin signaling pathway by various factors is critical for the anterior specification of head formation. We have revealed that Flop1 and Flop2 (Flop1/2) G protein-coupled receptors contribute to the regulation of head formation by inhibiting Wnt/β-catenin signaling in the amphibian *Xenopus*. Both the overexpression and knockdown of Flop1/2 resulted in abnormal head formation. More interestingly, overexpression of Flop1/2 in the absence of bone morphogenetic protein (BMP) signaling which is inhibitory to general neural differentiation, resulted in ectopic head formation in the ventral side. We have also found that Flop1/2 inhibits Wnt/β-catenin signaling by promoting β-catenin degradation. Although the ligand for the Flops is not identified, our findings suggest that Flop1 and Flop2 are novel inhibitory components of the Wnt/β-catenin signaling pathway and essential for proper head formation.

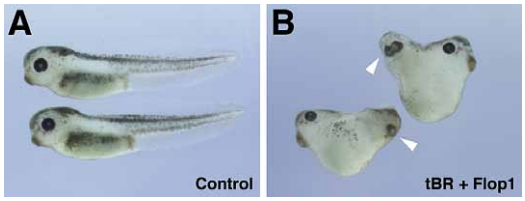

Figure 1. Ectopic head formation by Flop1 overexpression and BMP inhibition.
(A) Control larvae showing normal head and tail structures.
(B) Abnormal larvae in which Flop1 is overexpressed in the absence of BMP signals show duplicated heads with eyes at the expense of trunk and tail.

II. Mechanotransduction in *Xenopus* embryonic cells

During early vertebrate development, many dynamic morphogenetic movements occur, which include convergent extension of the axial mesoderm, collective migration of mesendoderm cells, epiboly of the ectoderm, and neural tube formation. These movements must generate physical forces between cells and tissues. There is accumulating evidence that forces generated by moving cells and tissues affect morphogenetic processes. The purpose of this project is to elucidate mechanisms that sense and respond to mechanical force in *Xenopus* embryonic cells, and to clarify the roles of mechanotransduction systems during development.

It is known that cells can sense mechanical stresses in several ways, for example, TRP channels, cell surface glycoproteins, flow-sensing cilia, cadherins, and focal adhesions. Physical stimuli sensed by these molecules may be converted to intracellular signaling and induce cellular response. Several studies using mammalian tissue culture cells have revealed that mechanical stresses induce activation of some protein kinases. Therefore we examined whether mechanical force might change protein phosphorylation in *Xenopus* embryonic cells. For this purpose, we used the proteomic approach to comprehensively analyze protein phosphorylation profiles. We have found that mechanical stress applied to *Xenopus* embryos changes phosphorylation states of several proteins, suggesting that *Xenopus* embryonic cells have a mechanism to sense mechanical forces, which involves protein phosphorylation/dephosphorylation.

III. Mathematical analysis of neural tube closure

During early development of the central nervous system, neuroepithelial cells decrease their apical surface area by actomyosin contractility. This cell shape change is called apical constriction, which converts the neural plate into a tubular structure, called the neural tube. We previously showed that intracellular calcium ion (Ca^{2+}) dynamically fluctuated throughout the *Xenopus* neural plate from the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).

single-cell to whole-tissue levels with distinct patterns. Live-imaging revealed that the Ca^{2+} fluctuations correlated with a remodeling of F-actin and the apical constriction, suggesting that the dynamic Ca^{2+} signaling positively regulates the neural tube closure. In this study, to further investigate the role of the Ca^{2+} fluctuations, we developed a two-dimensional vertex model, in which the natures of the apical constriction and the pulsed contraction of the apical junctions were introduced. Computational simulation of our model shows that the pulsed contraction accelerates the apical constriction and decrease of the tissue size independent of its frequency. However, although dense rather than sparse pulsation induces rapid tissue deformation, its overall effect throughout the entire course of the simulation is not effective when the total number of the pulses is constant. These data suggest that the distinct patterns of Ca^{2+}-induced local contraction always contribute to tissue deformation.

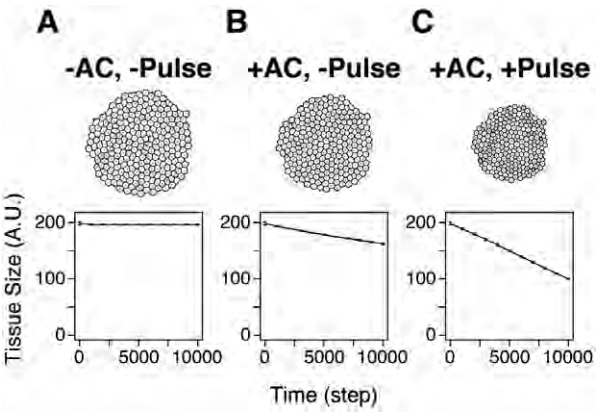

Figure 2. Effects of apical constriction (AC) and pulsed contraction (Pulse) on modeled epithelial sheets.
Representative images of modeled epithelial sheets at the end of each simulation (top) and average tissue sizes during the time course of the simulations (bottom) without the apical constriction and the pulsed contraction (A), with the apical constriction (B), and with the apical constriction and the pulsed contraction (C). Error bars depict s.e.m.

IV. Membrane invagination toward centrosome links the posterior ciliary positioning to spindle orientation in ascidian epidermal cells.

The role of the centrosome changes during cell cycle progression. Centrosomes form the basal bodies of cilia in interphase and the pole of the spindle in mitotic phase. Then, the positioning of the centrosome is important for the polarity of cilia and the orientation of the spindle.

During the last mitotic division of epidermal cells of the ascidian embryo, it is already reported that most cells divide along the anterior-posterior (A-P) axis. We found a unique filamentous membrane structure, which we call "invagination" elongated toward the centrosome in this division cycle. We have proposed a model, in which this novel membrane invagination maintains the position of the centrosome to the posterior of the cell with tensile force and is involved in spindle orientation along the A-P axis. Interestingly, we also found the formation of cilia on epidermal cells during the last division cycle. Therefore, we confirmed the position of cilia located to the posterior of the cell and its basal body was captured by the invagination with live-imaging analysis and Serial block face Scanning Electron Microscopy (SBF-SEM) observation. Posteriorly localized cilia are conserved among several organisms including ascidians for establishing the left – right (L-R) axis. Our investigations will provide a new model of not only spindle orientation, but also ciliary positioning.

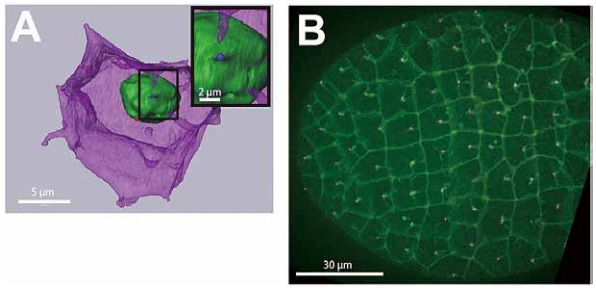

Figure 3. Membrane invagination associated with the epidermal cilia of acidian embryo.
(A) 3D image reconstructed from SBF-SEM data shows that tips of invagination approach the basal body. The basal body and daughter centriole are indicated as blue and red balls, respectively. The black-lined square is enlarged in upper right panel. Anterior is left. (B) A frame of a time lapse video of membrane invaginations reaching cilia. PH-tdTomato (green), plasma membrane probe; Arl13b-GFP (magenta), cilia probe. Anterior is left.

V. Notochord and evolution of chordates

The expression profile of *Brachyury* is highly conserved in metazoans from cnidarian to vertebrates. Specifically, *Brachyury* is expressed in the blastopore region of early embryos, and functions in invagination of the endomesodermal germ layer through the blastopore into the embryo. This "primary" function of *Brachyury* is associated with morphogenesis.

Comparison of *Brachyury* expression between nonchordate invertebrates and chordates clearly shows an additional expression domain seen only in chordates, namely, the dorsal midline of the blastopore, an embryonic region associated with the formation of the notochord. This "secondary" function arose during the evolution of chordates.

Therefore, it is now evident that the evolutionary origin of the notochord is essentially the question of the molecular mechanisms underlying how *Brachyury* acquired its secondary expression domain at the mid-dorsal region of the blastopore. To know how chordate ancestors gained the new expression domains, we used Amphioxus, the most ancestral chordates. We functionally analyzed the Amphioxus *Brachyury* promoter regions by reporter assay, using *Ciona* embryos.

VI. The biological function of invertebrate DNA methylation in pre-mRNA processing

DNA methylation at cytosine residues is an important epigenetic modification found in eukaryotes ranging from plants to humans. Invertebrates offer an interesting model for studying evolutionary changes in the targets and the function of DNA methylation. A marine invertebrate chordate *Ciona intestinalis* has a genome-wide mosaic methylation pattern comprising methylated and unmethylated genes. It has been

observed that DNA methylation is targeted to the transcribed region of ubiquitously expressed genes, and a constant targeting of the "gene body methylation" irrespective of cell types. To reveal the function of gene body methylation in gene transcription, we analyzed newly synthesized RNA from *C. intestinalis* embryos. By using 4sU labeling and sequencing methods, revealing global RNA processing kinetics at nucleotide resolution, we obtained snapshots of active transcription. Significant differences were seen in co-transcriptional splicing efficiency, in connection with methylation status of exons and introns. The splicing efficiency and DNA methylation status were also correlated to nucleosomal positions, suggesting that epigenetic states in the bodies of transcribed genes control the pre-mRNA processing through nucleosomal positioning.

VII. Cnidarian-symbiodinium Symbiosis

Corals are declining globally due to a number of stressors. Such stresses can lead to a breakdown of the essential symbiotic relationship between coral and *Symbiodinium*, a process known as coral bleaching. Although the environmental stresses causing this breakdown are largely known, the molecular and cellular mechanisms of symbiosis are still unclear. Corals are not very suitable as laboratory systems, because they are difficult to work with due to their slow growth, long generation times, and calcareous skeletons. To overcome these limitations, we focused on the small sea anemone *Aiptasia* as a novel experimentally tractable cnidarian model organism (Figure 4). *Aiptasia*, just as reef-building corals, establishes a stable but temperature-sensitive symbiosis with *Symbiodinium*. *Aiptasia* can be repeatedly bleached and repopulated with *Symbiodinium*, grows rapidly, and lacks a calcareous skeleton, allowing microscopic and cell biological analyses. In order to further elucidate the symbiotic mechanisms, it is necessary to establish molecular biological approaches. Therefore, we have attempted to develop a method of gene transfection to *Aiptasia*. Investigating symbiosis using *Aiptasia* should improve our understanding of the symbiotic mechanism.

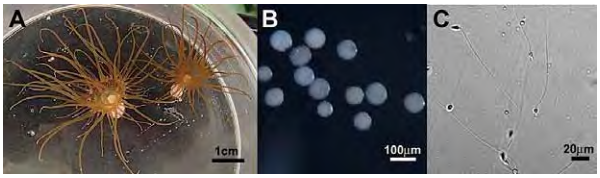

Figure 4. *Aiptasia* spawning can be induced under controlled laboratory conditions
(A) *Aiptasia* mature female (left) and male (right). (B and C) *Aiptasia* eggs and sperms, respectively.

Publication List:

〔Original papers〕

- Kai, M., Ueno, N., and Kinoshita, N. (2015). Phosphorylation-dependent ubiquitination of paraxial protocadherin (PAPC) controls gastrulation cell movements. PLoS One *10*, e0115111.
- Miyagi, A., Negishi, T., Yamamoto, T.S., and Ueno, N. (2015). G protein-coupled receptors Flop1 and Flop2 inhibit Wnt/β-catenin signaling and are essential for head formation in Xenopus. Dev. Biol. *407*, 131-144.
- Negishi, T., and Yasuo, H. (2015). Distinct modes of mitotic spindle orientation align cells in the dorsal midline of ascidian embryos. Dev. Biol. *408*, 66-78.
- Sakamaki, K., Iwabe, N., Iwata, H., Imai, K., Takagi, C., Chiba, K., Shukunami, C., Tomii, K., and Ueno, N. (2015). Conservation of structure and function in vertebrate c-FLIP proteins despite rapid evolutionary change. Biochem. Biophys. Rep. *3*, 175-189.
- Uno, Y., Nishida, C., Takagi, C., Igawa, T., Ueno, N., Sumida, M., and Matsuda, Y. (2015). Extraordinary diversity in the origins of sex chromosomes in anurans inferred from comparative gene mapping. Cytogenet. Genome Res. *145*, 218-229.

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

- Sekiguchi, T., Kuwasako, K., Ogasawara, M., Takahashi, H., Matsubara, S., Osugi, T., Muramatsu, I., Sasayama, Y., Suzuki, N., and Satake, H. Evidence for conservation of the calcitonin superfamily and activity-regulating mechanisms in the basal chordate *Branchiostoma floridae*: insights into the molecular and functional evolution in chordates. J. Biol. Chem. 2015 Dec 7.

DIVISION OF DEVELOPMENTAL GENETICS †

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Germ cells are specialized cells that can transmit genetic materials from one generation to the next in sexual reproduction. All of the other cells of the body are somatic cells. This separation of germ and somatic cells is one of the oldest problems in developmental biology. In many animal groups, a specialized portion of egg cytoplasm, or germ plasm, is inherited by the cell lineage which gives rise to germ cells. This cell lineage is called germline. The germline progenitors eventually migrate into the gonads, where they differentiate to form eggs and sperm when the organisms are physically matured. How this developmental transition from the juvenile stage to the adult reproductive stage is precisely regulated in response to developmental and environmental cues is a longstanding question in biology.

I. The mechanism regulating developmental transition from larvae to reproductive adults

In holometabolous insects, the steroid hormone ecdysone plays a pivotal role in metamorphosis. In *Drosophila*, ecdysone is produced in the prothoracic gland (PG) and then converted into its active form, 20-hydroxyecdysone (20E), in the peripheral organs. The activities of 20E terminate larval development and growth and initiate metamorphosis. Ecdysone biosynthesis is regulated in the PG by neuropeptides, enabling modulation of the timing of 20E pulses during development. The stimulator of ecdysone biosynthesis is prothoracicotropic hormone (PTTH) and Insulin-like peptides (Ilps), which activate the production of ecdysone biosynthetic proteins. In addition to these neuropeptides, the larval–prepupal transition is modulated by environmental cues such as nutritional conditions that influence larval body size. For example, early third-instar larvae attain the body-size checkpoint required for the transit from larva to prepupa. Although the checkpoint is believed to ultimately modulate ecdysone production in the PG, its downstream effectors and signaling pathway remain elusive.

We found that monoaminergic autocrine regulation of ecdysone biosynthesis in the PG is essential for metamorphosis. PG-specific knockdown of *Octβ3R*, resulted in arrested metamorphosis due to lack of ecdysone. Knockdown of tyramine biosynthesis genes expressed in the PG caused similar defects in ecdysone production and metamorphosis. Moreover, PTTH and Ilps signaling were impaired by *Octβ3R* knockdown in the PG, and activation of these signaling pathways rescued the defect in metamorphosis. Thus, monoaminergic autocrine signaling in the PG regulates ecdysone biogenesis in a coordinated fashion upon activation by PTTH and Ilps. We propose that monoaminergic autocrine signaling acts downstream of a body-size checkpoint that allows metamorphosis to occur when nutrients are sufficiently abundant.

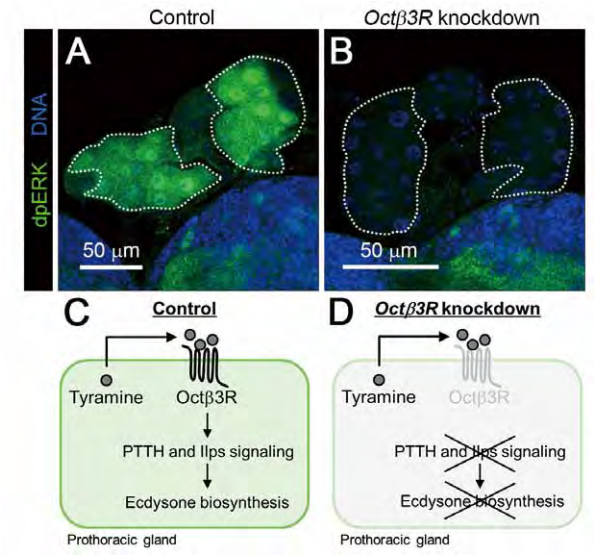

Figure 1. Tyramine-Octβ3R signaling is required for ecdysone biosynthesis.
(A and B) The PGs of control (A) and *Octβ3R* knockdown larvae (B) were labelled for the dephosphorylated form of ERK (dpERK, green) and DNA (blue). Expression of dpERK, a downstream signaling component of the PTTH pathway, was reduced in the PG of *Octβ3R* knockdown.
(C and D) Our model for the regulation of ecdysone biosynthesis. Tyramine is secreted from the PG after the attainment of body-size checkpoint. Tyramine-Octβ3R signaling activates PTTH and Ilps signaling, which leads to coordinated activation of ecdysone biosynthesis (C). In the PG of *Octβ3R* knockdown larvae, ecdysone biosynthesis is not activated due to loss of PTTH and Ilps signaling activity (D).

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〔Original papers〕

- Mukai, M., Hira, S., Nakamura, K., Nakamura, S., Kimura, H., Sato, M., and Kobayashi, S. (2015). H3K36 trimethylation-mediated epigenetic regulation is activated by Bam and promotes germ cell differentiation during early oogenesis in Drosophila. Biology Open 4, 119-124.
- Ohhara, Y., Shimada-Niwa, Y., Niwa, R., Kayashima, Y., Hayashi, Y., Akagi, K., Ueda, H., Yamakawa-Kobayashi, K., and Kobayashi, S. (2015). Autocrine regulation of ecdysone synthesis by β3-octopamine receptor in the prothoracic gland is essential for Drosophila metamorphosis. Proc. Natl. Acad. Sci. USA 112, 1452-1457.

†: This laboratory was closed on 31 March, 2015.

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The morphology of the body and tissues is established in spatio-temporarily regulated manners. A number of genes involved in morphogenesis have been identified, but it is still uncertain how the spatial and temporal information are established and converted to morphology during embryogenesis. Therefore, we are challenging to understand the mechanism by which the spatial information is established and that by which the temporal, or periodical, information is converted into morphology by several different approaches.

In the development of many tissues, secreted signal molecules are important for the formation of spatial information. These molecules are secreted from producing cells and transported to surrounding cells, resulting in the formation of concentration gradients. Given that their concentration decreases according to the distance from the source, the gradient of the signals defines relative positions of receiving cells in developing tissues. Many genetic studies revealed that a limited number of secreted signal proteins, including Wnt, BMP, and Hedgehog, function in the morphogenesis of tissues and embryos. In spite of the accumulation of genetic evidence, however, the molecular mechanism that regulates their spread in particular developing tissues remains to be elucidated. To this end, we started to visualize signal proteins and monitor their movement in tissues. In addition, we are examining structural and biochemical characteristics of these molecules, which appear to affect how they spread.

The segmental sub-regions of several specific tissues, including the paraxial mesoderm (or somites) and the pharyngeal pouches, by contrast, appear not to be simply directed by the gradient of the signal molecules, but by a unique mechanism proceeding periodically. For instance, somites are sequentially generated in an anterior-to-posterior order by converting the temporal periodicity created by a molecular clock into periodical structures. The molecular mechanism underlying this conversion and morphological segmentation, however, is not yet fully understood. Thus, another goal of our current studies is to reveal the molecular mechanism of this other and unique mode of patterning that underlies the periodical and sequential sub-division in the development of somites and pharyngeal arches.

I. Spatial regulation of secreted Wnt proteins in vertebrate development

The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis. In a classical view, it has been proposed that secreted signal proteins, like Wnt proteins, spread and function over a distance of several cells in many aspects of morphogenesis. In contrast, accumulating evidence also implies that Wnt proteins appear to transmit their signals locally in some particular cases. Therefore, their secretion and transport might be differently controlled depending on situation. Thus, for understanding the spatial regulation of tissue morphogenesis, the molecular mechanism underlying the spreading of Wnt proteins should be revealed.

For better understanding the spreading of Wnt proteins, we started to visualize Wnt proteins in the extracellular space by several different approaches. Our preliminary study indicated that Wnt proteins are not simply diffused during embryogenesis of the mouse. We precisely examined regulatory mechanisms and biological significance of Wnt protein distribution in mouse embryos. These analyses revealed a novel view of spatial regulation of Wnt signaling.

Extracellular molecules are known to regulate special distribution of Wnt proteins. Once Wnt proteins are secreted from the producing cell, their spread is regulated through interaction with these molecules. The heparan sulfate proteoglycan (HSPG), which is composed of a core protein with several chains of HS glycosaminoglycans, is a major component involved in this interaction. Genetic analyses with Drosophila mutants defective in the core proteins or GAG biosynthesis indicate the requirement of HSPG in Wnt signaling and gradients. In collaboration with Prof. Taira at the University of Tokyo, we found that HSPGs with different sugar chain modifications form distinct extracellular structures, which are called heparan sulfate nanostructures (HSNSs), in the early *Xenopus* embryo. Interestingly, *Xenopus* Wnt8 proteins were preferentially retained by HSNSs with N-sulfo modification (N-sulfo HSNSs). To understand the mechanism by which distinct distributions of HSNSs, especially N-sulfo HSNSs are formed, we focused on NDST1, an enzyme catalyzing N-sulfation of heparan sulfate. Gain and loss of function studies of NDST1 indicated that NDST1 is necessary and sufficient for the conversion of N-acetyl HSNSs into N-sulfo HSNSs. Extracellular accumulation of Wnt proteins and Wnt signaling were also affected by perturbation of NDST1 expression. These results indicated that N-sulfo modification of heparan sulfates are important for extracellular distribution of Wnt proteins and Wnt signaling (Figure 1).

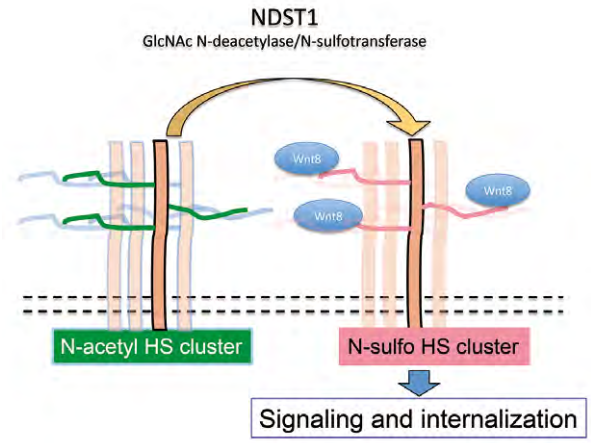

Figure 1. Schematic representation of the 2 different HSPG clusters of Wnt signaling.

II. Heterogeneity of secreted Wnt proteins secreted from culture cells

Although the structure of Wnt protein has already been revealed by X-ray crystallography, its higher order structure in extracellular space has not yet been fully understood. One of the proposed forms of Wnt proteins in extracellular space is binding to lipoprotein particles. On the other hand, recent studies indicated that Wnt proteins are secreted on another lipid-based carrier, called the exosome, which is an MVB (multivesicular body)-derived membrane vesicle. However, it remains unclear whether Wnt proteins are secreted only in these two forms, or also in some other forms from the same cells, or if different forms of Wnt proteins are secreted in a cell type specific manner.

To address this question, we systematically examined characteristics of Wnt proteins secreted from polarized MDCK (Madin-Darby Canine Kidney) cells. Although some Wnt3a proteins from mouse L cells were secreted together with lipoprotein particles, most of the Wnt3a proteins from either apical or basolateral side of MDCK cells did not. In contrast, secretion of a small amount of Wnt3a proteins via exosomes was detectable from the basolateral side of MDCK cells in a lipidation dependent manner. Interestingly, some apically secreted Wnt3a was secreted with an exosome marker, CD63, but its density was higher than typical exosomes. Signaling activity of Wnt proteins on basolaterally secreted exosomes and apically secreted exosome-like vesicles were lower than that of Wnt3a proteins in conditioned media. We also found that Wnt11, which is preferentially secreted from the apical side of MDCK cells, did not associate with exosomes, but with CD63-containing exosome-like vesicles. Our results indicate that Wnt are secreted with multiple carriers from polarized epithelia cells and suggest that Wnts are differently packaged depending on Wnt subtype and cellular context.

III. Molecular mechanism of somite development

Somites are segmental epithelial blocks located symmetrically on either side of the neural tube. Somites are periodically generated in an anterior to posterior manner from their precursor, the presomitic mesoderm (PSM), which is located posterior to newly formed somites. This periodic generation is achieved by a complex and dynamic mechanism in the PSM. First, the molecular clock, the so-called segmentation clock, creates oscillatory expression of particular genes, *hairy* and some *notch*-related genes, in the posterior PSM. Because the phase of oscillation is gradually shifted along the posterior-to-anterior axis, a wave of the oscillation appears to move in a posterior-to-anterior fashion. This oscillatory gene expression subsequently resulted in periodical generation of morphologically segmented somites.

The spatial pattern of somites is defined by positioning of intersomitic boundaries and rostro-caudal patterning within a somite. This spatial pattern is established in the anterior PSM by converting the oscillatory gene expression into spatially restricted patterns of gene expression. Several intra-cellular molecules, as well as FGF and Wnt signaling, are involved in this conversion. In the mouse, the prospective segmentation boundary is periodically established in the anterior PSM at the rostral border of the *Mesp2* expression domain. Mesp2, one of the key regulators in this conversion, is initially expressed at the most anterior region of the Tbx6 protein domain. This expression is not anteriorly extended beyond the anterior border of the Tbx6 protein domain because *Mesp2* expression requires Tbx6 proteins. Thus, the anterior border formation of the Tbx6 protein domain is a more fundamental process in the positioning of the segmentation boundary.

Importantly, this border is not consistent with the anterior border of *Tbx6* mRNA, rather it is regulated by a proteasome-mediated mechanism. Although the molecules directly executing this proteolysis are still unclear, *Mesp2*, as well as *Ripply1* and *Ripply2*, have shown to be required for the down-regulation of Tbx6 proteins by analysis of mouse embryos defective in these genes. Since expressions of *Ripply1* and *2* are eliminated in *Mesp2* deficient mouse embryos, we previously proposed the following model; *Mesp2,* whose expression is activated in the most anterior part of the Tbx6 domain, causes retreat of the Tbx6 protein domain through activation of *Ripply1* and *2* expression, and the retreated Tbx6 subsequently defines the next segmentation border and *Mesp2* expression. Recently, we showed that Ripply is a direct regulator of the Tbx6 protein level for the establishment of intersomitic boundaries. However, it is still to be elucidated whether *Mesp* is actually required for the formation of the boundaries in zebrafish.

To answer this question, we generated *mesp*-deficient zebrafish. Since four *mesp* genes have been identified in the zebrafish genome, we generated mutant fish carrying the frame shift mutation in all of the *mesp* genes using TALEN mediated mutagenesis and analyzed its phenotype (Figure 2). Unlike the mouse *Mesp2* mutant, zebrafish *mesps* quadruple embryo exhibited normally segmented somites and a normal boundary of the Tbx6 protein domain, although segmented expression of *epha4a* was disturbed in the anterior PSM, suggesting *mesps* and *mesps* dependent expression of *epha4a* are dispensable for somite positioning and segment boundary formation (Figure 3). Further analysis revealed zebrafish *mesp* genes are required for establishment of caudal identity

of somites similar to mouse *Mesp2*. *mesps* quadruple mutant embryos also showed disrupted superficial horizontal myoseptum formation probably resulting in the mis-migration of pigment cells and lateral line primordia in later development.

In contrast to *mesp*, double mutant for *ripply1* and *ripply2* revealed that these two genes are essential for segmental boundary formation. We found that *ripply1* and *2* expression is induced by a manner independent of *mesp*. Rather, striped expression of *ripply1* and *2* at the anterior PSM were severely disrupted in double mutant for *her1* and *7*, which are zebrafish clock genes encoding Hairy-related transcriptional repressors, suggesting proper regulation of *ripply1* and *2* expression by Hairy is essential for somite boundary formation. Furthermore, in *ripply1* and *2* double embryos, oscillatory expression of *her1* was expanded to the anterior paraxial mesoderm, indicating that *ripply1* and *2* are also required for termination of oscillation associated with mature somite formation.

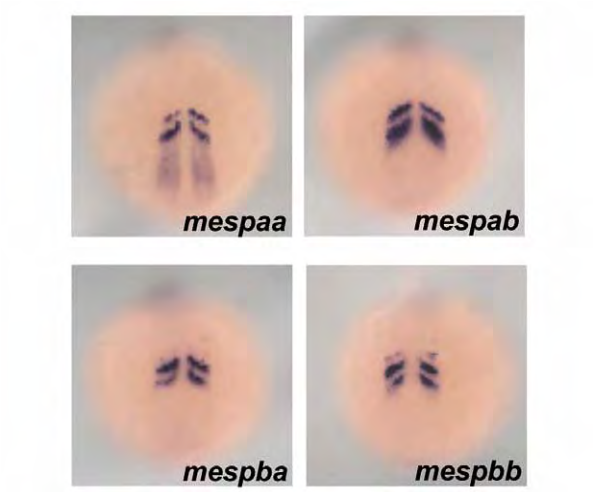

Figure 2. Expression of four *mesp* genes in zebrafish embryos. The zebrafish four *mesp* genes, *mespaa, maspab, mespba,* and *mespbb*, are similarly expressed in the anterior presomitic mesoderm during somitogenesis.

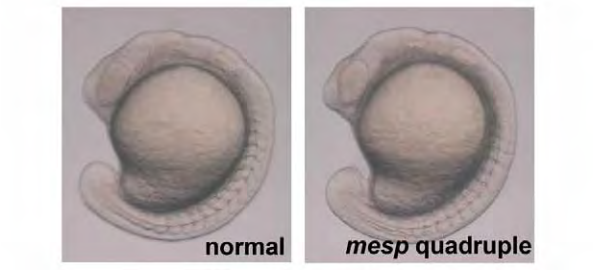

Figure 3. Phenotype of *mesp* quadruple mutant embryo. In contrast to the mouse, zebrafish *mesp* genes are dispensable for the formation of segmentation boundaries

IV. Molecular mechanism of the development of pharyngeal pouches

In addition to somites, metameric structures are observed in the pharyngeal region of vertebrates. Typical examples of such structures are skeletal elements of jaws, gills and cranial nerve projections. This metamerism is brought about by the segmental development of the pharyngeal pouches, which are generated by outpocketing of the pharyngeal endoderm. However, the molecular mechanisms underlying the segmentation of the pharyngeal pouches and the morphogenesis of the pharyngeal pouches still remained to be elucidated.

To understand these mechanisms, we are currently examining the function of several genes essential for the segmentation and morphogenesis of pharyngeal pouches using fish and mice as model systems.

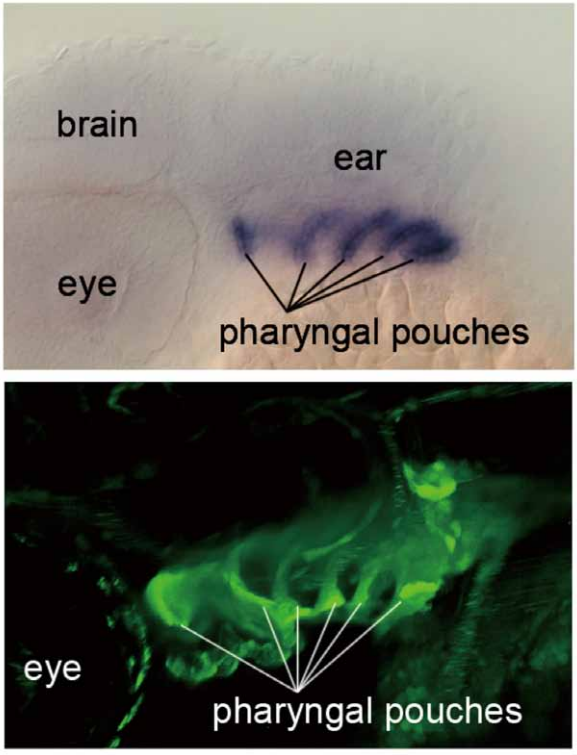

Figure 4. Segmental structure of the pharyngeal pouches in zebrafish embryos. Endodermal gene expression of *pax1* mRNA (Upper) and *sox17-egfp* (Lower) show the formation of segmental structures of the pharyngeal pouches.

Publication List:

〔Original papers〕

- Kametani, Y., Chi, N.C., Stainier, D.Y.R., and Takada, S. (2015). Notch signaling regulates venous arterialization during fin regeneration. Genes Cells *20*, 427-438.
- Okubo, T., and Takada, S. (2015). Pharyngeal arch deficiencies affect taste bud development in the circumvallate papilla with aberrant glossopharyngeal nerve formation. Dev. Dyn. *244*, 874-887.

Division of Embyology

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The aim of our research is to understand the events underlying early mammalian development during the period from pre-implantation to establishment of the body axes.

Early events during embryogenesis in mammals are relatively less understood, as compared to other animals. This is mainly due to difficulties in approaching the developing embryo in the oviducts and the uterus of the mother. Another characteristic of mammalian embryos is their highly regulative potential. The pattern of cell division and allocation of cells within an embryo during the early stages vary between embryos. The timing of the earliest specification events that control the future body axes is still under investigation. Functional proteins or other cellular components have not been found that localize asymmetrically in the fertilized egg. We want to provide basic and fundamental information about the specification of embryonic axes, differentiation of cell lineages, behaviors of cells, and the regulation of body shape in early mammalian development. We are also interested in the relationship between embryos and their environment, the oviducts and the uterus.

I. Establishment of a live imaging system for observation of early mouse development

To understand the mechanisms underlying embryonic development and morphogenesis, it has become common to observe the behavior of cells and gene expression in living embryos in a variety of animal species. Progress in GFP technologies, genetic engineering and embryonic manipulation concomitantly with the progress in optics and microscope technologies, including development of highly sensitive photo-detectors, has allowed us to observe developing embryos live, even in mammals. We have established a series of transgenic mouse lines for live imaging, which is part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CLST. These mouse lines have been used to visualize nuclei, cell shapes, the cytoskeleton and other organelles to observe cell behaviors in living mouse embryos in many laboratories over the world. We also established mouse lines to monitor the cell cycle.

We have also been establishing several reporter mouse lines in the lab to study gene expression patterns during the peri-implantation stage of mouse development. In these mice, cDNA encoding fluorescent proteins are connected to the mouse genomic DNA sequences of the enhancer/promoter region of important genes encoding factors regulating cell differentiation in these stages. Combining these two types of reporter transgenic mouse lines, we have been analyzing behaviors of cells comparing gene expression properties at the single cell level.

For the live imaging of early mammalian embryos, a combined microscope and incubation system is an important tool. Conventional CO_2 incubators provide better conditions compared to microscope top incubation chambers, including stability of temperature and humidity, to support embryonic development *in vitro*. Incubation microscopes have also recently become commercially available, however, these are expensive for personal use. We have modified an incubation microscope with wide field fluorescent illumination, which is relatively inexpensive. We added a spinning disc confocal system and sensitive EM-CCD camera for observation of developing mouse embryos with less photo-toxicity and higher spatial resolution.

We have been trying to observe and reveal aspects of cell shape, morphogenesis, cell lineage, gene expression and cell differentiation in developing embryos and in other tissues by combining these techniques. We reported multiple phases in transcriptional regulation of the *Nanog* gene, which is known to be one of the major pluripotent regulators, during the pre-implantation stages of development.

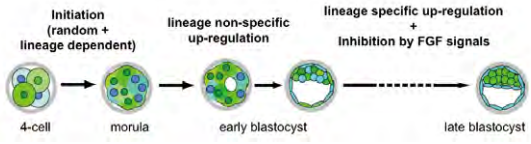

Figure 1. Multiple phases in *Nanog* gene expression during preimplantation mouse development. Weak expression of Nanog starts around the 4-cell stage, and is random. From the 4-cell stage to the morula, the up-regulation of Nanog expression is not lineage specific. Lineage specific differences appear by the early blastocyst stage, and inhibition by FGF signaling becomes evident in the late blastocyst respectively. Images are adapted from Komatsu and Fujimori, 2015.

II. Histological observation of mouse embryos developing in the uterus

One of the characteristics of mammalian development is that embryos develop in the uterus after implantation. Direct interaction between embryos and the cells of the uterus is important. In studies on developmental biology of mammalian embryos, embryos are usually removed from the uterus, and isolated embryos are analyzed. We have been analyzing early embryonic development of mouse comparing the changes in the uterine epithelium. To observe the morphogenesis of embryos within the uterus, serial sections of implanted uteruses were made, stained with hematoxylin and eosin, and images of the embryos within the uteruses were captured to make high resolution three-dimensional re-constructions. Figure 2 shows an example of a section. We are preparing samples obtained from various developmental stages. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic

development will be examined using these images. To obtain three dimensional images of embryos within the uterus, we have been developing a system to automatically extract regions of the uterus where embryos localize by utilizing image analysis after images of serial sections are captured using a slide scanner.

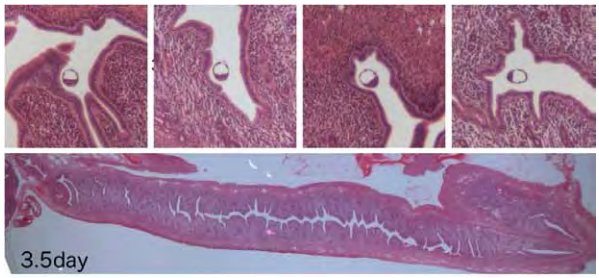

Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

III. Polarity formation in the mouse oviduct epithelium

The oviduct (Fallopian tube) is the organ connecting the periovarian space and the uterine horn. The ova released from the ovary are transported through the oviduct, where the ova are fertilized by spermatozoon entering from the uterus. We are focusing on the region of the oviduct close to the opening to the ovary.

The epithelium of the mouse oviduct consists of multi-ciliated cells and secretory cells. Ovulated oocytes are transported in oviducts by unidirectional motility of multi-cilia and the resultant secretory fluid flow from ovary to uterus. This cellular orientation in the oviduct is one example of planar cell polarity (PCP). PCP is seen in many animals and tissues, where cells sense global axes of the tissue to which they belong and orient themselves to fulfill specialized functions.

Many evolutionally conserved genes are required for the formation of PCP, and several of those encode proteins that are localized in polarized manners within cells. We found that Celsr1, a PCP core member, was strongly concentrated only at the specific domains of cell boundaries which are perpendicular to the ovary-uterus axis and that this polarized localization appeared to precede the directional movement of cilia.

In *Celsr1*-deficient mutant oviducts, cilia were generated and those within each cell appeared to beat as in the wild type oviduct. However, the beating direction was not coordinated along the ovary-uterus axis throughout the epithelia; some cells were oriented orthogonally to the axis, while some others exhibited reverse polarity. In addition to the ciliary polarity phenotype, cellular shape polarity was also impaired in the *Celsr1*-deficient mice. We found a new feature of cellular polarity in the wild type oviduct, e.g. that the apical surface of epithelial cells was elongated along the ovary-uterus axis. In *Celsr1*-deficient mice, epithelial cells showed less elongation and randomized orientation.

Furthermore, we also found that the three-dimensional architecture of oviductal epithelia was dramatically disrupted. In adult wild-type mice, the epithelial cell sheet is bent multiple times, which forms around twenty longitudinal folds parallel to the organ axis. However, in the mutant oviducts, epithelial folds no longer run parallel to the organ axis. The direction of the folds was randomized, and ectopic branches of the folds were observed. This suggests that Celsr1 is important for the formation of multi-scale polarities in the mouse oviduct ranging from the cellular to the tissue scale.

Currently, we have been trying to reveal the mechanisms of oviduct epithelial morphogenesis by integrating the molecular functions of PCP factors, cellular shape changes, tissue morphology, and involvement of mechanical forces. We are also focusing on some other PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain their polarity.

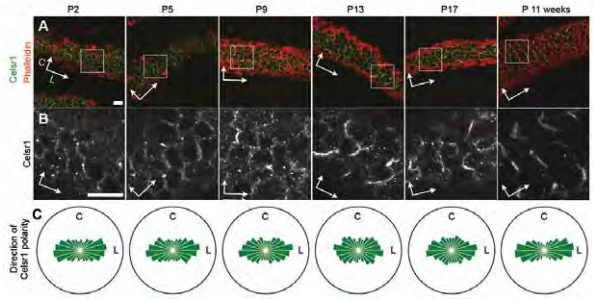

Figure 3. Celsr1 localization in mouse oviduct epithelium. (A-B) Confocal microscopy images of the oviduct epithelium stained for Celsr1 and F-actin (Phalloidin) at several developmental stages. The ovary side is to the left and the uterus side is to the right. Higher magnification of Celsr1 images are shown in (B). P: postnatal days. Arrows indicate the axes; L, longitudinal axis (the ovary-uterus axis); C, circumferential axis. (C) Distribution and orientation of the cellular polarity of Celsr1 localization quantified by image processing. Scale bars: 10 micron. Images are adapted from Shi et al., 2014.

IV. Analysis of mechanical properties of cells and tissues during embryonic development

Mechanics is one of the essential components of biological processes, including cell shape transformation and tissue morphogenesis etc. However, how mechanical states such as force and material stiffness regulate these processes is poorly understood. To approach this problem, measuring cellular and tissue geometric information and mechanical states is necessary. We developed image processing based techniques to measure cellular and tissue geometric information from fluorescent microscopic images and frameworks to theoretically estimate the mechanical states.

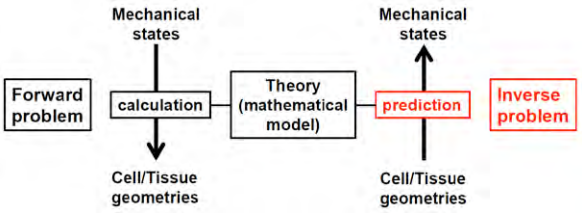

Figure 4. Theoretical estimation of cellular/tissue mechanical states. Schematic illustration of estimation.

By employing image processing techniques, we have extracted geometric information, shapes, and movements of early embryogenesis in *C. elegans* and mice. In the framework for estimating mechanical states, geometric informa-

tion was combined with a mechanical simulation, which was based on data assimilation (Figure 4). We successfully estimated the spatio-temporal dynamics of cellular and tissue mechanical states by systematically fitting the *in vivo* geometric states to the mechanical simulation. The mechanical information will be useful to investigate physical mechanisms of early embryonic development and morphogenesis during organogenesis in late stages in development.

Publication List:

〔**Original papers**〕

- Fujii, S., Nishikawa-Torikai, S., Futatsugi, Y., Toyooka, Y., Yamane, M., Ohtsuka, S., and Niwa, H. (2015). Nr0b1 is a negative regulator of Zscan4c in mouse embryonic stem cells. Sci. Rep. *5*, 9146.
- Horikawa, S., Ishii, Y., Hamashima, T., Yamamoto, S., Mori, H., Fujimori, T., Shen, J., Inoue, R., Nishizono, H., Itoh, H., Majima, M., Abraham, D., Miyawaki, T., and Sasahara, M. (2015). PDGFRα plays a crucial role in connective tissue remodeling. Sci. Rep. *5*, 17948.
- Komatsu, K., and Fujimori, T. (2015). Multiple phases in regulation of Nanog expression during pre-implantation development. Dev. Growth Differ. *57*, 648-656.
- Nakayama, S., Arima, K., Kawai, K., Mohri, K., Inui, C., Sugano, W., Koba, H., Tamada, K., Nakata, Y.J., Kishimoto, K., Arai-Shindo, M., Kojima, C., Matsumoto, T., Fujimori, T., Agata, K., and Funayama, N. (2015). Dynamic transport and cementation of skeletal elements build up the pole-and-beam structured skeleton of sponges. Curr. Biol. *25*, 2549-2554.
- Reyes de Mochel, N.S., Luong, M., Chiang, M., Javier, A.L., Luu, E., Fujimori, T., MacGregor, G.R., Cinquin, O., and Cho, K.W. (2015). BMP signaling is required for cell cleavage in preimplantation-mouse embryos. Dev. Biol. *397*, 45-55.

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Overview of our research

Production of numerous sperm for a long period in testis is fundamental for continuity of life across generations. This process of spermatogenesis is supported by the robust function of "stem cells", which both maintain the undifferentiated cells, while generating differentiated cells in a nicely balanced manner. The Division of Germ Cell Biology aims to fully understand the mouse sperm stem cell system under the context of *in vivo* testicular tissue. Our particular interests have been laid on the "undifferentiated spermatogonia", which are responsible for the stem cell functions. Our study has revealed several key properties of this interesting population.

First, we found that this stem cell system includes a functional hierarchy. It is comprised of an "actual" stem cell compartment that is prone to self-renew, and a differentiation-primed, "potential" stem cell compartment. Regarding the "actual" stem cells, we have been investigating their cellular identity, their *in vivo* behavior at a single-cell resolution, and the underlying mathematical principles. This lead to the discovery of neutral competition between the stem cells. We are currently investigating the mechanisms underlying the constant size of this population, and its connection to its tissue environment. "Potential stem cells" are also of our enthusiastic interest: In undisturbed, steady-state spermatogenesis, they largely contribute as transient amplifying cells and differentiate rather than self-renew. However, when tissues are insulted or testicular cells are transplanted to a host testes, their probability of self-renewal jumps up and they effectively replenish the lost "actual" stem cells. Such a flexible feature of stem cell dynamics has been found paradigmatic for many other stem cell-supported tissues.

Key references for these studies that are currently public include Nakagawa et al., Dev. Cell 2007; Yoshida et al., Science 2007; Nakagawa et al., Science 2010; Klein et al., Cell Stem Cell 2010; and Hara et al., Cell Stem Cell 2014.

I. The stem cell dynamics

Morphologically, the population of A_{undiff} includes singly isolated cells (A_s), or syncytia consisting mainly of 2 (A_{pr}), 4 (A_{al-4}), 8 (A_{al-8}), or 16 (A_{al-16}) cells. The formation of syncytia is due to incomplete cell division, a germline-specific cell division process where cytokinesis does not complete and the cytoplasmic connection between daughter cells persists via intercellular bridges. The prevailing stem cell theory proposed in 1971 states that stem cell activity is restricted to the population of A_s cells, while interconnected A_{pr} and A_{al} syncytia are irreversibly committed to differentiation and no longer contribute to the stem cell pool (Huckins, 1971; Oakberg, 1971), known as the "A_s model".

Figure 1 represents our latest model for the functional structure of the spermatogenic stem cell system, which indeed proposes an alternative for the "A_s model". This is the simplest interpretation of the results of our functional analyses of GFRα1$^+$ spermatogonia, which act as the "actual" stem cells. These include intravital live-imaging experiments, clonal fate analysis of pulse-labeled cells, and biophysical modeling analysis of the results.

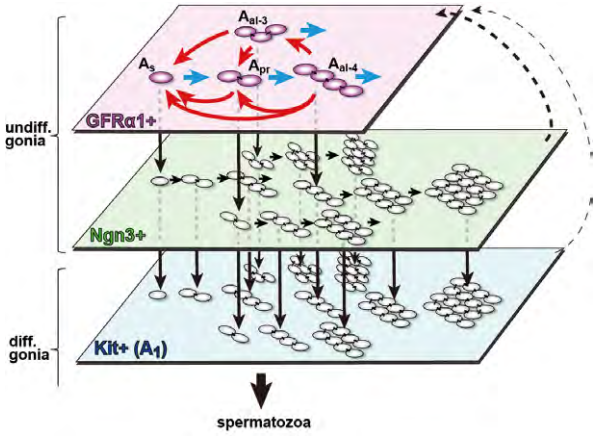

Figure 1. A proposed stem cell dynamics. On the top of the differentiation hierarchy, GFRα1$^+$ spermatogonia comprise a single stem cell pool, in which cells continually and reversibly interconvert between states of A_s, A_{pr} and A_{al} spermatogonia through incomplete cell division (blue arrows) and syncytial fragmentation (red arrows), while giving rise to Ngn3$^+$ cells. After leaving the GFRα1$^+$ compartment, differentiation-destined cells follow a series of transitions (GFRα1$^+$→Ngn3$^+$→Kit$^+$; downward black arrows) that accompany the extension of syncytial length (rightward black arrows). Ngn3$^+$ and, to a lesser extent, Kit+ cells retain the capacity to revert back into the GFRα1+ compartment in a context-dependent fashion (broken arrows). (Reprinted from Hara et al., Cell Stem Cell 2014.)

As crystalized in this model, our results suggest that the GFRα1+ sub-population of A_{undff} spermatogonia, which include both A_s cells and syncytia (A_{pr} and A_{al}) comprises a single stem cell pool, in which cells continually interconvert between these morphologically heterogeneous states through stochastic incomplete division and fragmentation of syncytia. The incomplete division and syncytial fragmentation causes the expansion of this population, while the excess cells over a particular "quota" would overflow to become the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).

Ngn3$^+$ state of A_{undff}, which then further differentiate into Kit$^+$ "differentiating" spermatogonia that are largely devoid of self-renewing potential. Currently, we are investigating the mechanism that determines the quota or tissue capacity of GFRα1$^+$ cells, as well as the detailed nature of the heterogeneity of this population.

II. Mechanisms of the different behavior of "actual" and "potential" stem cells

In 2015, we challenged one key question: Why a particular "actual" stem cell population of A_{undiff} (i.e., GFRα1$^+$ cells) remain undifferentiated, while the other, "potential" stem cells (Ngn3$^+$) differentiate, despite that both cells are located in the same tissue "open niche" environment and exposed to the same differentiation-inducing signal? (Ikami et al., Development 2015)

2-1. Architecture of the mouse testis – the "open niche"

It was generally considered that the behavior of stem cells is regulated by a tissue microenvironment, or the stem cell niche. In some tissues, like *Drosophila* gonads or mammalian small intestines, stem cells cluster in an anatomically specialized ("closed" or "definitive") niche that determines the stem cell fates. Because niche-derived signals appear to be spatially restricted, cells that are located within the niche can be maintained in an undifferentiated state, and their displacement from the niche leads to differentiation. In mouse testis, in contrast, spermatogenic stem cells appear to be distributed over the basal compartment and intermingled with differentiating

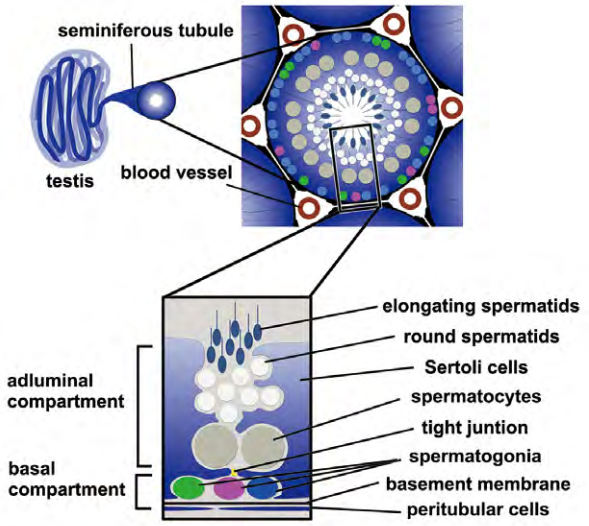

Figure 2. Anatomy of seminiferous tubules and seminiferous epithelium. GFRα1$^+$ and Ngn3$^+$ cells and KIT$^+$ differentiating spermatogonia, reside in the basal compartment (between the basement membrane and the tight junction of Sertoli cells). Modified from Ikami *et al*., Development (2015).

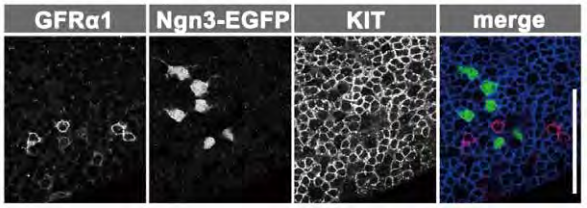

Figure 3. Intermingling of GFRα1$^+$, Ngn3$^+$, and KIT$^+$ spermatogonia. Representative images of triple immuno-stained whole-mount seminiferous tubules of an *Ngn3-EGFP* mouse. Scale bars = 50 μm. Reproduced from Ikami *et al*., Development (2015)

progeny, designated as an "open" niche. Here, the details of the mechanisms that determine whether stem cells differentiate or remain undifferentiated are unknown.

Mouse spermatogenesis occurs in seminiferous tubules and represents a typical example of an open niche-supported stem cell system (Figure 2) (Russell *et al*., 1990; Stine and Matunis, 2013). Here, GFRα1$^+$ (roughly corresponding to "actual" stem cells), Ngn3$^+$ (nearly "potential" stem cells), and Kit$^+$ ("differentiating" spermatogonia that have lost most stem cell potential) populations of spermatogonia are intermingled with each other in the basal compartment of the seminiferous tubules (Figure 3). These cells are uniformly exposed to retinoic acid (RA), an essential differentiation-inducing signaling molecule that is synthesized in a spatially ubiquitous but temporally periodic manner. Intriguingly, however, all of A_{undiff} cell do not differentiate, rather some A_{undiff} always remain undifferentiated. Although this process is extremely important for the long-term maintenance of the stem cells and the integrity of spermatogenesis, the underlying mechanism has been largely unknown.

2-2. Heterogeneous differentiation competence in response to retinoic acid

To address this issue, we tested the response of Ngn3$^+$ and GFRα1$^+$ spermatogonia to RA by pulse-labeling these populations using the tamoxifen-inducible cre-loxP system. The results indicated that Ngn3+ cells rapidly and efficiently differentiated into KIT$^+$ cells in response to RA. In contrast, GFRα1$^+$ cells did not show effective differentiation induced by RA, but they generated Ngn3$^+$ cells in an RA-independent manner. Thus, GFRα1$^+$ and Ngn3$^+$ cells show distinctive differentiation competence in response to RA.

Next, through DNA microarray gene expression analysis of FACS-sorted GFRα1$^+$ and Ngn3$^+$ fractions, we found that the expression of Retinoic acid receptor gamma (RARγ) was highly specific in Ngn3$^+$ cells compared to GFRα1$^+$ cells (Figure 4). In contrast, the levels of other transcripts related to the RA signaling pathway were similar between these cells, Further, we found that enforced expression of RARγ provided the GFRα1$^+$ cells with differentiation competence to differentiate to KIT$^+$ cells in response to RA.

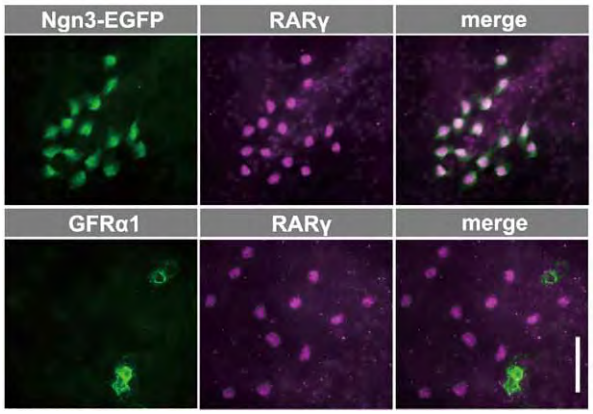

Figure 4. Predominant expression of RARγ in NGN3$^+$ spermatogonia compared with GFRα1$^+$ cells.
Representative images of whole-mount seminiferous tubules from *Ngn3-EGFP* mice stained for EGFP, GFRα1 and RARγ. Scale bars = 50 μm. Reproduced from Ikami *et al*., Development (2015)

2-3. Differential dynamics of GFRα1$^+$ and Ngn3$^+$ spermatogonia

To summarize, these results inferred that, when exposed to a high concentration of RA, RARγ drives the Ngn3$^+$ cells to differentiation, while the absence of RARγ allows the GFRα1$^+$ cells to remain undifferentiated. This heterogeneous differentiation competence, combined with the periodically but ubiquitously distributed RA, appears to be important for homeostatic spermatogenesis in the open niche environment of seminiferous tubules (Figure 5). This scenario would be paradigmatic for other open niche-supported tissue stem cells, since it would allow the stem cell population to maintain the undifferentiated pool while periodically producing differentiating progeny.

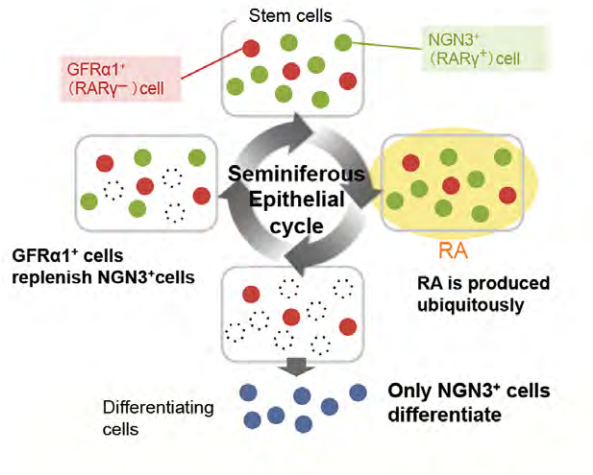

Figure 5. A model of stem cell dynamics during the seminiferous epithelial cycle.
Before the elevation of RA, the entire A_{undiff} population is composed of a mixture of GFRα1$^+$ (RARγ$^-$) and Ngn3+ (RARγ$^+$) cells (top). When the RA amount increases, to which both GFRα1$^+$ and Ngn3$^+$ spermatogonia appear to be equally exposed (right), only Ngn3+ cells that express RARγ respond and differentiate into Kit$^+$ cells, while GFRα1$^+$ cells remain undifferentiated (bottom). Then, the GFRα1$^+$ cells replenish Ngn3$^+$ cells through a RA-independent mechanism (left). Modified from Ikami *et al*., Development (2015)

Publication List:

〔Original paper〕

- Ikami, K., Tokue, M., Sugimoto, R., Noda, C., Kobayashi, S., Hara, K., and Yoshida, S. (2015). Hierarchical differentiation competence in response to retinoic acid ensures stem cell maintenance during mouse spermatogenesis. Development *142*, 1582-1592.

〔Review Article〕

- Yoshida, S. (2015). From cyst to tubule: innovations in vertebrate spermatogenesis. WIREs Developmental Biology, doi: 10.1002/wdev.204

Laboratory of Molecular Genetics for Reproduction †

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Laboratory scope

Reproduction is a universal and fundamental system for organisms to produce new generations. To accomplish this purpose, organisms have developed their own sexual strategies, which allow them to adapt to their environment, thereby progressing toward maximum efficiency of reproduction. During the embryo and larval terms, organisms develop many cell-lineages that have special and essential roles in each different process of reproduction. These lineages are mostly conserved among vertebrates.

Vertebrates, however, exhibit a variety of reproductive systems. The mechanisms of sex determination and sex differentiation are some of the components of the reproductive system which produce this variety. Actually, there are many modes of sex determination. Sex determination genes are different among vertebrates. Sex determination does not even have to be controlled genetically. This variety is allowed by the different employment and different emergence of the cell lineages during embryogenesis. Therefore, it is important to address the roles of each cell lineage for understanding the fundamental mechanism underlying a variety of reproductive systems. Currently, our lab focuses on the core mechanisms which are independent of sex determination genes and which produce and maintain the sex. The core mechanism can be referred to as cellular interaction between germ cells and surrounding somatic cells, wherein germ cells have the ability to feminize somatic cells while the surrounding somatic cells are predisposed to male development. These characters of each set of cells are totally independent of the sex determination gene on the medaka Y chromosome. We are addressing the details of this core mechanism by analyzing each cell lineage in the context of sex differentiation.

To accomplish the purpose of our study, we use medaka fish (*Oryzias latipes*). We have been generating transgenic medaka enabling us to analyze how different cell lineages are involved in the process of gonad formation and sex differentiation *in vivo*. Additionally, in order to identify the genes essential for reproduction, we carried out a mutational screening of medaka with defective phenotypes and disrupted several candidate genes. With these two unique analytical methods (visualizing cells, and mutants), we are attempting to unveil both the fundamental mechanisms and the specific mechanisms that produce a variety of reproductive systems.

Through these analyses, we have been revealing the presence of germline stem cells in the ovary. This was the first proof of this system in vertebrates (Nakamura et al 2010 Science). The fluorescently labeled germline stem cells keep producing eggs with fluorescence during the entire period of medaka reproduction, which is a conclusive indication of the presence of germline stem cells. The tricks employed in this experiment are transgenic medaka that allow heat-inducible gene expression.

I. An issue of sex determination of germ cells

In the core mechanism of sex, germ cells are responsible for feminization and somatic cells are for masculinization. An important thing here is that we can determine the sex intentionally, if the core mechanism is to be modified, without any effect from the sex determination gene. Then a big issue is how the sex of germ cells, in other words, the fate decision of germ cells to become sperm or eggs, is determined. Few people have addressed this issue in vertebrates.

One way of looking at this issue is that there is no germ cell-intrinsic mechanism of sex. Somatic cells might send a signal(s) to germ cells during each developmental stage of spermatogenesis/oogenesis. In this case, it would be difficult to discern the germ cell-intrinsic mechanism of sex. Alternatively, we might assume that the sexual fate of germ cells is determined at the initial commitment into gametogenesis. Somatic cells may only regulate the timing and/or supply an essential material(s) for germ cells to develop into the next stage of gametogenesis. In this case, once the somatic cells give a cue to germ cells for which way to go, the path toward oogenesis or spermatogenesis is already determined. But nobody has shown which way of understanding is right in germ cell sex determination.

Since transplanted germ cells from testis into females develop into eggs and the germ cells from ovaries become sperm in testis, It is generally accepted that germline stem cells are sexually indifferent or unfixed. No morphological difference between germ cells from females or those from males exist during early stages of gametogenesis, until they reach the pachytene stage in meiosis. At the pachytene stage, female germ cells are larger than male germ cells. These observations have collectively suggested that germ cell-sex determination occurs at some point between the germline stem cell stage and that of the pachytene stage (figure 1).

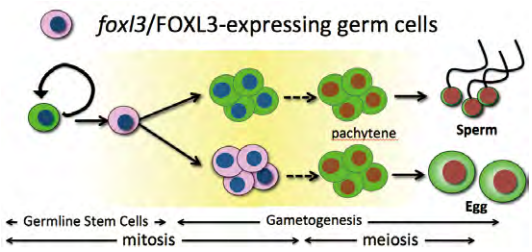

Figure 1. Simplified cellular processes of gametogenesis. Given that an intrinsic mechanism of sex determination is present in germ cells, it should be between the stage of germline stem cells and that of pachytene in meiosis (yellow graded area). *foxl3* is found to be expressed only in female germ cells (pink germ cells) in the yellow graded term.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).
†: This laboratory was closed on 31 May, 2016.

II. Identification of a sexually different gene in early germ cells (Nishimura et al 2015 Science)

We have prepared all XX or all XY medaka larva and have purified germ cells during early gametogenesis. Different stages of germ cells were successfully purified using fluorescent activated cell sorter (FACS) and the transcriptomes of different stages and different sexes of germ cells were analyzed by next-generation sequencing.

One gene that attracted us was *foxl3*, a gene encoding a transcriptional factor with the forkhead domain. The gene is initially expressed in both female and male developing gonads but male developing gonads lose its expression while *foxl3* expression persists in female germ cells. Interestingly, some populations of germline stem cells express this gene and, as oogenesis proceeds, expression in germ cells diminishes by the time they enter the pachytene stage. The expression pattern matched the theoretically expected expression pattern of the sex determination gene in germ cells (figure 1).

III. *foxl3* represses initiation of spermatogenesis in germ cells

To understand the function of *foxl3*/FOXL3, we generated *foxl3* mutant medaka using the TALEN-method.

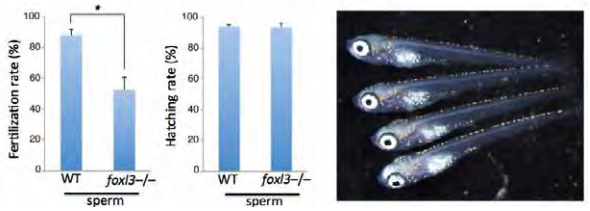

Figure 2. Although the rate is relatively low compared with normal sperm, sperm from mutant ovaries have the ability to fertilize eggs (left graph). Once the eggs are fertilized with sperm from the mutant ovary, they develop to hatch as efficiently as normal embryos (middle graph). Hatched larva grow into fertile medaka. The picture on the right shows medaka larva hatched from eggs fertilized with sperm from a mutant ovary.

Very interestingly, homozygous female medaka initiate development of sperm in the early larval stage. Together with the fact that *foxl3* is expressed specifically in female germ cells, this indicates that *foxl3* represses initiation of spermatogenesis in germ cells in female gonads.

IV. Functional sperm develop in the ovary

We artificially inseminated sperm from female mutants into normal eggs, and found that the fertilized eggs developed into normal embryos. The embryos hatched and grew into fertile adult medaka. This clearly demonstrates that sperm in the female mutants is functional (figure 2).

Then, we analyzed the structure of the gonads in the female mutants. The gonads display a typical ovarian structure with an ovarian cavity separated from the stromal compartment by a germinal epithelium. In normal ovaries, the germline stem cells with ovarian niches, Germinal Cradles, are located within the germinal epithelium and, as described, germline stem cells express *foxl3*. In the female *foxl3* mutants, sperm is developed in the germinal epithelium. Probably because there is no exit for the sperm to leave the germinal epithelium, the germinal epithelium with functional sperm expands towards the stromal compartment. Therefore the mutant ovary looks as if sperm fully fills an entire ovary (figure 3).

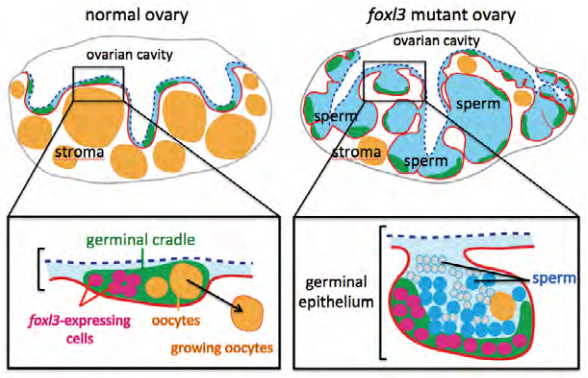

Figure 3. Schematic comparison of normal ovary with ovary producing sperm in the mutant female. Sperm develop in the germinal cradles in the germinal epithelium in the *foxl3* female mutant

These results reveal several important points:

1. An intrinsic mechanism of sex determination is present in germ cells. This mechanism can function against the direction of the sex determination gene and direct germ cells to develop to a sex opposite from the somatic sex.
2. Repression of initiation of spermatogenesis is a main component of the sexual switch of germ cells (the fate decision to develop into eggs or sperm) and involves *foxl3*.
3. Spermatogenesis does not require the testicular environment. It can occur in the ovarian structure and environment. This suggests that the testicular environment is required for the coordinated regulation of spermatogenesis with other parts of the body. This also suggests that germline stem cells with *foxl3* are ready for spermatogenesis.

Publication List:

〔Original paper〕

● Nishimura, T., Sato, T., Yamamoto, Y., Watakabe, I., Ohkawa, Y., Suyama, M., Nakamura, S., Saito, T.L., Yoshimura, J., Morishita, S., Kobayashi, S., and Tanaka, M. (2015). *foxl3* is a germ cell-intrinsic factor involved in sperm-egg fate decision in medaka. Science *349*, 328-331.

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We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using mice. This research covers many developmental events including the patterning of the retina, neuronal terminal differentiation, axonal navigation, branching and targeting, synapse formation, refinement and plasticity. The scope of our interests also encompasses the mechanisms for various functions of the mature brain, including body-fluid regulation, behavior control, learning, and memory.

I. Mechanisms for neural circuit formation

Adenomatous polyposis coli 2 (APC2) is preferentially expressed in the nervous system from early developmental stages through to adulthood. The knockdown of *Apc2* in chick retinas reduced the stability of microtubules in retinal axons, and yielded abnormal growth cone behaviors including a reduced response to ephrin-A2 and misprojection in the tectum without making clear target zones. In *Apc2*-deficient mice, robust defects in neuronal lamination were observed in the cortex, hippocampus, cerebellum, and olfactory bulb. These laminary abnormalities are a result of dysregulated neuronal migration by a cell-autonomous mechanism. APC2 is distributed along actin fibers as well as microtubules in neurons. Our investigation suggests that APC2 is involved in the signaling pathway from membrane receptors for extracellular guidance factors to the intracellular migration machinery.

Very recently, two siblings with Sotos syndrome-like features caused by homozygous frame shift mutation in the *APC2* gene were found by whole exome sequencing. Sotos syndrome (OMIM #117550) is characterized by varying degrees of mental retardation and a combination of typical facial features (prominent forehead with a receding hairline, downslanting palpebral fissures, and a pointed chin) and large head circumference its estimated incidence is 1/14,000 live births. Sotos syndrome has been known to be caused by haploinsufficiency in the *NSD1* gene. However, these patients had no mutations in *NSD1* or other known susceptibility genes.

We performed an etiological study on these patients. Cell-based functional assays indicated that the mutant APC2 of these patients was a functionally null protein. *Apc2*-deficient mice exhibited significantly impaired learning and memory abilities, together with an abnormal brain structure and head shape, indicating a high degree of concordance between the phenotypes observed in human patients and knockout mice. We demonstrated for the first time that the expression of *APC2* was under the control of *NSD1*. The impaired migration of cortical neurons was observed when *Nsd1* was knocked down in the embryonic mouse brain, as in the *Apc2*-deficient brain, and this defect was rescued by the forced expression of *Apc2* (Figure 1). Our study explains molecular mechanisms of the intellectual disability associated with Sotos syndrome.

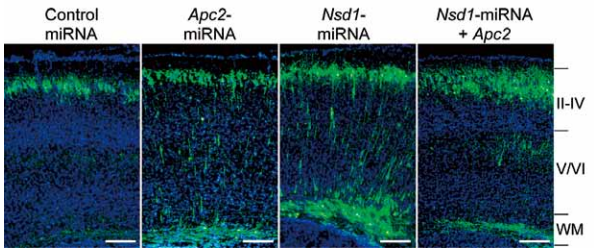

Figure 1. Effects of *Nsd1* knockdown on the migration of cortical neurons during development. The control miRNA, *Apc2*-miRNA, or *Nsd1*-miRNA construct was electroporated into the ventricular region of the forebrain at E15.5. For the rescue experiment, *Nsd1*-miRNA was co-electroporated with an *Apc2*-expressing plasmid. At P7 the location of transfected cells (GFP+) was assessed in coronal sections of the cortex. Nuclei were stained with DAPI. The layers of the cortex are indicated on the right side. WM, white matter. Scale bars, 100 μm.

II. Physiological roles of receptor-like protein tyrosine phosphatases

Protein tyrosine phosphorylation plays crucial roles in various biological events such as cellular proliferation, differentiation, survival, migration, and metabolism. Cellular tyrosine phosphorylation levels are governed by the opposing activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). The physiological functions and regulatory mechanisms of receptor-like PTPs (RPTPs) are not fully elucidated. We have been making efforts to reveal the functional roles of RPTPs, especially of the R3 and R5 subfamilies.

2-1 R3 RPTP subfamily

The R3 RPTP subfamily, which is comprised of PTPRB, PTPRH, PTPRJ, and PTPRO, reportedly plays pivotal roles in the development of several tissues including the vascular and nervous systems. We performed a large scale examination of the enzyme-substrate interaction between the R3 RPTP members and representative RPTKs covering

all RPTK subfamilies. We revealed that multiple RPTKs are recognized as substrates by the R3 RPTPs. Among the enzyme-substrate relationships identified, we examined the interaction between the R3 RPTPs and insulin receptor (IR) in detail.

Co-expression of R3 RPTPs with the IR in HEK293T cells suppressed the insulin-induced tyrosine phosphorylation of the IR. *In vitro* assays using synthetic phosphopeptides revealed that R3 RPTPs preferentially dephosphorylates particular phosphorylation sites of the IR; Y960 in the juxtamembrane region and Y1146 in the activation loop. It has been revealed that phosphorylation of Y1146 and Y960 are required for the full activation and the signal transduction of the IR, respectively. Therefore, R3 RPTPs are thought to effectively dephosphorylate these sites of IR, and thereby suppress insulin signaling.

RT-PCR and *in situ* hybridization showed that only PTPRJ was expressed together with IR in major insulin target tissues, such as skeletal muscle, liver, and adipose tissue. Expectedly, *Ptprj*-deficient mice exhibited enhanced activation of IR and Akt by insulin, and improved glucose and insulin tolerance (Figure 2). These results indicate that PTPRJ is a physiological enzyme attenuating insulin signaling *in vivo*, and selective inhibitors of PTPRJ could be insulin-sensitizing agents.

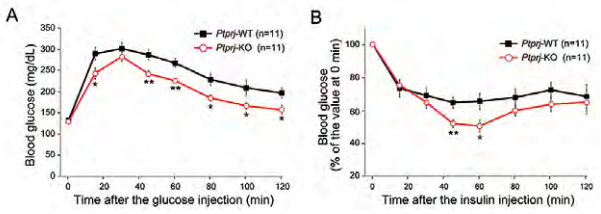

Figure 2. Improved glucose and insulin tolerance in *Ptprj*-KO mice. In glucose (A) and insulin (B) tolerance tests, glucose (2 g/kg body weight) and insulin (1 unit/kg body weight) were administered by intraperitoneal injection to fasted mice, respectively, and blood glucose levels were assayed. Values are shown as the mean ± SEM. The asterisks indicate significant differences from WT mice by Student's *t*-test (*$p < 0.05$, **$p < 0.01$).

2-2 R5 RPTP subfamily

Protein-tyrosine phosphatase receptor type Z (PTPRZ) is predominantly expressed in glial and neuronal cells during development in the central nervous system (CNS). Oligodendrocyte precursor cells (OPCs) are the principal source of myelinating oligodendrocytes. Deficiencies in myelination in diseases such as multiple sclerosis (MS) lead to serious neurological disorders. Two animal disease models have been widely accepted for studying the clinical and pathological features of MS lesions. Experimental autoimmune encephalomyelitis (EAE) is a T-cell-mediated inflammatory CNS demyelination model, and the cuprizone model of demyelination is induced by a T-cell-independent mechanism through feeding of the copper chelator cuprizone. We previously showed that adult *Ptprz*-deficient mice were less susceptible to the induction of EAE than wild-type controls.

This year, we revealed that cuprizone-fed *Ptprz*-deficient mice exhibited severe demyelination and axonal damage in the corpus callosum, similar to wild-type mice, whereas remyelination in *Ptprz*-deficient mice after cuprizone-induced demyelination was significantly accelerated. Accelerated remyelination was attributed to the higher differentiation potential of OPCs, because the number of oligodendrocyte-lineage cells recruited to the demyelinated area was not altered. Importantly, pleiotrophin, one of the inhibitory ligands for PTPRZ, was transiently expressed in the brain upon demyelination, and gradually disappeared with remyelination. Pleiotrophin was detected in affected cortex neurons and their axon fibers in the cuprizone model. Since pleiotrophin colocalized with a synaptic vesicle marker, the relevant neurons are presumed to release pleiotrophin from their demyelinated axons (Figure 3). The treatment of a primary culture of wild-type mouse brain cells with pleiotrophin itself did not induce oligodendrocyte maturation, but enhanced thyroid hormone-induced oligodendrocyte differentiation. Of note, the differentiation of *Ptprz*-deficient cells was not further potentiated by pleiotrophin. Pleiotrophin released from demyelinated fibers may stimulate the differentiation of OPCs recruited in the demyelinated area *in vivo*.

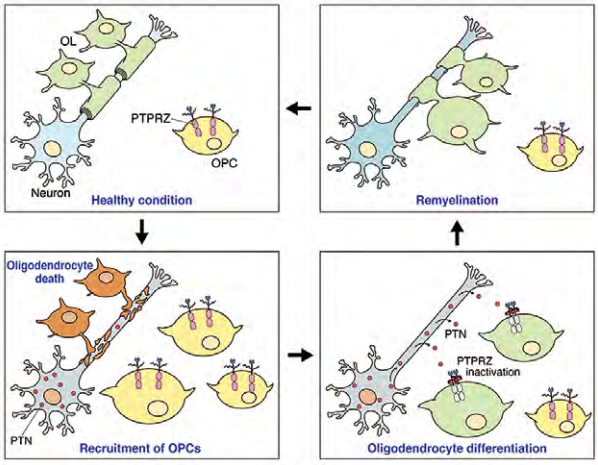

Figure 3. Proposed mechanism for remyelination after demyelinating lesions. OPCs, but not mature oligodendrocytes (OLs), abundantly express PTPRZ-A/B receptor proteins as chondroitin sulfate (CS) proteoglycans. The CS moiety of PTPRZ is important for achieving the high-affinity binding of PTN to the core protein. PTPRZ activity is requisite to maintaining the immature state of OPCs. After cuprizone-induced oligodendrocyte death and demyelination, the expression of PTN is transiently upregulated in damaged neurons. PTN may be released from demyelinated axons and bind to PTPRZ at the cell surface of OPCs that are recruited to lesions, probably through PTPRZ-independent mechanisms. The binding of PTN results in receptor dimerization or oligomerization, thereby inhibiting its catalytic activity. PTPRZ inactivation releases the block of differentiation in OPCs, so the remyelination of neighboring axons is initiated.

We established immature oligodendrocytes (OL1 cells) from *p53*-deficient mice. They were strongly positive for the two receptor isoforms of PTPRZ-A and PTPRZ-B with chondroitin sulfate chains; however, their expression gradually decreased with differentiation, with only PTPRZ-B being weakly detectable in mature oligodendrocytes. The treatment of immature OL1 cells with pleiotrophin enhanced the phosphorylation of p190 RhoGAP, which is a substrate molecule of PTPRZ. We found that pleiotrophin reduced the expression of NG2 proteins in OL1 cells. Therefore, it is

conceivable that the catalytic activity of PTPRZ functions to maintain OPCs in an undifferentiated state, and the pleiotrophin-induced inactivation of PTPRZ releases this blockage (Figure 3).

III. Brain systems for body-fluid homeostasis

Sodium (Na) is a major electrolyte of extracellular fluids and the main determinant of osmolality. Na homeostasis is essential to life and Na^+ concentrations in plasma and cerebrospinal fluid (CSF) are continuously monitored to maintain a physiological level of Na^+ in body fluids. We have previously shown that Na_x, which structurally resembles voltage-gated sodium channels ($Na_v1.1$–1.9), is a Na^+-concentration ($[Na^+]$)-sensitive Na channel with a gating threshold of ~150 mM for extracellular $[Na^+]$ ($[Na^+]_o$) *in vitro*. Na_x is preferentially expressed in glial cells of sensory circumventricular organs including the subfornical organ, and is involved in $[Na^+]$ sensing for the control of salt-intake behavior.

Although Na_x is also expressed in neurons of some brain regions including the amygdala and cerebral cortex, the channel properties of Na_x in neurons have not yet been adequately characterized. To investigate the properties of Na_x expressed in neurons, we established an inducible cell line of Na_x using the mouse neuroblastoma cell line, Neuro-2a, which is endogenously devoid of the expression of Na_x (N2a-Mf1 cell). Functional analyses of this cell line revealed that the $[Na^+]$-sensitivity of Na_x in neuronal cells was similar to that expressed in glial cells.

Furthermore, we found that Na_x bound to postsynaptic density protein 95 (PSD95) through its PSD95/Disc-large/ZO-1 (PDZ)-binding motif at the C-terminus. Na_x co-localized with PSD95 clusters along dendrites of lateral amygdala neurons. PSD95 is known as an anchoring and scaffolding protein for receptors and other postsynaptic proteins. The interaction between Na_x and PSD95 may be involved in promoting the surface expression of Na_x channels by suppressing their endocytosis, because the depletion of endogenous PSD95 resulted in a decrease in Na_x at the plasma membrane (Figure 4). These results show for the first time that Na_x functions as a $[Na^+]$-sensitive Na channel in neurons as well as in glial cells.

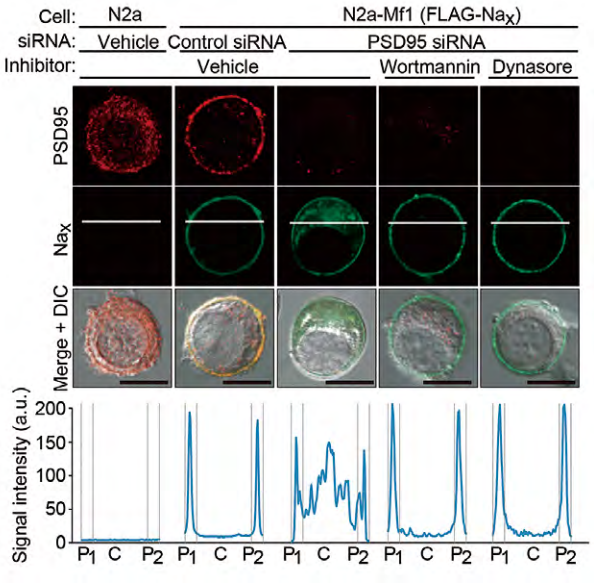

Figure 4. Subcellular distribution of Na_x in non-treated Neuro-2a (N2a) cells, or N2a-Mf1 cells transfected with control or *PSD95* siRNA. Upper panels: Immunostaining with anti-PSD95 and anti-Na_x antibodies. In order to inhibit endocytosis, cells expressing Na_x were treated with 100 nM wortmannin or 200 μM dynasore for 6 h. Scale bars, 10 μm. Lower graphs: Fluorescence intensity profiles along the white lines in the upper panels. The profile was divided into three parts (P1 : C : P2 = 15 : 70 : 15 in length). a.u., arbitrary unit.

Publication List:

〔Original papers〕

- Almuriekhi, M.*, Shintani, T.*, Fahiminiya, S.*, Fujikawa, A., Kuboyama, K., Takeuchi, Y., Nawaz, Z., Nadaf, J., Kamel, H., Kitam, A.K., Samiha, Z., Mahmoud, L., Ben-Omran, T., Majewski, J., and Noda, M. (2015). Loss-of-function mutation in *APC2* causes Sotos Syndrome features. Cell Repotrs *10*, 1585-1598. *Co-first authors.
- Fujikawa, A., Matsumoto, M., Kuboyama, K., Suzuki, R., and Noda, M. (2015). Specific dephosphorylation at Tyr-554 of Git1 by Ptprz promotes its association with Paxillin and Hic-5. PLoS One *10*, e0119361.
- Kuboyama, K., Fujikawa, A., Suzuki, R., and Noda, M. (2015). Inactivation of protein tyrosine phosphatase receptor type Z by pleiotrophin promotes remyelination through activation of differentiation of oligodendrocyte precursor cells. J. Neurosci. *35*, 12162-12171.
- Matsumoto, M., Hiyama, T.Y., Kuboyama, K., Suzuki, R., Fujikawa, A., and Noda, M. (2015). Channel properties of Na_x expressed in neurons. PLoS One *10*, e0126109.
- Shintani, T., Higashi, S., Takeuchi, Y., Gaudio, E., Trapasso, F., Fusco, A., and Noda, M. (2015). The R3 receptor-like protein-tyrosine phosphatase subfamily inhibits insulin signaling by dephosphorylating the insulin receptor at specific sites. J. Biochem. *158*, 235-243.
- Sun, L.O., Brady, C.M., Cahill, H., Al-Khindi, T., Sakuta, H., Dhande, O.S., Noda, M., Huberman, A.D., Nathans, J., and Kolodkin, A.L. (2015). Functional assembly of accessory optic system circuitry critical for compensatory eye movements. Neuron *86*, 971-984.

〔Review articles〕

- Noda, M., and Hiyama, T.Y. (2015). The Na_x channel: What it is and what it does. Neuroscientist *21*, 399-412.
- Noda, M., and Hiyama, T.Y. (2015). Sodium sensing in the brain. Pflügers Arch. – Eur. J. Physiol. *467*, 465-474.

DIVISION OF BRAIN BIOLOGY †

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This year we have reported the following results: (1) Long-term two-photon calcium imaging, (2) *in vivo* dendritic spines in marmoset neocortex, and (3) expression patterns of immediate early genes in the cerebellum.

I. Long-Term Two-Photon Calcium Imaging of Neuronal Populations with Subcellular Resolution in Adult Non-human Primates.

Two-photon imaging with genetically encoded calcium indicators (GECIs) enables long-term observation of neuronal activity in vivo. However, there are very few studies of GECIs in primates. Here, in collaboration with Professor Matsuzaki's laboratory, we report a method for long-term imaging of a GECI, GCaMP6f, expressed from adeno-associated virus vectors in cortical neurons of the adult common marmoset (Callithrix jacchus), a small New World primate. We used a tetracycline-inducible expression system to robustly amplify neuronal GCaMP6f expression and up- and downregulate it for more than 100 days. We succeeded in monitoring spontaneous activity not only from hundreds of neurons three-dimensionally distributed in layers 2 and 3 but also from single dendrites and axons in layer 1. Furthermore, we detected selective activities from somata, dendrites, and axons in the somatosensory cortex responding to specific tactile stimuli. Our results provide a way to investigate the organization and plasticity of cortical microcircuits at subcellular resolution in non-human primates (Published in Cell Rep. 13, 1989-1999, 2015).

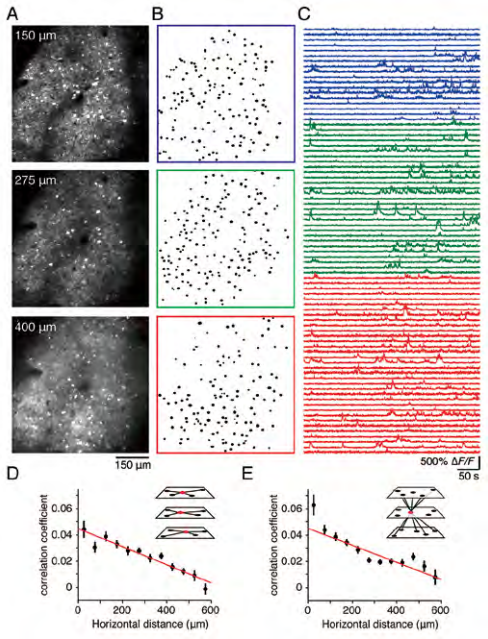

Figure 1. Three-Dimensional Imaging of Neuronal Population Activity in L2 and L3

(A) Representative two-photon images of GCaMP6f at a depth of 150 µm (top), 275 µm (middle), and 400 µm (bottom) from the cortical surface. These fields have the same horizontal location.

(B) ROI for activity analysis at each depth.

(C) Δ*F*/*F* traces of all active neurons over 400 s. Blue, green, and red indicate the traces of neurons at a depth of 150 µm, 275 µm, and 400 µm, respectively.

(D) Relation between pairwise correlation coefficient and horizontal cellular distance for 1,120 pairs of active neurons located at the same depth (from the same color traces shown in C). Red line indicates the linear regression line. Pairs were grouped every 50 µm. The error bars denote SEM.

(E) Relation between pairwise correlation coefficient and horizontal cellular distance for 1,508 pairs of active neurons located at different depths (from blue and green traces and from green and red traces; shown in C). (Cited from Sadakane et al., Published in Cell Rep. 13, 1989-1999, 2015)

II. In Vivo Two-Photon Imaging of Dendritic Spines in Marmoset Neocortex

Two-photon microscopy in combination with a technique involving the artificial expression of fluorescent protein has enabled the direct observation of dendritic spines in living brains. However, the application of this method to primate brains has been hindered by the lack of appropriate labeling techniques for visualizing dendritic spines. Here, we developed an adeno-associated virus vector-based fluorescent protein expression system for visualizing dendritic spines in vivo in the marmoset neocortex. For the clear visualization of each spine, the expression of reporter fluorescent protein should be both sparse and strong. To fulfill these requirements, we amplified fluorescent signals using the tetracycline transactivator (tTA)-tetracycline-responsive element system and by titrating down the amount of Thy1S promoter-driven tTA for sparse expression. By this method, we were able to visualize dendritic spines in the marmoset cortex by two-photon microscopy in vivo and analyze the turnover of spines in the prefrontal cortex. Our results demonstrated that short spines in the marmoset cortex tend to change more frequently than long spines. The comparison of in vivo samples with fixed samples showed that we did not detect all existing spines by our method. Although we found glial cell proliferation, the damage of tissues caused by window construction was relatively small, judging from the comparison of spine length between samples with or without window construction. Our new labeling technique for two-photon imaging to visualize in vivo dendritic spines of the marmoset neocortex can be applicable to examining circuit reorganization and synaptic plasticity in primates (Published in eNeuro. 2(4) ENEURO.0019-15, 2015).

†: This laboratory was closed on 31 March, 2015.

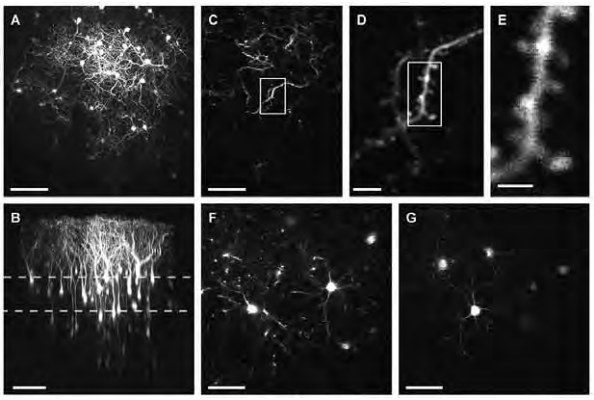

Figure 2. Dendritic spines imaged by *in vivo* two-photon microscopy. ***A***, Maximum intensity projection of the images acquired by *in vivo* two-photon imaging of marmoset cortex. ***B***, Side view of three-dimensional reconstruction of the images of the same site shown in ***A***. The depths of the areas shown in ***F*** and ***G*** are indicated by dashed lines. ***C***, Image plane near pial surface. ***D***, Magnified image of the boxed area in ***C***. ***E***, Magnified image of boxed area in ***D*** showing dendritic spines. ***F***, Image plane at a depth of 220 μm showing soma and basal dendrites. ***G***, Image plane at a depth of 330 μm. Scale bars: ***A***, ***B***, 100 μm; ***C***, 50 μm; ***D***, 5 μm; ***E***, 2 μm; ***F***, ***G***, 50 μm (Published in eNeuro. 2(4) ENEURO.0019-15, 2015)

III. Expression pattern of immediate early genes in the cerebellum of D1R KO, D2R KO, and wild type mice under vestibular-controlled activity

We previously reported the different motor abilities of D1R knockout (KO), D2R KO and wild-type (WT) mice. To understand the interaction between the cerebellum and the striatal direct and indirect pathways, we examined the expression patterns of immediate early genes (IEG) in the cerebellum of these three genotypes of mice. In the WT naive mice, there was little IEG expression. However, we observed a robust expression of c-fos mRNA in the vermis and hemisphere after running rota-rod tasks. In the vermis, c-fos was expressed throughout the lobules except lobule 7, and also in crus 1 of the ansiform lobule (Crus1), copula of the pyramis (Cop) and most significantly in the flocculus in the hemisphere. jun-B was much less expressed but more preferentially expressed in Purkinje cells. In addition, we observed significant levels of c-fos and jun-B expressions after handling mice, and after the stationary rota-rod task in naive mice. Surprisingly, we observed significant expression of c-fos and jun-B even 30 min after single weighing. Nonetheless, certain additional c-fos and jun-B expressions were observed in three genotypes of the mice that experienced several sessions of motor tasks 24 h after stationary rota-rod task and on days 1 and 5 after rota-rod tasks, but no significant differences in expressions after the running rota-rod tasks were observed among the three genotypes. In addition, there may be some differences 24 h after the stationary rota-rod task between the naive mice and the mice that experienced several sessions of motor tasks. (Published in Front Cell Dev Biol. 3:38, 2015).

Publication List

〔Original papers〕

- Nakamura, T., Sato, A., Kitsukawa, T., Sasaoka, T., and Yamamori, T. (2015). Expression pattern of immediate early genes in the cerebellum of D1R KO, D2R KO, and wild type mice under vestibular-controlled activity. Front. Cell Dev. Biol. *3*, 38.
- Sadakane, O., Masamizu, Y., Watakabe, A., Terada, S., Ohtsuka, M., Takaji, M., Mizukami, H., Ozawa, K., Kawasaki, H., Matsuzaki, M., and Yamamori, T. (2015). Long-term two-photon calcium imaging of neuronal populations with subcellular resolution in adult non-human primates. Cell Rep. *13*, 1989-1999.
- Sadakane, O., Watakabe, A., Ohtsuka, M., Takaji, M., Sasaki, T., Kasai, M., Isa, T., Kato, G., Nabekura, J., Mizukami, H., Ozawa, K., Kawasaki, H., and Yamamori, T. (2015). In vivo two-photon imaging of dendritic spines in marmoset neocortex. eNeuro doi: 10.1523/ENEURO.0019-15.2015

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Various firing patterns of many cortical neurons represent information processing in the brain. The microarchitecture of synaptic connections control information processing in cortical circuits. The structure and location of synapses determine and modify the strength of this information processing. The aim of our laboratory is to reveal how information is formed, maintained, selected, and decoded in the brain at the levels of single cells and of single synapses. To do so, we mainly use two-photon microscopy that allows us to see fluorescence signals from deep within living tissue, developing novel photostimulation methods and animal behavioral tasks. The goals of our recent studies are to reveal how voluntary movement is memorized and represented in cortical circuits. In addition, we are working to apply two-photon microscopy to non-human primates in order to understand information processing in the brain, which is relevant to high cognitive functions.

I. Cortical modules for discrete & rhythmic movements (Hira et al., J Neurosci., 2015).

Animal behavior has discrete and rhythmic components, such as reaching and locomotion. It is unclear how these movements with distinct dynamics are represented in the cerebral cortex.

We examined the dynamics of complex movements of freely movable forelimbs in awake ChR2 transgenic mice with the skull coated by transparent resin (Hira et al., 2009). The left cortical surface was illuminated by a blue laser (500-ms train of 2- or 4-ms duration pulses delivered at 50 Hz) focused through the objective lens (Fig. 1). We call this method pTOS. The pTOS-induced trajectory of the right forepaw was tracked in three-dimensional space by two high-speed cameras at 100 or 200 frames/s.

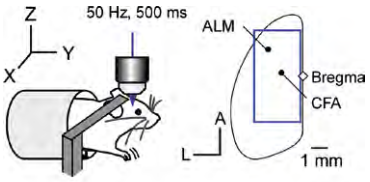

Figure 1. pTOS of an awake, head-restrained, ChR2 mouse. Right, the dorsal view of the left cerebral cortex. The blue square indicates the mapped area.

pTOS induced one of two major types of right forelimb movement. During pTOS of the anterior lateral part of the motor cortex (ALM; Fig. 1), the right forepaw moved to a narrow space in every trial. By contrast, during pTOS of the caudal forelimb motor area (CFA; Fig. 1), the right forepaw moved circularly for more than one cycle and its end point varied. We categorize the former right-forelimb movement as a discrete movement, and the latter as a rhythmic movement. To map the representation of the discrete and rhythmic forelimb movements, we divided a 6 × 3 mm area of the left dorsal cerebral cortex into 16 × 8 sites (inter-site distance of 400 μm) and performed pTOS at each site (Fig. 1). For the first analysis, the direction and distance from the mean initial point to the mean end point of the forepaw were calculated for each pTOS site. The direction and distance had clear topography in the cortex (arrows in Fig. 2). Long arrows on the map indicate the sites that induced forelimb movements with a limited target space and long distance. The ALM and the hindlimb motor area (HA), which is caudal to the CFA, had long arrows with almost opposite directions.

For each pTOS site, the tangential velocity profile of the averaged movement was fitted with a Gaussian function and the coefficient of determination was defined as the 'discreteness index'. The domains with a large discreteness index (magenta in Fig. 2) corresponded to the ALM and the HA, which is consistent with the finding that these areas had long arrows on the map showing the direction and distance of the pTOS-induced movement (green in Fig. 2). We found that rhythmic movement with forward rotation was represented by the domain spanning from the medial part of the RFA to the CFA. The domain to induce the rhythmic movement was sandwiched by two domains to induce the discrete movements in forward and backward directions (forward discrete movement and backward discrete movement) (Fig. 2). When the photostimulation frequency was optimal, the time to reach maximum tangential velocity in the discrete movement was 136 ± 43 ms (n=4 mice) and the frequency with the maximum power of the rhythmic movement was 8.7 ± 0.28 Hz (n=3 mice). Thus, low-frequency photostimulation of the ALM generated a discrete movement with an intrinsic bell-shaped velocity profile, whereas high-frequency photo-

Figure 2. Maps of the discreteness index (magenta) and the rhythmicity index (green) overlain on the map of the direction and distance of the forelimb movement (arrows). The solid line indicates the FDR border.

†: This laboratory was closed on 31 March, 2016.

stimulation of the CFA generated a rhythmic movement with a relatively stable, intrinsic resonance property.

To determine whether the cortical domains to induce the discrete and rhythmic movements were hierarchical or parallel, we applied a mixture of AMPA/kainate receptor antagonist and NMDA receptor antagonist to the cerebral cortex. The size of the movement induced by pTOS of the ALM or CFA was not significantly reduced after the application of the blockers to the photostimulated areas. In addition, unsupervised clustering of the sites with cortico-cortical synaptic interactions obtained by the Allen Mouse Brain Connectivity Atlas (Oh et al., 2014) showed that the border

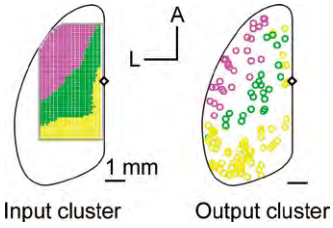

Figure 3. Circles and grids with the same color share cortico-cortical synaptic output and input patterns, respectively.

separating the two different clusters in the frontal cortex roughly corresponded to the FDR border (Fig. 3). Thus, the domains to induce the forward discrete movement and the rhythmic movement were functionally and anatomically distinct. We call such domains 'modules'.

Photostimulation of either of the CFA or ALM suppressed spontaneous activity at the other site. The distance of the discrete movement induced by the simultaneous pTOS of the CFA and ALM was significantly shorter than that induced by pTOS of the ALM alone. Likewise, the power of the rhythmic movement was significantly smaller than that induced by pTOS of the CFA alone. Thus, the two distinct modules possess the ability to mutually weaken the neuronal activity and the movement induction elicited by the other module.

II. Cortical representation of ethologically relevant movements and lever-push/pull movements (Hira et al., J Neurosci., 2015).

Discrete and rhythmic movements might be a part of coordinated movements that involve multiple parts of the body. We extracted three types of ethologically relevant movements by detecting movements of other parts of the body as well as the right forelimb. These were termed 'forepaw-to-mouth', 'defensive-like', and 'locomotion-like' movements. Forepaw-to-mouth and locomotion-like movements were mainly elicited by pTOS of the ALM and the CFA, respectively. Defensive-like movement was induced by pTOS of the anterior and middle parts of the rhythmic module. The border between forepaw-to-mouth and defensive-like movements was almost identical to the FDR border. These results are consistent with the observation that the forepaw-to-mouth movement had a constant goal position and that defensive- and locomotion-like movements included oscillatory components.

Although the medial part of the RFA and the CFA were involved in the rhythmic module, neuronal activity in these areas is strongly related to a voluntary lever-pull movement that is not rhythmic (Hira et al., 2013; Masamizu et al., 2014). Therefore, these areas may not function only for rhythmic movement. To assess this possibility, we conducted pTOS when a lever was located near the right forepaw in four mice.

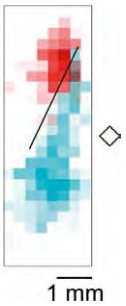

Figure 4. Maps of lever-push (red) and lever-pull (cyan) movements induced by pTOS. The solid line indicates the FDR border.

This stimulation exclusively induced a lever-push movement (red in Fig. 4). On the other hand, pTOS of the CFA preferentially induced a lever-pull movement (cyan in Fig. 4), which was sometimes followed by the rhythmic movement. The medial part of the RFA and the HA almost exclusively induced a lever-pull movement. The border of the lever-push and lever-pull movements roughly corresponded to the FDR border (Fig. 4). These results indicate that the distinct cortical modules adapted to distinct lever movements, and that the module for the rhythmic movement could induce a non-rhythmic movement.

Our results indicate that motor cortical neurons may not exclusively encode unique purposes or specific complex movements. Instead, a population of neurons in a motor cortical area may evolve to encode various purposeful movements depending on the environmental constraints and intrinsic dynamics.

III. Long-term two-photon calcium imaging of neuronal populations with subcellular resolution in adult non-human primates (Sadakane et al., Cell Rep., 2015).

We developed a new method for long-term imaging of a genetically encoded calcium indicator (GECI), GCaMP6f, expressed from adeno-associated virus (AAV) vectors in cortical neurons of the adult common marmoset (*Callithrix jacchus*), a small New World primate. We first cloned two components of the TET-Off system, namely, the tetracycline-controlled transactivator (tTA) under the control of the Thy1S promoter and GCaMP6f under the control of the tetracycline response element (TRE3) promoter, into separate AAV vectors (Fig. 5).

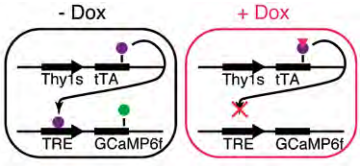

Figure 5. Schematic illustration of the TET-Off gene expression system.

In the absence of Dox, tTA constitutively activates expression of a transgene under the TRE3 promoter. Dox prevents the binding of the tTA to the TRE3, and inhibits transgene expression (Fig. 5).

For fluorescence imaging after AAV injection to the marmoset neocortex, marmosets were placed under the

microscope and lightly anesthetized by isoflurane inhalation. We detected strong epifluorescence signals around the injection site through the imaging window after only 10 days of AAV injection (Fig. 6). In many neurons, spontaneous calcium transients were clearly detected (Fig. 6).

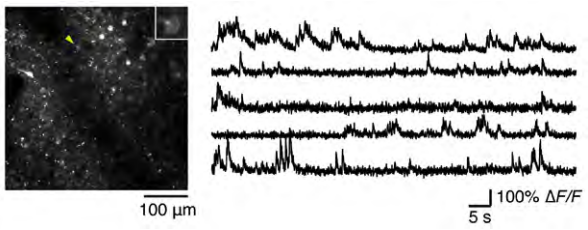

Figure 6. Left, representative two-photon image of GCaMP6f on post injection day 10. Right, representative Δ*F/F* traces from five ROIs in the field shown in left.

To test the effectiveness of Dox control in marmosets injected with AAV-TET-Off vectors, we administered Dox in drinking water to the animals for several days, and examined an image of the same area repeatedly over time. Twenty eight days after the start of the 5-day Dox administration, we were able to identify the same population of neurons that we observed before Dox administration. Spontaneous calcium transients were detected from the same neuron both before and after Dox administration. We observed a set of neurons with very similar configurations at an interval of more than 100 days after three trials of Dox administration, which are most likely to represent identical neuronal populations (Fig. 7).

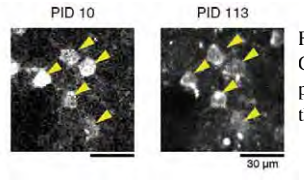

Figure 7. Representative images of GCaMP6f fluorescent signals on post injection days 10 and 118 from the same filed.

Due to a high signal-to-noise ratio of the fluorescence signals, we were able to monitor the activity of neuronal somata located up to 400 µm from the cortical surface. This depth range is supposed to cover L1 and L2, as well as the upper part of layer 3 (L3). Using a piezoelectric objective mount, we continuously recorded the activity of multiple neurons with single-cell resolution from a relatively broad area (625 × 625 µm) at three depths: 150 µm, 275 µm, and 400 µm (from L2 to L3). In this experiment, 445 putative neuronal somata were determined and 81 of them were spontaneously active neurons.

Next, we tested whether neuronal responses evoked by tactile stimulation could be detected in the somatosensory cortex. For tactile stimulation, we attached vibrators to the arm and leg of marmoset B that were contralateral to the hemisphere with the imaging window, and stimulated each body part alternately for 1 s with an interval of 7.5 s. We found some neurons that responded to the tactile stimulation. Figure 8*A* shows two examples of neuronal soma in L3 whose fluorescence changes were clearly time-locked to the stimuli.

Consistent with the existence of stimulus-selective neurons, we also observed stimulus-selective responses from dendrites in L1 (Figure 8*B*) and axonal boutons in L1 (Figure 8*C*). The responses of these dendrites and axons to each stimulus were as robust as those of the somata.

Thus, our results demonstrate that the spatial and temporal profile of each neuron, dendrite, and axon in the marmoset cerebral cortex can be correlated with a variety of sensory stimuli. Our new technique removes a major obstacle to studying the non-human primate cortex using calcium imaging. In future studies, the technique, combined with various cognitive tasks, should shed light on the organization and plasticity of the primate cerebral cortex.

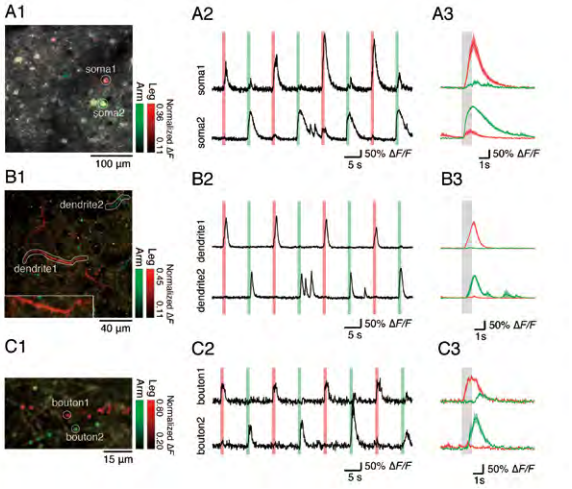

Figure 8. Selective sensory responses to tactile stimulation from neuronal somata (A), dendrites (B), and axonal boutons (C). Middle (2), the green and red lines indicate the timing of tactile stimulation to the left arm and the left leg, respectively. Right (3), the responses to nine stimuli to each body part were averaged. The gray band indicates the timing of the left arm and the left leg stimulation.

Publication List:

〔Original papers〕

- Hira, R., Terada, S., Kondo, M., and Matsuzaki, M. (2015). Distinct functional modules for discrete and rhythmic forelimb movements in the mouse motor cortex. J. Neurosci. *35*, 13311-13322.
- Sadakane, O., Masamizu, Y., Watakabe, A., Terada, S., Ohtsuka, M., Takaji, M., Mizukami, H., Ozawa, K., Kawasaki, H., Matsuzaki, M., and Yamamori, T. (2015). Long-term two-photon calcium imaging of neuronal populations with subcellular resolution in adult non-human primates. Cell Rep. *13*, 1989-1999.

LABORATORY OF NEUROPHYSIOLOGY

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Postdoctoral Fellow:	*NAKAYASU, Tomohiro**
Visiting Scientist:	*NAKAYASU, Tomohiro*

In order to interact successfully with the environment, animals must deduce their surroundings based on sensory information. The visual system plays a particularly critical role in such interactions with the environment.

"Why can we see?" This question is fundamental for a thorough understanding of vision-dependent animals, including human beings. In order to better understand the visual system of animals, we are researching animal behaviors through psychophysical and computational methods.

I. Psychophysical study of Medaka fish

One of our major subjects is the psychophysical and computational study of medaka (*Oryzias latipes*). Recently, we made progress in studies of prey-predator interaction using medaka and zooplankton. Visual motion cues are one of the most important factors for eliciting animal behavior, including predator-prey interactions in aquatic environments. To understand the elements of motion that cause such selective predation behavior, we used a virtual plankton system where the predation behavior in response to computer-generated prey was analyzed. Virtual prey models were programmed on a computer and presented to medaka, which served as predatory fish. Medaka exhibited predation behavior against several characteristic virtual plankton movements, particularly against a swimming pattern that could be characterized as pink noise motion. Analyzing prey-predator interactions via pink noise motion will be an interesting research field in the future (Matsunaga & Watanabe, 2012).

In recent years, we have made progress in studies of the schooling behaviors of medaka. Many fish species are known to live in groups. Visual cues have been shown to play a crucial role in the formation of shoals. Using biological motion stimuli, depicting a moving creature by means of just a few isolated points, we examined whether physical motion information is involved in the induction of shoaling behavior. We found that the presentation of virtual biological motion can prominently induce shoaling behavior. We have shown what aspects of this motion are critical in the induction of shoaling behavior. Motion and behavioral characteristics can be valuable in recognizing animal species, sex, and group members. Studies using biological motion stimuli will enhance our understanding of how non-human animals extract and process information which is vital for their survival (Nakayasu & Watanabe, 2014).

This year, we have developed a novel method for behavior analysis using 3D computer graphics. The fine control of various features of living fish have been difficult to achieve in studies of fish behavior. However, computer graphics allow us to manipulate morphological and motion cues systematically. Therefore, we have constructed 3D computer graphic animations of medaka based on tracking coordinate data and photo data obtained from real medaka. These virtual 3D models will allow us to represent medaka faithfully and to undertake a more detailed analysis of the properties of the visual stimuli that are critical for the induction of various behaviors.

Simultaneously, we began studying "behavioral lateralization" as a characteristic which can affect the formation of shoals. Like humans, fishes have lateral differences in their movement and perception (Bisazza & Brown, 2011). However, there have been few studies to examine the influence of behavioral lateralization on their interaction. If each individual has a laterally biased response to surrounding companions or predators, how is the united movement as shoals realized? This study is expected to bring a new viewpoint for understanding the structure and behavior of schooling.

Figure 1. Virtual Medaka fish (Male Models) constructed of 3D polygonal models and photo textures.

II. Psychophysical study of Human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual illusions experienced by human beings (*Homo sapiens*). One recent focus of this debate is the flash-lag effect, in which a moving object is perceived to lead a flashed object when both objects are aligned in actual physical space. We developed a simple conceptual model explaining the flash-lag effect (Delta model, Watanabe *et al*., 2010: https://www.youtube.com/eijwat). In recent years, we have made more developed novel visual illusions, such as the shelf-shadow illusion (http://eijwat.blogspot.jp/).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).

DIVISION OF EVOLUTIONARY BIOLOGY

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	KAWASHIMA, Takeshi
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	LI, Ping
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	KAJIKAWA, Ikumi
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	MATSUZAKI, Yoko
	NISHI, Tayo
	OOI, Shoko
Secretary:	*KOJIMA, Yoko*

I. Evolution of Complex Adaptive Characters

The theory of natural selection and the neutral theory of molecular evolution are powerful concepts in evolutionary biology. However, even with such theories, there still remain unexplained phenomena, one of which is the evolution of complexity. It is difficult to explain the mechanisms needed to evolve complex adaptive traits at cellular and organismal levels, such as cell division machinery, regeneration, novel organ development, host race change, and mimicry. Such traits comprise many components and become adaptive only when all components are gathered together. However, based on evolutionary theory, each component should evolve one by one according to the accumulation of mutations. We aim to reveal the genetic networks regulating these complex traits and to infer the mechanisms needed to evolve complex characters.

II. Evolution of Regeneration: Reprogramming of Differentiated Cells to Pluripotent Stem Cells

Different species have different morphology and also cellular characters vary between species. Stem cells self-renew and repeatedly produce differentiated cells during development. Conversely, differentiated cells can be converted into stem cells in some organisms. In plants, regeneration of a stem cell can lead to generation of a new individual, which is an effective strategy for propagation. The ability in reprogramming is different from species to species but the reason is unknown. The moss *Physcomitrella patens* has a rapid reprogramming ability and is feasible for use in experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours. We found that *P. patens COLD SHOCK DOMAIN PROTEIN* genes (*PpCSP*s), homologous to mammal induced pluripotent stem (iPS) cell factor LIN28, are positive regulators of the stem-cell formation in Physcomitrella. Characterization of *PpCSP*s are ongoing by Chen Li and Yosuke Tamada.

We also found that the stem cell formation required light signaling mediated by red and blue light receptors. In addition, the stem cell formation was facilitated in the quadruple deletion mutant of the *P. patens SQUAMOSA promoter binding protein* (*PpSBP*) genes, some of which are known to be repressed by the blue-light receptor CRYPTOCHROME. In addition, the histone chaperone genes, *Histone gene repressor A* (*HIRA*) were found to regulate chromatin modification of *PpSBP*s. Characterization of these genes are in progress mainly by Yukiko Kabeya to investigate the cross talk between light and chromatin modification pathways in the process of the stem cell formation.

III. Evolution of Regeneration: Master Regulator for Reprogramming *STEMIN*

Animal somatic cells can be reprogrammed to iPS cells by introducing four transcription factors, while such factors have not been identified in plants. We have previously identified a gene encoding a member of a plant-specific transcription factor, STEM CELL-INDUCING FACTOR 1 (STEMIN1) that was able to induce direct reprogramming of differentiated leaf cells into chloronema apical stem cells without wounding signals. *STEMIN1* and its two paralogous genes (*STEMIN2* and *STEMIN3*) were activated in leaf cells that underwent reprogramming. In addition, deletion of the three *STEMIN* genes delayed reprogramming after leaf excision, suggesting that these genes redundantly function in the reprogramming of cut leaves. We also found that three *STEMIN* genes function redundantly in chloronema apical stem cell formation from chloronema cells during chloronema development. On the other hand, in contrast to *STEMIN1*, induction of *STEMIN2* or *STEMIN3* in gametophores and chloronemata did not induce formation of chloronema apical stem cells. These results indicate that *STEMIN1* has sufficient ability to change intact leaf cells to stem cells, but its paralogous genes do not. Masaki Ishikawa and Mio Morishita were this study's main researchers.

IV. Evolution of Regeneration: Lateral inhibition by stem cells to surrounding cells to be stem cells

Singly isolated leaf cells are reprogramed into stem cells in *P. patens*. However, only one cell of two longitudinally

isolated adjacent cells becomes a stem cell and the other appears to be laterally inhibited by the cell to be a future stem cell. Liechi Zhang is investigating the factors involved in the lateral inhibition.

V. Evolution of Regeneration: Other pathways

Nan Gu is interested in DNA damage and reprogramming, and is working with the mechanisms connecting DNA damage and reprogramming of differentiated cells to stem cells.

We found that INHIBITOR OF GROWTH (ING) proteins are involved in the stem cell formation of cut leaves. The ING proteins are known to regulate an apoptosis pathway in animals but plants do not have the corresponding pathway. Akihiro Imai is mainly investigating the molecular function of ING.

VI. Cell Cycle Reentry from the Late S Phase in Physcomitrella

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a mitotic spindle, which is composed of microtubules. Differentiated cells are in a non-dividing, quiescent state, but some differentiated cells can reenter the cell cycle in response to appropriate stimuli. Quiescent cells are generally arrested at the G0/G1 phase, reenter the cell cycle, and progress to the S phase to replicate their genomic DNA. On the other hand, some types of cells are arrested at different phases and reenter the cell cycle from there. In the moss *Physcomitrella patens*, the differentiated leaf cells of gametophores formed in the haploid generation contain approximately 2C DNA content, and DNA synthesis is necessary for reentry into the cell cycle, which is suggested to be arrested at late S phase. Masaki Ishikawa reviewed various cell-division reactivation processes in which cells reenter the cell cycle from the late S phase, and discussed possible mechanisms of such unusual cell cycle reentries with special emphasis on Physcomitrella (Ishikawa and Hasebe 2015).

VII. Evolution of Elaborated Cell Division Machinery: Spindle body

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a bipolar mitotic spindle, which is composed of microtubules. The centrosomes, which act as microtubule organizing centers, induce formation of the two poles in metazoan cells. In contrast, the cells of land plants and their sister group, zygnematales green algae, form the bipolar spindle in the absence of centrosomes. For understanding the mechanism of acentrosomal spindle formation, the steps of microtubule reorganization during spindle formation should be visualized. We collaborated with Prof. Tomomi Nemoto in Hokkaido University and developed a two-photon spinning disk confocal microscope, which enables 3-dimensional imaging of living cells with high temporal and spatial resolution. We found that spindle microtubules elongate from a prospindle, that is, a microtubule cage with two poles on the nuclear envelope. Our data suggests that the prospindle organizes the bipolar spindle, as centrosomes do in metazoan cells. In contrast to the metazoan centrosomes, however, the prospindle disappears before metaphase. To understand the mechanism how the bipolar spindle is maintained in the absence of the organizer, we established a minispindle system, which involves a bipolar microtubule complex composed of an isolated chromosome and microtubules in tobacco cells. Analyses of microtubule behavior in the minispindle are in progress. Daisuke Tamaoki and Takashi Murata were this study's main researchers.

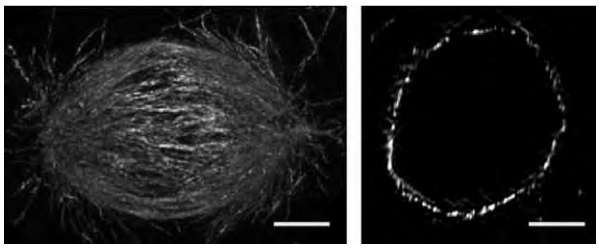

Figure 1. Prospindle of a tobacco BY-2 cell. Left: projection image, Right: cross section image of the same cell. Bar, 5 μm.

VIII. Molecular mechanisms of Plant Movement using *Mimosa pudica*

The sensitive plant *Mimosa pudica* has long attracted the interest of researchers due to its spectacular leaf movements in response to touch or other external stimuli. Although various aspects of the seismonastic movement have been elucidated by physiological approaches, the lack of genetic tools has hampered the investigation of molecular mechanisms involved in these processes. To overcome this obstacle, we developed an efficient genetic transformation method for *M. pudica* (Mano *et al*., 2014) and then established a CRISPR/Cas9-mediated gene knock-out system. By using these new techniques, we succeeded in generating immotile *M. pudica* mutants. Further characterization of these mutants will provide insights into the mechanism of leaf movements as well as their adaptive meanings. Another promising approach based on the transgenic technique is molecular imaging using fluorescent reporter proteins. We generated transgenic plants expressing fluorescent proteins which can visualize actin cytoskeleton and calcium ions, respectively. We are now attempting live-imaging of these factors, which participate in the seismonastic movement, but their roles still remain unclear. This study was conducted mainly by Hiroaki Mano.

IX. Molecular mechanisms of mimicry

An excellent example of mimicry is the flower-mimicry of the orchid mantis *Hymenopus coronatus* with pink and white coloration and petal-like legs. Biochemical analyses indicated that the reduced form of xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink color. We also analyzed the ultrastructure of pigment granules by transmission electron microscopy. We found that integumentary cells of *H. coronatus* contain a numerous number of electron-dense granules with 60-nm diameters. This result suggests that this special intracellular structure may contribute to the unique pink color of *H. coronatus*. This work was mainly done by Hiroaki Mano.

X. Oriented cell division shapes carnivorous pitcher leaves of *Sarracenia purpurea*

Complex morphology is one of the major evolutionary outcomes of phenotypic diversification. In some carnivorous plants, the planar leaves of common ancestors have been modified to form a pitcher shape. However, how leaf development was altered in the evolution of pitcher leaves remains unknown. Here we show that the pitcher leaves of *Sarracenia purpurea* develop through cell division patterns of adaxial tissues that are distinct from those in bifacial and peltate leaves, subsequent to regular expression of adaxial and abaxial marker genes. Differences in the orientation of cell divisions in the adaxial domain cause bifacial growth in the distal region and adaxial ridge protrusion in the middle region. These different growth patterns in a leaf establish the pitcher morphology. A computer simulation suggests that the cell division plane is critical for the acquisition of the novel pitcher morphology. Our results imply that tissue-specific changes in the orientation of cell division underlie the development of a morphologically complex leaf (Fukushima et al. 2015).

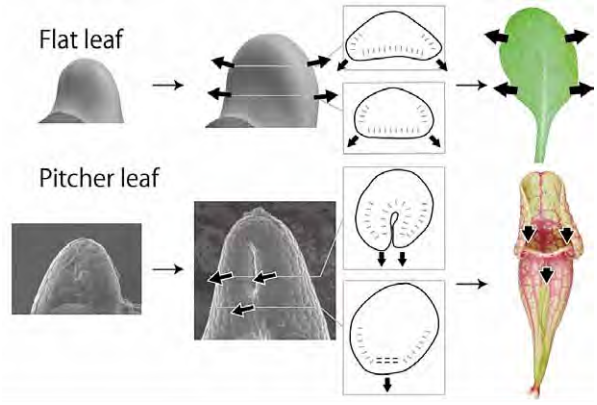

Figure 2. Schematics of leaf development in flat leaf and pitcher leaf. Growth directions are indicated by arrows. Lines show cell division planes.

To further investigate the evolution of pitcher leaves, we selected the Australian pitcher plant *Cephalotus follicularis*, which produces both carnivorous pitcher and non-carnivorous flat leaves, enabling us to deduce carnivory-related genes by comparative approaches in a single species. To understand the genomic changes associated with the evolution of carnivory, we sequenced 2-Gbp genome of *C. follicularis*. Whole-genome shotgun data corresponding to 100x coverage were produced by Illumina paired-end/mate-pair sequencing with 180-bp to 5-kb insert sizes, and *de novo* assembly yielded 15.6 kb of contig N50 and 78 kb of scaffold N50. 14 Gbp of PacBio reads with 2-kb mean max subread length were produced for gap filling. Transcript-based gene prediction with RNA-seq reads found 45,469 gene models. This study was mainly conducted by Kenji Fukushima.

XI. Evolution of plant development

To investigate evolution of novel complex traits, the following studies are ongoing: Chiharu Kamida studies genes involved in movable tentacle development in the sandew *Drosera spatulata*. Shizuka Koshimizu is interested in the evolution of floral homeotic genes and investigates the function of MADS-box genes in non-flowering plants *Physcomitrella patens*. The pseudanthium is a flower-like inflorescence, the molecular mechanisms of the development of which are unknown. Tomomi Sugaya succeeded in transferring the *FT* gene from *Arabidopsis thaliana* into the pseudanthium *Houttuynia cordata*. Furthermore, introduction of the *FT* gene successfully induced flowers.

Publication List:

〔Original papers〕

- Fukushima, K., Fujita, H., Yamaguchi, T., Kawaguchi, M., Tsukaya, H., and Hasebe, M. (2015). Oriented cell division shapes carnivorous pitcher leaves of Sarracenia purpurea. Nat. Commun. *6*, 6450.
- Kinoshita, A., ten Hove, C.A., Tabata, R., Yamada, M., Shimizu, N., Ishida, T., Yamaguchi, K., Shigenobu, S., Takebayashi, Y., Iuchi, S., *et al*. (2015). A plant U-box protein, PUB4, regulates asymmetric cell division and cell proliferation in the root meristem. Development *142*, 444-453.
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- Teh, O.K., Hatsugai, N., Tamura, K., Fuji, K., Tabata, R., Yamaguchi, K., Shingenobu, S., Yamada, M., Hasebe, M., Sawa, S., *et al*. (2015). BEACH-domain proteins act together in a cascade to mediate vacuolar protein trafficking and disease resistance in Arabidopsis. Mol. Plant *8*, 389-398.

〔Review article〕

- Ishikawa, M., and Hasebe, M. (2015). Cell cycle reentry from the late S phase: implications from stem cell formation in the moss Physcomitrella patens. J. Plant Res. *128*, 399-419.

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Rhizobium–legume symbiosis is one of the most successful mutually beneficial interactions on earth. In this symbiosis, soil bacteria called rhizobia supply the host legumes with ammonia produced through bacterial nitrogen fixation. In return, host plants provide the rhizobia with their photosynthetic products. To accomplish this biotic interaction, leguminous plants develop nodules on their roots. On the other hand, more than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. Despite marked differences between the fungal and bacterial symbioses, common genes are required for both interactions. Using a model legume *Lotus japonicus*, we are trying to unveil the molecular mechanisms of both symbiotic systems.

I. AM symbiosis

Arbuscular mycorrhiza is a mutualistic plant-fungus interaction that confers great advantages to growth and survival on the land. During the symbiosis development, AM fungi enter into the host root and elongate the hyphae between the root cells. The intraradical hyphae finely branch in the cortical cells and form a symbiotic structure called an arbuscule. AM fungi supply inorganic materials, especially phosphate, to the host plant through arbuscules and in return, they obtain photosynthetic products form the host.

1-1 Strigolactone-induced putative secreted protein 1 in AM fungus *Rhizophagus irregularis* is required for symbiosis

Strigolactones (SLs) are a kind of phytohormone that were identified as a signal factor for AM fungi. SLs enhancement increases hyphal branching and elongation in the AM fungi. This process facilitates direct contact between the AM fungi and host plants. After hyphal entrance into the host root, AM fungi form a characteristic densely-branched hypha 'arbuscule' in the cortical tissue of the root, where the nutrient exchange is undertaken. To provide novel insights into the molecular mechanisms of AM symbiosis, we screened and investigated genes of the AM fungus *Rhizophagus irregularis* that contribute to the infection of host plants.

To compare comprehensive gene expression profiles of *R. irregularis* between the pre-symbiotic and symbiotic stages, RNA-seq analysis was performed in non-symbiotic (control), SL-treated, and symbiotic hyphae. The RNA-seq analysis revealed that 19 genes are up-regulated by both treatment with SL and symbiosis. Interestingly, 11 of the 19 genes were predicted to encode small proteins with secretory signal peptides at their N-terminal ends. Among the 11 putative secreted protein genes, *SL-induced putative secreted protein 1* (*SIS1*) showed the largest induction under both conditions. To analyze the functions of SIS1 during AM symbiosis, *SIS1* was then characterized by a reverse genetic approach using host-induced gene silencing (HIGS), which leads to RNAi in the fungus via the host plant. We designed a SIS1-HIGS construct and transformed *Medicago truncatula* hairy roots with this construct. In the hairy root lines, *SIS1* expression is knocked down by HIGS, resulting in significant suppression of colonization compared with an empty vector (EV) control root line. Futhermore, the arbuscules in EV control hairy roots were mature and finely branched (Figure 1A), whereas the majority of arbuscules in the SIS1-HIGS hairy roots displayed defective morphology (Figure 1B). The stunted arbuscules are an indication of incomplete AM symbiosis. These results suggest that SIS1 contributes to the establishment of AM symbiosis in *R. irregularis*.

We concluded that SIS1 is a novel putative secreted protein that is induced by both SL treatment and AM symbiosis in *R. irregularis*, and that it positively regulates AM colonization.

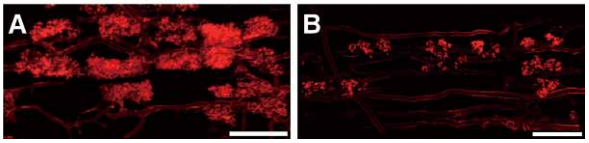

Figure 1. Arbuscules labeled with wheat germ agglutinin (WGA)-Alexa Fluor 594 dye at 8 weeks after inoculation. (A) Empty vector control. (B) SIS1-HIGS hairy roots. Bars represent 50 μm.

1-2 Gibberellin signaling interferes with AM signaling and regulates AM-induced gene expression

We isolated the AM mutant *nsp1*, which has a defect in its infection processes, through screening of root nodule symbiosis mutants of *L. japonicus*. The *nsp1* mutant is known to show a decrease of strigolactone biosynthesis, in addition, our transcriptome analysis of the *nsp1* mutant revealed that expression of gibberellin biosynthesis genes are disturbed. We have already reported that gibberellin signaling interacts with the symbiotic signaling pathway and changes the expression of AM-induced genes. Furthermore, we have found that some AM-induced genes were induced in the *nsp1* mutant without AM fungal infection. Hormonome analysis showed that the mutant contains a decreased amount of gib-

berellins, suggesting that the AM-induced genes are induced in low gibberellin conditions. We treated *L. japonicus* with gibberellin biosynthesis inhibitors, or introduced gibberellin signaling suppressor protein delta-GAI, and confirmed significant induction of the AM-induced genes in the treated or transformed plants. These results demonstrated that the AM-induced genes are also under the control of gibberellin signaling and the promoter of these genes is the junction point between symbiotic and gibberellin signaling.

We expected that alternation of the gibberellin signaling conditions may suppress the *nsp1* mutant phenotypes. Actually, treatment with gibberellin biosynthesis inhibitor or introduction of delta-GAI to the *nsp1* mutant suppresses the low infection phenotype of the *nsp1* mutant. This result indicated that abnormal gibberellin conditions caused *nsp1* phenotypes and NSP1 functions in gibberellin homeostasis and regulation for AM fungal infection in the host plant.

Gibberellin conditions in host plants are altered by various environmental stimuli. The close interaction between both signaling pathways suggests that a part of regulation of AM development in the field might be controlled through alternation of gibberellin conditions. We continue to study the regulation mechanisms by gibberellin and symbiotic signaling and, moreover, will investigate the relation between AM symbiosis, gibberellin signaling, and various environmental responses to understand actual regulation mechanisms of AM in natural environments.

II. Long-distance control of nodulation

To establish symbiotic associations with rhizobia, a group of nitrogen-fixing bacteria, leguminous plants form nodules on their roots in response to rhizobial infection. The rhizobia colonize these nodules, supplying host plants with fixed atmospheric nitrogen while receiving photosynthates in turn. While such symbiotic relationship generally is beneficial to both partners, the formation of excessive numbers of nodules inhibits the growth of the host plants. To avoid this effect, plants perform autoregulation of nodulation (AON), which systemically controls the number of nodules. AON is a long-distance negative-feedback system involving root-shoot communication. In *L. japonicus*, two leucine-rich repeat receptor-like kinases, HYPERNODULATION ABERRANT ROOT FORMATION 1 (HAR1) and KLAVIER, have been identified as key components of AON that function in shoots. The two proteins are orthologous to *Arabidopsis* CLAVATA1 and RPK2, respectively, which are involved in the maintenance of stem cell populations in shoot apical meristems via short-range cell-to-cell communication. As an underlying mechanism of AON, it has been postulated that signaling substances are produced in roots upon rhizobial infection which then are transported to the shoot. The perception of these primary signals in the shoot generates secondary signals. These shoot-derived signals, also called shoot-derived inhibitors (SDI), are transported to the roots where they inhibit the initiation of new nodule development. In *L. japonicus*, the two peptides, CLE-RS1 and CLE-RS2, are strong candidates for root-derived mobile signaling molecules. Expression of the corresponding genes is induced specifically in infected roots, and CLE-RS2 glycopeptides are transported to the shoot where they directly bind to HAR1. Application of arabinosylated CLE-RS peptides to shoots suppresses nodulation in an HAR1-dependent manner. Furthermore, the TOO MUCH LOVE (TML) F-box protein recently has been identified as a root-acting AON factor that inhibits nodulation downstream of HAR1. Although these results provide some insight into signaling mechanisms between root and shoot, the mechanism and regulation of AON-inhibition of nodule development remains unclear. In particular, the shoot-derived inhibitor, SDI, has remained unidentified for a long time.

We focused on downstream events of the CLE-RS1/2-HAR1 signaling pathway and attempted to identify SDI. We show that the production of cytokinins in shoots is activated by the CLE-RS1/2-HAR1 pathway, and that application of exogenous cytokinins to shoots can inhibit nodulation in a TML-dependent manner. Our results suggest that shoot-derived CKs systemically regulate root nodulation in AON.

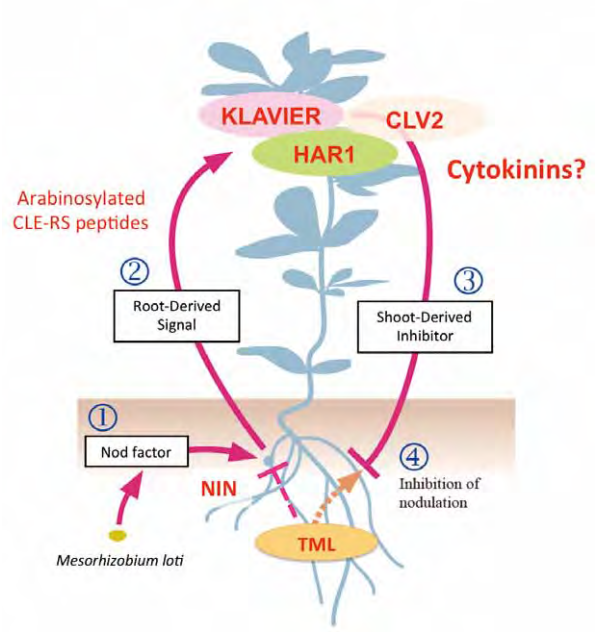

Figure 2. Schematic illustration of the proposed AON model. AON is mediated by two long-distance signal molecules, arabinosylated CLE peptides and SDI. Cytokinins may act as SDI and play a central role in AON.

III. Evolutionary dynamics model of the Legume–Rhizobia symbiosis

In symbiotic relationships, the participating organisms provide mutual benefits to each other. One of the most famous symbioses occurs between legumes and rhizobia, in which rhizobia extract nutrients (or benefits) from legume plants while supplying them with nitrogen resources produced by nitrogen fixation. However, the nitrogen fixation reaction consumes a lot of energy (or costs), and ineffective rhizobia that colonize their host plants without undertaking nitrogen fixation are ubiquitous in nature.

Here, we constructed a mathematical model to investigate how benefit and cost influence the evolution of this symbiosis, and the conditions required for establishing the symbiotic relationship. (Figure 3A). According to our model, stable

mutualism depends on the cost–benefit balance (Figure 3B). That is, a tight symbiotic relationship emerges when the beneficial effect is much stronger than the cost (Figure 3F), but is dissolved under the opposite condition of relatively strong cost (Figure 3C). In the intermediate condition, where benefit is approximately offset by cost, more complicated behaviors emerge such as imperfect symbiotic interactions (Figure 3D) and the coexistence of symbionts and cheaters (Figure 3E). Our findings can explain why ineffective rhizobia are widely distributed in nature. Our model provides a theoretical basis for understanding how the legume–rhizobia symbiosis evolves.

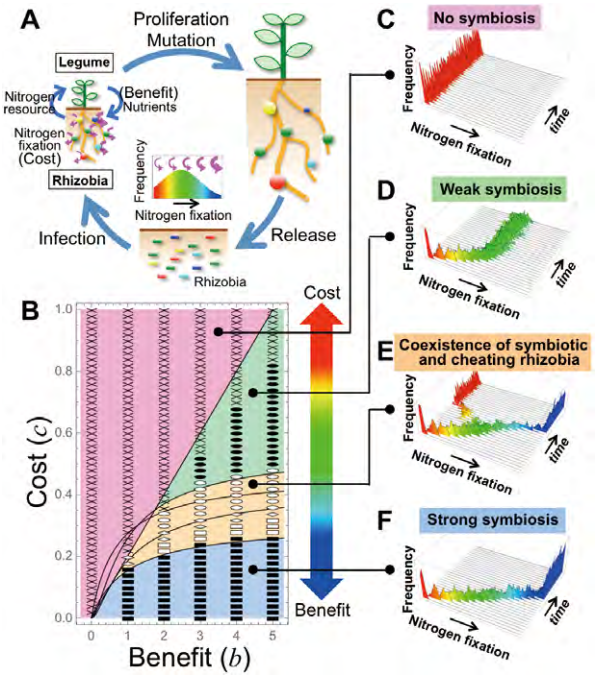

Figure 3. Evolutionary model of the Legume-Rhizobia symbiosis. (A) Model framework. (B-F) The evolution of the Legume-Rhizobia symbiosis depends on the cost-benefit balance. As the benefit strengthens relative to the cost, the evolutionary outcome shifts in the order of "No symbiosis" (C), "Weak symbiosis" (D), "Coexistence of nitrogen-fixing and cheating rhizobia" (E), and "Strong symbiosis" (F).

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- Fukushima, K., Fujita, H., Yamaguchi, T., Kawaguchi, M., Tsukaya, H., and Hasebe, M. (2015). Oriented cell division shapes carnivorous pitcher leaves of Sarracenia purpurea. Nat. Commun. *6*, 6450.
- Handa, Y., Nishide, H., Takeda, N., Suzuki, Y., Kawaguchi, M., and Saito, K. (2015). RNA-seq transcriptional profiling of an arbuscular mycorrhiza provides insights into regulated and coordinated gene expression in *Lotus japonicus* and *Rhizophagus irregularis*. Plant Cell Physiol. *56*, 1490-1511.
- Horst, R.J., Fujita, H., Lee, J.S., Rychel, A.L., Garrick, J.M., Kawaguchi, M., Peterson, K.M., and Torii, K.U. (2015). Molecular framework of a regulatory circuit initiating two-dimensional spatial patterning of stomatal lineage. PLoS Genet. *11*, e10 05374.
- Okamoto, S., Suzuki, T., Kawaguchi, M., Higashiyama, T., and Matsubayashi, Y. (2015). A comprehensive strategy for identifying long-distance mobile peptides in xylem sap. Plant J. *84*, 611-620.
- Sugiyama, A., Fukuda, S., Takanashi, K., Yoshioka, M., Yoshioka, H., Narusaka, Y., Narusaka, M., Kojima, M., Sakakibara, H., Shitan, N., Sato, S., Tabata, S., Kawaguchi, M., and Yazaki, K. (2015). Molecular characterization of LjABCG1, and ABC-binding cassette protein in *Lotus japonicus*. PLoS ONE *10*, e0139127.
- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2015). Gibberellin regulates infection and colonization of host roots by arbuscular mycorrhizal fungi. Plant Signal. Behav. *10*, e1028706.
- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2015). Gibberellins interfere with symbiosis signaling and gene expression and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol. *167*, 545-557.

〔Review articles〕

- Nakagawa, T., and Imaizumi-Anraku, H. (2015). Rice arbuscular mycorrhiza as a tool to study the molecular mechanisms of fungal symbiosis and a potential target to increase productivity. Rice *8*, 32.
- Shinya, T., Nakagawa, T., Kaku, H., and Shibuya, N. (2015). Chitin-mediated plant-fungal interactions: catching, hiding and handshaking. Curr. Opin. Plant Biol. *26*, 64-71.
- Suzaki, T., Yoro, E., and Kawaguchi, M. (2015). Leguminous plants: inventors of root nodules to accommodate symbiotic bacteria. Int. Rev. Cell Mol. Biol. *316*, 111-158.

DIVISION OF EVOLUTIONARY DEVELOPMENTAL BIOLOGY

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Visiting Graduate Student:	*KONISHI, Yusuke*
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Secretary:	*SAITO, Eiko*

The Division of Evolutionary Developmental Biology was started in June 2015. We focus on the evolutionary novelties acquired by insects through evolution, in order to elucidate the molecular and evolutionary mechanisms leading to the large variety of traits that they display. From this wealth of exciting traits, our lab currently focuses on promoting research into (1) the origin and diversification of insect wings, (2) wing color patterns and mimicry of ladybird beetles, and (3) acquisition and diversification of beetle horns.

I. Origin and diversification of insect wings

Of the various flying animals on the earth, insects have evolved a unique flight organ. Despite over two centuries' debate, the evolutionary origin of the insect wing is still an enigma. We try to approach this issue using evo-devo methods. In *Drosophila melanogaster*, the wing master gene *vestigial* (*vg*) and its interaction partner *scalloped* (*sd*) play pivotal roles in the formation of wing field identity. For this reason, these genes are ideal candidates for investigating wing origin and evolution.

One way to identify the structure from which insect wings first evolved is to explore the function of "wing" genes in ancestral wingless (apterygote) species. We chose the firebrat, *Thermobia domestica*, as a model (Figure 1). *T. domestica* belongs to Thysanura, phylogenetically the closest extant relative of winged (pterygote) insects, making it ideal for elucidating wing origin. We cloned *vg* and *sd* orthologs from *T. domestica* (*Td-vg* and *Td-sd*). To examine the functions of these genes, we developed RNA interference (RNAi) based methods for *T. domestica*. We are currently testing for functional effects of altered transcription of each of these wing genes in the ancestrally wingless firebrats. In addition, we are performing comparative analyses of the function of these same genes in "primitively winged" (hemimetabolous) insects, to obtain additional clues relevant to understanding the origin and evolution of insect wings.

Figure 1. The firebrat, *Thermobia domestica*.

II. Wing color patterns and mimicry of ladybird beetles

Insect wing color patterns demonstrate a tremendous range of diversity and have evolved to fulfill various ecologically important functions such as intraspecific sexual signaling, mimesis, mimicry, and warning against predators. The molecular mechanisms responsible for generating such patterns, however, remain unknown for most species. To investigate the developmental mechanisms of color pattern formation, we chose the multicolored Asian ladybird beetle, *Harmonia axyridis*, which has conspicuous and variable wing color patterns consisting of black and red pigments (Figure 2A). Vivid wing color patterns of ladybirds function as a warning to predators that they are distasteful, and ladybird beetles are mimicked by various insect species. Mimicry provides an exciting opportunity to study how independent lineages of insect converge on similar color patterns. For exploring color pattern formation in a mimic, we use the leaf beetle, *Argopistes coccinelliformis*, which has color patterns similar to *Harmonia*, and which is thought to be a Batesian mimic of ladybird beetles (Figure 2B). To elucidate the molecular mechanisms underlying these wing color patterns, we have established a technique for germline transformation using a *piggyBac* vector and RNAi in the lady birds. Recently, we also designed a TALEN-based method

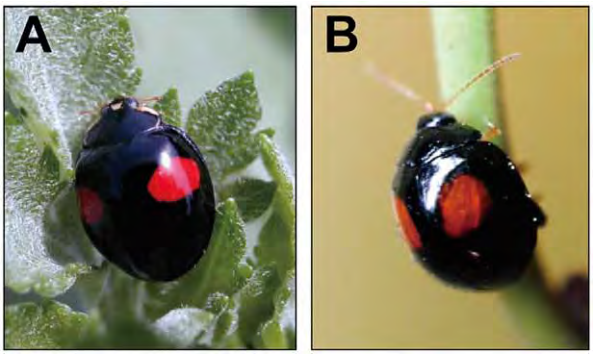

Figure 2. The ladybird beetle, *Harmonia axyridis* (A) and the leaf beetle, *Argopistes coccinelliformis* (B).

for genome editing in *H. axyridis*. Based on the knowledge obtained from *H. axyridis*, we are trying to understand how the similar wing-color patterns of model and mimic are generated – for example, do they use conserved or divergent mechanisms?

III. Acquisition and diversification of beetle horns

Insects show a tremendous range of diversity in "horns", rigid body outgrowths that function as weapons. Horns are exciting for evo-devo studies because they have arisen multiple times *de novo*, as evolutionary "novelties".

However, the molecular mechanisms involved in sexually dimorphic horn formation are still poorly understood. To investigate the developmental mechanisms of horn formation, we focus on the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera), which exhibits remarkable sexual dimorphisms in head and thoracic horns (Figure 3). The male-specific horns of *T. dichotomus* are one of the best models to study how an extreme, sex-specific morphology is formed. We have developed a technique for larval RNAi in *T. dichotomus,* permitting us to rigorously and systematically test the functional roles of a large suite of candidate developmental genes, revealing for the first time the molecular mechanisms responsible for growth of male rhino beetle horns.

To understand how *sexual dimorphism* in exaggerated horn growth arises, we are currently employing two main approaches: a candidate gene approach and a high throughput approach. To identify novel genes involved in the sexually dimorphic horn development in *T. dichotomus*, we are assessing mRNA of the developing horn discs using deep-sequencing transcriptome analysis. Furthermore, to begin to understand how, molecularly, beetle horns have diversified, we are extending our analyses to include additional beetle species with different types of exaggerated horns, including rhinoceros beetles with diverse horn structures as well as horned beetles in other phylogenetic groups.

Figure 3. The Japanese rhinoceros beetle, *Trypoxylus dichotomus*. Adult male (Left) and female (Right).

Publication List:

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

- Hatakeyama, M., Yatomi, J., Sumitani, M., Takasu, Y., Sekiné, K., Niimi, T., and Sezutsu, H. Knockout of a transgene by transcription activator-like effector nucleases (TALENs) in the sawfly, *Athalia rosae* (Hymenoptera) and the ladybird beetle, *Harmonia axyridis* (Coleoptera). Insect Mol. Biol. 2015 Oct 26.

LABORATORY OF MORPHODIVERSITY

Associate Professor
KODAMA, Ryuji

Visiting Scientist: *YOSHIDA, Akihiro*

The aim of this laboratory is to observe the variety of morphogenetic processes that occur in the course of the ontogenesis of multicellular organisms and to analyze the mechanisms of such processes, mainly by morphological methods. The accumulation of such analyses of the embryogenetic processes of related species is expected to provide an insight into the evolution of morphogenetic processes. This laboratory uses the wings of lepidopteran insects as the main target of morphological studies.

I. Wing outline shape formed by cell death

The wings of the lepidopteran insects (butterflies and moths) develop from the wing imaginal disc, which is a hollow sac made of simple epithelium. Due to its simple construction, this tissue is a good material for studying cellular interactions in the course of morphogenesis.

The outline shape of the adult wing is often different from that of the pupal wing. This difference is brought about by the programmed cell death of the marginal area of the pupal wing. The marginal dying area is called “the degeneration region” and the internal area which develops into the adult wing is called “the differentiation region”.

Cell death in the degeneration region proceeds very rapidly and finishes in a half-day to one-day period in *Pieris rapae* and in several other examined species. It was shown that the dying cells in the degeneration region have characteristics in common with the apoptotic cell death in mammalian cells, such as fragmented and condensed nuclei containing short DNA fragments detected by TUNEL staining. The cells in the degeneration region are actively engulfed by the macrophages in the cavity beneath the wing epithelium. The macrophages seem to be concentrated beneath the degeneration region by the strong adhesion between the basal surfaces of the dorsal and ventral epithelium in the differentiation region, which occurs at the time of prominent cell death and excludes macrophages from the differentiation region. Thus realized concentration of macrophages seems to accelerate the shrinkage of the degeneration region.

A possible physiological role of the cell degeneration at the wing margin is to make space for the growth of the marginal scales. Marginal scales are extremely elongated scales that grow densely on the edge of the wing. These scales are considered to be important in stabilizing the turbulence occurring posterior to the wing. The movements of the marginal scales are closely monitored by sensory scales and bristles growing among them (Yoshida and Emoto, Zool. Sci. *28*, 430-437, 2011).

Trachea and tracheoles are both important in delivering air into the wing and their patterns coincide with that of the boundary of degeneration and differentiation zones at the distal end of the wing. According to previous observations, the pattern formation of wing epithelium is often dependent on tracheal and tracheole patterns. Basic research on the development of tracheal pattern formation is being done by scanning and transmission electron microscopy to describe the exact pathway and time course of the formation of the elaborate pattern of trachea and tracheoles and to establish the cytological and developmental relationship between the formation of the tracheal pattern and the epithelial cell pattern.

II. Wing morphogenesis and the growth of marginal scales in small moths

In the small moths which have very long scales along their wing margins, the cuticle of the pupal wings does not appear to be large enough to house these scales. We examined the developmental process of the pupal wings of three species of the small gelechiid moths and found that, concomitant with the programmed cell deaths at the wing margin, there occurs a shrinkage of the differentiation region which has not been observed in large winged butterflies and moths. The shrinkage concomitant with the cell deaths causes the space between the pupal wing and the cuticle to extend, which appears to contribute to the growth of long marginal scales.

Microscopic observation of the long marginal scales revealed that they have a novel morphology and we are studying the effect this morphology may have on the mechanical interaction between marginal scales and further on the air flow over the wing during flight.

Figure 1. Adult specimen of *Metzneria lappella*, one of the gelechiid moths examined, with long scales along the wing margin.

III. Other research activities

This laboratory also conducts morphological observation of various animal tissues by scanning and transmission electron microscopy and immuno-electron microscopic analyses in collaboration with other laboratories of NIBB. Training in specimen preparation and instrument operation for such observations is also given.

LABORATORY OF BIORESOURCES

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Secretary:	*SUZUKI, Tokiko*

Medaka is a small egg-laying "secondary" fresh water fish found in brooks and rice paddies in Eastern Asia. This species has a long history as an experimental animal, especially in Japan. Our laboratory has conducted studies on evolution of the sex determination system using medaka and relatives, identification of the causal gene of mutants for PGC migration and pigment cell development, and the gonadal development of medaka. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

I. Evolution of the sex chromosome and sex determination genes in *Oryzias* fish

Recent studies have demonstrated that *Oryzias* species have different genetic sex-determination systems (XX/XY and ZZ/ZW) (Figure 1). Furthermore, the sex chromosomes differ in their origin and degree of differentiation. These findings suggest the repeated creation of new sex chromosomes from autosomes during evolution of *Oryzias* fishes, possibly in association with the formation of new sex-determining genes. We are now trying to positionally clone the novel sex-determining genes in these species. Identification of these genes would provide a clue to understand the evolutionary process underlying frequent turnover of sex determination mechanisms.

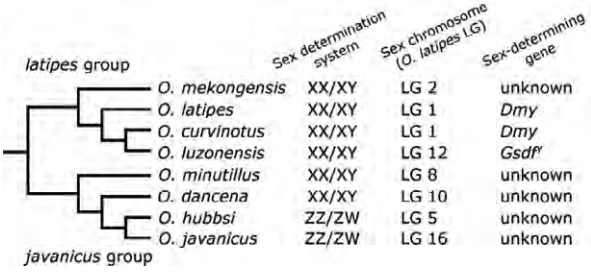

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

II. Genetic dissection of migration of primordial germ cells in medaka

Germ cells are responsible for the sustainability of life over generations in many multicellular animal species. To elucidate the mechanisms underlying the development of primordial germ cells, we identified multiple mutations affecting the migration and development of the primordial germ cells in medaka in a prior large-scale mutagenesis screening project, and have analyzed a set of them to date. We focused on three mutants that have defects in primordial germ cell migration, *kamigamo*, *shimogamo,* and *naruto* that were isolated in the screening project. Positional cloning and analysis of the genes carrying the mutations are now in progress. In addition, two mutations, *kamigamo* and *shimogamo*, cause cystic pronephric ducts simultaneously with abnormal positioning of the primordial germ cells. Therefore, the analysis of these mutations will be important in giving basal knowledge underlying the mechanisms of human cystic kidney diseases.

III. The study of type 2 diabetes using leptin receptor knockout medaka

Leptin in mammals is a peptide hormone secreted by adipose tissue. It has been shown to play a key role in the maintenance of energy homeostasis through the regulation of food intake and a range of physiological functions. Mice with a deficiency of leptin or its receptor exhibit hyperphagia (an increase in food intake). The hyperphagia causes obesity leading to type 2 diabetes-like symptoms, which is consistent with Caucasian patients. Leptin has also been isolated from fish, including medaka, however, the amino acid sequence is poorly conserved between fish and mammals (11-30%), and fish leptins are expressed mainly in the livers. To clarify the function of leptin on fish, we generated leptin receptor knockout (LepRKO) medaka by the TILLING method. The phenotypic analyses allowed us to reveal an appetite suppressive function of leptin signaling on medaka as well as mammals, and to find new value in medaka as a novel animal model for studying type 2 diabetes. As for appetite suppressive functions; LepRKO medaka showed high expression of the mRNA of NPY (3.5-fold) and AgRP (6-fold), which are known to be orexigenic peptides, and an increase in food intake (1.7-fold). Next, as for glucose metabolism; adult mutants showed signs of diabetes, such as fasting hyperglycemia (Figure 2) and impaired insulin secretion, which is a late-onset disorder caused by excessive feeding during post-juvenile stages. Furthermore, they showed hyperglycemia even with the same fat level in the blood, muscle, and liver as WT medaka (Figure 3). The symptom is consistent with those of Asian patients, not but Caucasian patients and mice with leptin signaling deficiencies. Now, we are investigating the gene expression associated with dysfunction of pancreatic tissues under various feeding conditions. This will allow us to identify the factors of diabetes that are sensitive to food intake, regardless of obesity.

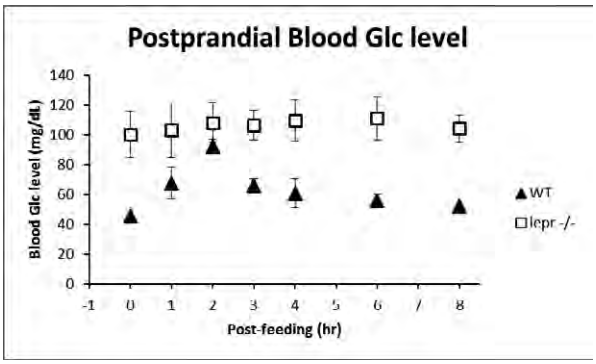

Figure 2. Postbrandial blood glucose levels of WT and LepRKO medaka.

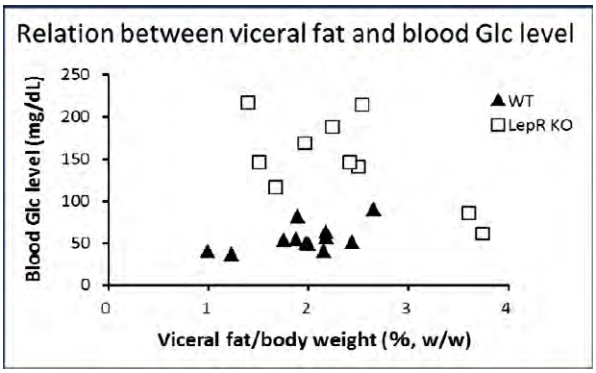

Figure 3. Relation between visceral fat and blood glucose levels of WT and LepRKO medaka.

V. National BioResource Project Medaka (NBRP Medaka) (http://www.shigen.nig.ac.jp/medaka/)

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA, BAC/ Fosmid clones, and hatching enzymes, as well as integrated information on medaka (Figure 4). We have been providing BAC clones of medaka related species, a library screening system employing a 3D PCR strategy for evolutionary studies, and the TILLING screening system for promoting the reverse genetic approach.

Figure 4. NBRP Medaka website.

NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

Publication List:

〔Original papers〕

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LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Among various nutrients amino acids are the major nitrogen source; therefore, perception of the amino acid environment is essential for cells. The cellular amino acid sensing system employs Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC1 (Tor complex1) and TORC2. TORC1 is involved in amino acid sensing, regulating protein synthesis, the cell cycle, and autophagy. On the other hand, TORC2 is responsible for actin organization and cell integrity. So far, it is not clear whether TORC2 also perceives nutrient signals.

The aim of our research group is to reveal the molecular mechanisms of how TORC1 receives amino acid signals and how the TORC1/2 pathways regulate each phenomenon. We have been studying Tor signaling in the budding yeast *Saccharomyces cerevisiae*, and have found three novel branches of the TOR signaling pathways (Figure 1).

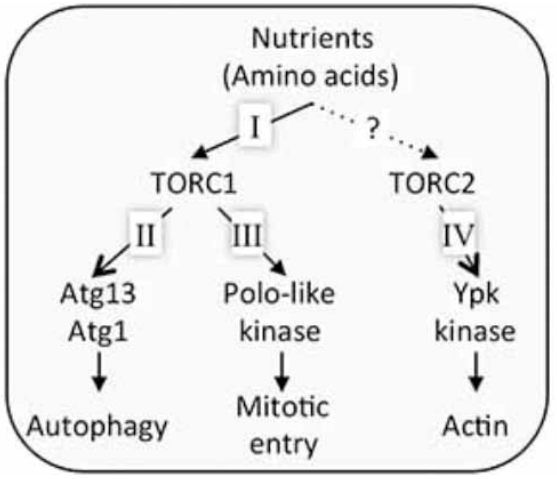

Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. How do amino acids regulate TORC1?

It is confirmed that TORC1 is the master regulator in amino acid sensing. However, little is known how TORC1 perceives amino acid signals.

Since both amino acid sensing and TORC1 have essential cellular functions, we assume that factor(s) transmitting amino acid signals to TORC1 should be encoded by essential gene(s). We identified several essential genes as candidates of TORC1-regulators.

II. TORC1 phosphorylates Atg13, the molecular switch of autophagy

TORC1 negatively regulates autophagy, a protein degradation system induced by nutrient starvation and rapamycin (a TORC1 inhibitor).

We found the TORC1-mediated regulatory mechanism of autophagy. Under nutrient-rich conditions, TORC1 directly phosphorylates Atg13, a component of the Atg1 kinase complex. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced in response to TORC1 inactivation. Activation of Atg1 requires formation of the Atg1 complex which consist of Atg1, Atg13, Atg17, Atg29, and Atg31. Phosphorylation of Atg13 by TORC1 plays a pivotal role in Atg1 complex formation; phosphorylated Atg13 loses its affinity to Atg1 resulting in disassembly of the Atg1 complex and repression of autophagy. On the other hand, dephosphorylation of Atg13 triggers formation of the Atg1 complex, activation of Atg1 kinase, and consequently induction of autophagy.

We further determined eight phosphorylation sites of Atg13 and generated an unphosphorylatable Atg13 mutant (Atg13-8SA). Expression of Atg13-8SA induces autophagy bypassing inactivation of TORC1, suggesting that Atg13 acts as a molecular switch for autophagy induction.

III. TORC1 regulates mitotic entry via polo-like kinase

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at the G1 phase.

We demonstrated that TORC1 is also involved in another stage of the cell cycle, mitotic entry. We generated a temperature-sensitive allele of *KOG1 (kog1-105),* which encodes an essential component of TORC1, and found that TORC1 plays an important role in mitotic entry (G2/M transition). Since Cdc5, the yeast polo-kinase is mislocalized and inactivated in *kog1-105* mutant cells, TORC1 mediates G2/M transition via regulating polo-kinase. In addition, we discovered a physiological role of TORC1 in mitosis; autophagy negatively controlled by TORC1 plays an important part in maintenance of genome stability under starvation conditions.

IV. Ypk kinase acts directly downstream of TORC2 to control actin organization

TORC2 has an essential function controlling polarity of the actin cytoskeleton.

We identified Ypk2, a member of the AGC kinase family which acts directly downstream of TORC2 using molecular genetics. The activated allele of *YPK2* can rescue a lethality caused by TORC2 dysfunction, suggesting that Ypk kinase is the major downstream protein of the TORC2 pathway. We also demonstrated that Ypk2 is directly phosphorylated by TORC2 through a biochemical assay.

LABORATORY OF BIOLOGICAL DIVERSITY

MANO Group

Assistant Professor: *MANO, Shoji*
Postdoctoral Fellow: *KANAI, Masatake*
Visiting Scientist: *WATANABE, Etsuko*
KAMIGAKI, Akane
Technical Assistant: *HIKINO, Kazumi*
YAMAGUCHI, Chinami
NAKAI, Atsushi
KATO, Kyoko
Secretary: *UEDA, Chizuru*

Plant cells can induce, degenerate and differentiate their organelles to adapt to environmental changes. This flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aims of our research group are to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, and to understand the integrated functions of individual plants through organelle dynamics.

I. Molecular mechanisms of peroxisome dynamics and functions in plant cells

Peroxisomes are single-membrane bounded organelles, which are ubiquitously present in eukaryotic cells, and they are involved in various biological processes such as lipid metabolism and photorespiration. To understand peroxisome dynamics and functions, we have been analyzing a number of Arabidopsis mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants). Based on analyses using these mutants a part of the mechanism of division, protein transport, and degradation of peroxisomes were revealed. In addition, we revealed that the physical interaction between peroxisomes and chloroplasts is dependent on photosynthesis (Figure 1).

Recently, we found that peroxisome functions are required for the reproductive process. Therefore, peroxisomes in gametes and gametophytes were visualized, and their dynamics are currently under investigation.

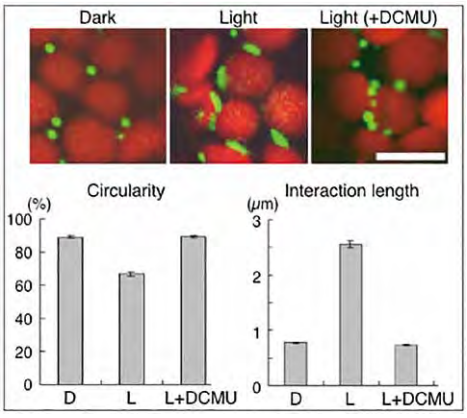

Figure 1. Photosynthesis-dependent chloroplast-peroxisome interaction. Chloroplasts and peroxisomes are visualized with autofluorescence (red) and GFP (green). Morphology of peroxisomes and interaction length between chloroplasts and peroxisomes are different in dark and light conditions. Even in light conditions, the photosynthesis inhibitor, DCMU, causes the dark-dependent phenotype. Bar: 10 μm.

II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves as foods and industrial materials. Storage reserves are different among different plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybean contains proteins as a major reserve. We are analyzing the mechanisms controlling oil and protein contents in seeds, and trying to apply our knowledge and techniques for increasing beneficial storage reserves.

III. Construction of The Plant Organelles Database 3 (PODB3)

PODB3 was built to promote a comprehensive understanding of organelle dynamics. PODB3 consists of six individual units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. Through these databases, users can obtain information on plant organelle responses to environmental stimuli of various tissues of several plant species, at different developmental stages. We expect that PODB3 will enhance the understanding of plant organelles among researchers.

Publication List:

〔Original papers〕

- Motomura, K., Le, Q.T.N., Hamada, T., Kutsuna, N., Mano, S., Nishimura, M., and Watanabe, Y. (2015). Diffuse DCP2 accumulates in DCP1 foci under heat stress in *Arabidopsis thaliana*. Plant Cell Physiol. *56*, 107-115.
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〔Original papers (E-publication ahead of print)〕

- Kanai, M., Mano, S., Kondo, M., Hayashi, M., and Nishimura, M. Extension of oil biosynthesis during the mid-phase of seed development enhances oil content in Arabidopsis seeds. Plant Biotechnol. J. 2015 Oct 26.
- Kimori, Y., Hikino, K., Nishimura, M., and Mano, S. Quantifying morphological features of actin cytoskeletal filaments in plant cells based on mathematical morphology. J. Theor. Biol. 2015 Nov 10.
- Ueda, H., Yokota, E., Kuwata, K., Kutsuna, N., Mano, S., Shimada, T., Tamura, K., Stefano, G., Fukao, Y., Brandizzi, F., Shimmen, T., Nishimura, M., and Hara-Nishimura, I. Phosphorylation of the C-terminus of RHD3 has a critical role in homotypic ER membrane fusion in Arabidopsis. Plant Physiol. 2015 Dec 18.

〔Review article〕

- Goto-Yamada, S., Mano, S., Yamada, K., Oikawa, K., Hosokawa, Y., Hara-Nishimura, I., and Nishimura, M. (2015). Dynamics of the light-dependent transition of plant peroxisomes. Plant Cell Physiol. *56*, 1252-1263.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

The aim of this laboratory is to research reproductive hormones in invertebrates and to analyze the mechanisms by which they work. The comparisons of such molecules and mechanisms in various species are expected to provide insights into the evolution of reproductive hormone systems.

I. Gonadotropins in the starfish, *Asterina pectinifera*

Gonadotropins play important regulatory roles in reproduction in both vertebrates and invertebrates. The vertebrate gonadotropins, LH and FSH are structurally and functionally conserved across various species, whereas no such molecule has been identified in invertebrates. The insect parsin hormones are assumed to be the physiological counterpart of LH and FSH in mammals. Some gonadotropic hormones, such as the egg development neurosecretory hormone of the mosquito, the egg-laying hormone of the sea hare, and the androgenic gland hormone of the terrestrial isopod, have been found in invertebrate species. More recently, an insulin-like peptide was reported to be responsible for the regulation of egg maturation in the mosquito, *Aedes aegypti*, demonstrating the involvement of insulin signaling in egg maturation among invertebrates.

The gonad-stimulating substance (GSS) of an echinoderm, the starfish, was the very first gonadotropin to be identified in invertebrates. GSS mediates oocyte maturation in starfish by acting on the ovary to produce the maturation-inducing hormone (MIH), 1-methyladenine, which in turn induces the maturation of the oocytes. In this sense, GSS is functionally identical to vertebrate LH, especially piscine and amphibian LHs, acting on the ovarian follicle cells to produce MIH to induce the final maturation or meiotic resumption of the oocyte. Considering the functional similarity that GSS shares with vertebrate LH, it is very important from an evolutionary point of view to know the chemical and molecular structure of GSS. We cloned the gene encoding GSS referred to amino acid sequence of purified GSS from radial nerves of the starfish, *Asterina pectinifera*. Interestingly, phylogenetic analyses revealed that it belonged to the insulin/insulin-like growth factor (IGF)/relaxin superfamily and, more precisely, to the subclass of relaxin/insulin-like peptides (Figure 1).

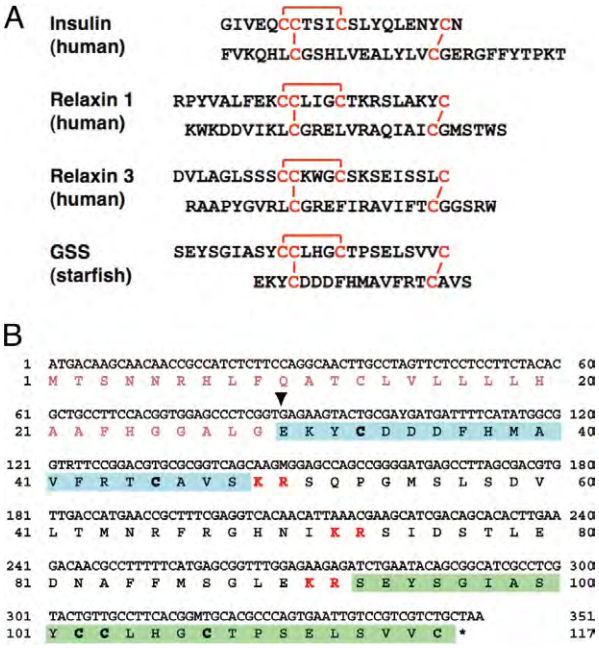

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of A and B chains are shown in green and blue boxes, respectively. Characters shown in red boldface indicate basic dipeptides that are the sites of proteolytic cleavage. Inverted triangle shows the deduced cleavage site of the signal peptide.

II. Search for reproductive hormones in invertebrates

In a collaborative effort with Prof. Yoshikuni's Laboratory of Kyushu Univ. and Dr. Yamano and Dr. Awaji of the National Research Institute of Aquaculture, Fisheries Research Agency (NRIA), we are searching for reproductive hormones in invertebrates; sea urchin, sea cucumber, oyster, and shrimp. While the collaborators are partially purifying physiological materials which induce egg maturation from nerve extracts and analyzing them with a tandem mass spectrometer, we are creating and analyzing EST libraries from nerve tissues and developing a database of the mass analysis performed in this laboratory.

LABORATORY OF BIOLOGICAL DIVERSITY

KOMINE Group

Associate Professor: *KOMINE, Yuriko*

We have been interested in the developmental and evolutional aspects of the structure of mammalian brains. In a comprehensive analysis of homeobox genes expressed in the developing mouse neocortex, we isolated a novel gene *Zfhx2*, which encodes a transcription factor containing three homeobox domains and 18 Zn-finger motifs. *Zfhx2* is highly expressed in the developing mouse brain, particularly in differentiating neurons, and continues to be expressed throughout adulthood at a low level. Two other phylogenically related genes, *Zfhx3* and *Zfhx4*, have been identified. The former was reported to be expressed in a manner dependent on neural differentiation, and the latter is a candidate gene causing congenital bilateral isolated ptosis. Although these three genes are expressed in substantially similar patterns in the developing brain, common functional features have not been clarified. Currently we have been focusing on *Zfhx2* to reveal its function and mechanisms of expression control in the developing brain.

I. Expression of *Zfhx2* is negatively regulated by its own antisense RNA

We found that the antisense strand of *Zfhx2* is also expressed in the mouse brain in a manner complementary to the expression of *Zfhx2* mRNA (Figure 1). Although most neurons express *Zfhx2* mRNA immediately after their final mitosis, several types of neuron (e.g., granule cells in the olfactory bulb and pyramidal and granule cells in the hippocampus) express antisense RNA prior to *Zfhx2* mRNA during the early phase of their differentiation. By generating a gene-targeting mouse line in which *Zfhx2* sense RNA is expressed but not antisense RNA, we showed that this antisense RNA has a negative regulatory role in the expression of *Zfhx2* mRNA. These observations suggest that the ZFHX2 protein might have a role in a particular step of neuronal differentiation, and in some types of neuron, this step might be delayed by the expression of antisense RNA.

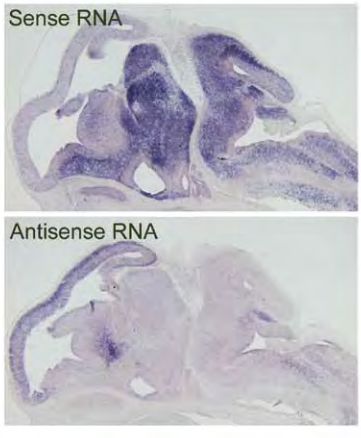

Figure 1. Expression of *Zfhx2* sense RNA (mRNA) and antisense RNA in the embryonic mouse brain. The antisense RNA was expressed where mRNA was not.

II. ZFHX2 might play roles in controlling emotional aspects

To elucidate the function of ZFHX2, we have also generated a *Zfhx2*-deficient mouse line. Although the production of the ZFHX2 protein is completely abolished in the homozygous mutant mice, the mice appear grossly normal and healthy. No anatomical abnormality has been observed in the mutant mouse brains so far examined. We hence subjected the *Zfhx2*-deficient mice to a comprehensive battery of behavioral tests to explore the physiological function of ZFHX2 in the nervous system. The homozygous *Zfhx2* deficient mice showed several behavioral abnormalities, namely, hyperactivity (Figure 2), enhanced depression-like behaviors, and an aberrantly altered anxiety-like phenotype. These behavioral phenotypes suggest that ZFHX2 might play roles in controlling emotional aspects through the function of monoaminergic neurons where ZFHX2 is expressed.

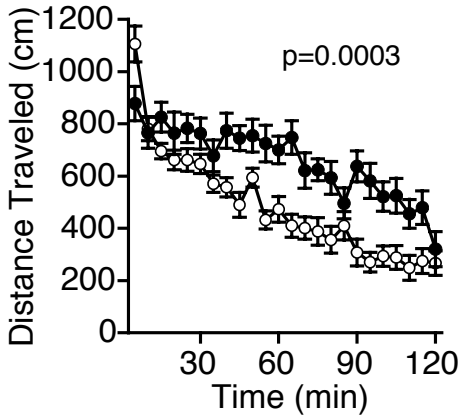

Figure 2. Locomotor activity of the *Zfhx2*-deficient mice. Mice were transferred into a novel environment and the distance traveled of each animal was measured for 2 hours. The *Zfhx2*-deficient mice (●, n=19) were significantly more active than the wild-type mice (○, n=21).

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

Assistant Professor: *HOSHINO, Atsushi*
Technical Assistant: *NAKAMURA, Ryoko*
TAKEUCHI, Tomoyo
ITO, Kazuyo

While genomic structures as well as their genetic information appear to stably transmit into daughter cells during cell division, and also into the next generation, they can actually vary genetically and/or epigenetically. Such variability has had a large impact on gene expression and evolution. To understand these genome dynamics in eukaryotes, especially in plants, we are characterizing the flower pigmentation of morning glories.

I. Flower pigmentation patterns of the morning glories

Ipomoea nil (Japanese morning glory), *I. purpurea* (the common morning glory), and *I. tricolor* have been domesticated well as floricultural plants, and their various spontaneous mutations have been isolated. The wild type morning glories produce flowers with uniformly pigmented corolla, whereas a number of mutants displaying particular pigmentation patterns have been collected. Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

Margined, *Rayed* and *Blizzard* of *I. nil* are dominant mutations. While these mutants show distinct flower pigmentation patterns, the same pigmentation gene is repressed by non-coding small RNAs in the whitish parts of the corolla. It is suggested that distinct regulation of these small RNAs cause the difference in pigmentation patterns. The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate their flower pigmentation. We are currently characterizing detailed molecular mechanisms of these mutations.

II. *de novo* sequencing of the Japanese morning glory genome

Although morning glories are studied worldwide, especially in plant physiology and genetics, no whole nuclear genome sequences of any *Ipomoea* species are available. To facilitate the studies of our group as well as all morning glory researchers, we are conducting *de novo* genome sequencing of *I. nil*. We chose a standard line for genome sequencing, and employed shotgun sequencing using a single molecule real time sequencing system. We recently obtained a draft genome sequence consisting of 15 pseudo-chromosomes with reasonable size, and are going to characterize more details of the genome sequence.

III. BioResource of morning glories

NIBB is the sub-center for the National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* has been one of the most popular floricultural plants since the late Edo era in Japan. It has an extensive history of genetic studies and also has many advantages as a model plant; simple genome, large number of mutant lines, and efficient self-pollination. Our collection includes 235 lines and 157,000 DNA clones.

IV. Pale- and dull-colored flower formation

Anthocyanin is responsible for the colors of many flowers and is usually glucosylated by 3GT (UDP-glucose:flavonoid 3-*O*-glucosyltransferase). We first demonstrated that absence of 3GT results in pale and dull flower coloration by using the recessive *3GT* mutants of *I. nil* and *I. purpurea* (Figure 1). Anthocyanin analysis revealed that 3GT is essential for maintaining proper production quantity, acylation, and glucosylation of anthocyanin. Incomplete acylation and glucosylation of anthocyanin results in dull flower coloration in *Ipomoea*. One of the *I. nil* mutants produces flower variegations that are thought to be epigenetically regulated (Figure 1c). We are currently studying a molecular mechanism of the flower variegation.

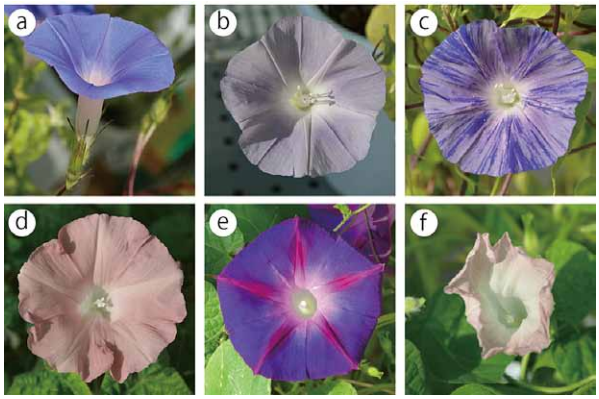

Figure 1. Wild type (a) and *3GT* deficient mutant of Japanese morning glory (b-d). Wild type (e) and *3GT* deficient mutant of common morning glory (f).

Publication List:

〔Original paper〕

- Morita, Y., Ishiguro, K., Tanaka, Y., Iida, S., and Hoshino, A. (2015). Spontaneous mutations of the UDP-glucose: flavonoid 3-*O*-glucosyltransferase gene confers pale and dull colored flowers in the Japanese and common morning glories. Planta *242*, 575-587.

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

- Azuma, M., Morimoto, R., Hirose, M., Morita, Y., Hoshino, A., Iida, S., Oshima, Y., Mitsuda, N., Ohme-Takagi, M., and Shiratake, K. A petal-specific InMYB1 promoter from Japanese morning glory: a useful tool for molecular breeding of floricultural crops. Plant Biotechnol. J. 29 Apr 2015.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*

Although transposons occupying large portions of the genome in various plants were once thought to be junk DNA, they play an important role in genome reorganization and evolution. Active DNA transposons are important tools for gene functional analysis. The endogenous non-autonomous transposon, *nDart1-0*, in rice (*Oryza sativa* L.) is expected to generate various transposon-insertion mutants because *nDart1-0* elements tend to insert into genic regions under natural growth conditions. The transpositions of *nDart1-0* were promoted by an active autonomous element, *aDart1-27*, on chromosome 6. By using the endogenous *nDart1*/*aDart1-27* system in rice, a large-scale *nDart*-inserted mutant population was easily generated under normal field conditions, and the resulting tagged lines were free of somaclonal variation.

I. A Gain of Function Mutant

The *nDart1*-promoted gene tagging line was developed using the endogenous *nDart1*/*aDart1* system to generate various rice mutants effectively. While the dominant mutants were occasionally isolated from the tagging line, it was unclear what causes dominant mutations. Efficient selection and analysis of dominant mutants to analyze the gene functions in rice is very useful. A semidominant mutant, *Bushy dwarf tiller1* (*Bdt1*), which has the valuable agronomic traits of multiple tillering and dwarfism, was obtained from the tagging line. The *Bdt1* mutant carried a newly inserted *nDart1* at 38-bp upstream of the transcription initiation site of a non-protein-coding gene, *miR156d*. This insertion caused an upstream shift of the *miR156d* transcription initiation site and, consequently, increased the functional transcripts producing mature microRNAs. These results indicate

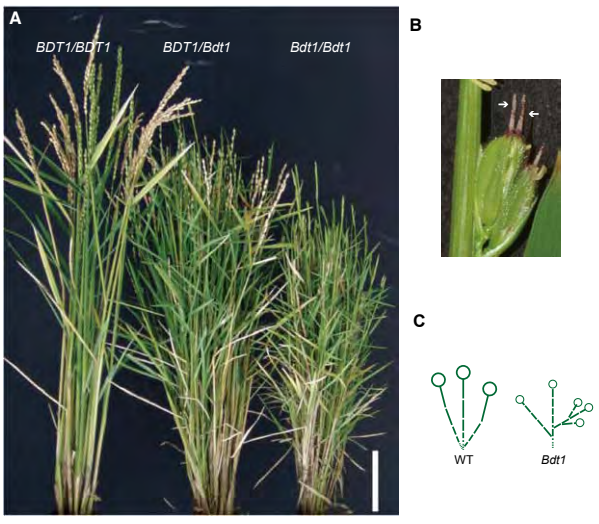

Figure 1. Phenotype of *Bushy dwarf tillers1* (*Bdt1*). (A) Three month old plants, (B) Abnormal panicles of *Bdt1*/*Bdt1* plants. White arrowheads indicate overgrown bracts and leaf-like structures, respectively. (**C**) Morphological phenotypes of WT and *Bdt1* plants. Each broken line and each circle represents an internode and a panicle, respectively.

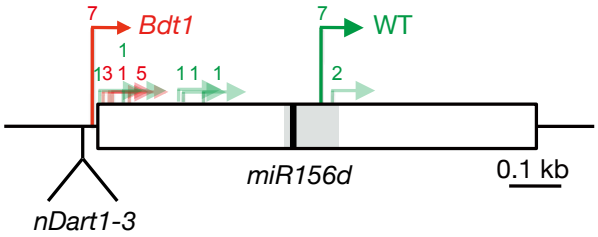

Figure 2. Transcription initiation sites of *BDT1* gene. Major transcription initiation sites of *miR156d* in WT and *Bdt1*plants. Red and green arrows indicate transcription initiation sites of miR156d in WT and *Bdt1* plants, respectively. Numbers above the arrows represent the numbers of clones that correspond to the transcription initiation site. The left end of the white box indicates the reported 5' terminal of the full-length cDNA (AK073452) in Nipponbare (http://rapdb.dna.affrc.go.jp/). The gray and black boxes show the corresponding positions of the pre-miR156d and miR156d sequences.

that the total amount of miR156d is controlled not only by transcript quantity but also by transcript quality. Furthermore, transgenic lines introduced an *miR156d* fragment that flanked the *nDart1* sequence at the 5' region, suggesting that insertion of *nDart1* in the gene promoter region enhances gene expression as a cis-element. This study demonstrates the ability of *nDart1* to produce gain-of-function mutants as well as further insights into the function of transposable elements in genome evolution.

Publication List:

〔Original paper〕

● Hayashi-Tsugane, M., Maekawa M., and Tsugane, K. (2015). A gain-of-function *Bushy dwarf tiller* 1 mutation in rice microRNA gene *miR156d* caused by insertion of the DNA transposon *nDart1*. Sci. Rep. *5*, 14357.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: *JOHZUKA, Katsuki*
Technical Staff: *ISHINE, Naomi*

Chromosome condensation is a basic cellular process that ensures the faithful segregation of chromosomes in both mitosis and meiosis. This process is required not only for shrinking the length of chromosome arms, but also for resolving entanglements between sister-chromatids that are created during DNA replication. Any abnormality in this process leads to segregation errors or aneuploidy, resulting in cell lethality. Chromosome condensation is mainly achieved by condensin, a hetero-pentameric protein complex, widely conserved from yeast to humans. Despite its conservation and importance for chromosome dynamics, how condensin works is not well understood. Recent studies reveal that condensin functions are not restricted to chromosome condensation and segregation during cell divisions. It is required for diverse DNA metabolism such as genome stability, transcriptional regulation, and cell differentiation.

Our research interest is to understand the mechanism and regulation of chromosome condensation. We have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. Microscopic observation indicated the nucleolar localization of condensin. Consistent with this, the ribosomal RNA gene (rDNA) repeat is the most condensed region in the genome during mitosis. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. To date, the best characterized condensin binding region is the rDNA repeat on the right arm of chromosome XII in budding yeast. We further discovered the multiple protein network required to recruit condensin to the RFB site.

I. Dynamic relocalization of condensin during meiosis

Our genetic screening indicated that two proteins, Csm1 and Lrs4, were required for condensin recruitment to the RFB site. Physical interactions between Csm1/Lrs4 and subunits of condensin are important for recruitment of condensin to the RFB site. These proteins are known as components of the monopolin complex that are required for faithful segregation of homologous chromosomes during meiotic division I. During meiosis I, the monopolin complex re-localizes from the rDNA repeat to the centromere and acts for ensuring sister-chromatid co-orientation. Re-localization of Csm1/Lrs4 proteins suggested the re-localization of condensin from rDNA repeat to centromere. As expected, chromatin-IP experiments indicated that condensin re-localizes to the centromere during meiosis I. Condensin might clamp sister-chromatids together during meiosis I.

II. Condensin-dependent chromatin folding

The RFB site, which consists of a ~150bp DNA sequence, is functioning as a cis-element for recruitment of condensin to chromatin in the yeast genome. If the RFB site is inserted into an ectopic chromosomal locus, condensin can associate with the ectopic RFB site. To explore the role of condensin in chromosome organization, we have constructed a strain in which two RFB sites are inserted on an ectopic chromosome arm with an interval of 15kb distance in the cell with complete deletion of the chromosomal rDNA repeat. Using this strain, condensin-dependent chromatin interaction between two RFBs was examined by chromosome conformation capture (3C) assay. We found condensin-dependent chromatin interaction between the two RFB sites on the chromosome arm. This result indicates that condensin plays a role in chromatin interaction between condensin binding sites, and this interaction leads to creation of a chromatin loop between those sites (Figure 1). It is thought that condensin-dependent chromatin folding is one of the basic molecular processes of chromosome condensation. During the cell cycle stages, the RFB - RFB interaction signal increases in metaphase and reaches its maximum level in anaphase. In addition to the RFB – RFB interaction, the chromatin interactions between internal regions of two RFBs increases in anaphase. Thus, the configuration of chromatin fiber changes from a simple loop into a complicated twisted shape as the cell cycle progresses from metaphase to anaphase.

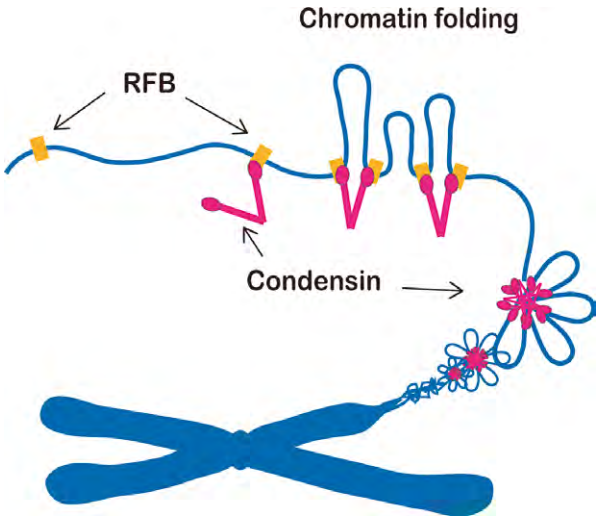

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

KATO Group

Specially Appointed Assistant Professor:
KATO, Kagayaki

Organogenesis is accomplished by a series of deformations of the planar cell sheet into a three-dimensional shape during embryogenesis. This drastic structural change is an integrated result of individual cell behaviors in response to spatio-temporally controlled mechanisms.

To better understand the programs underlying organ formation, it is required to analyze individual cells' morphology and dynamics quantitatively. However, due to the massive images generated by 4D microscopy and their ambiguity, this made it difficult to perform these analyses.

To unveil organogenesis from the point of view of distinct cell behaviors, we are developing application software that is capable of describing cell dynamics out of 4D time-lapse imaging data sets by employing image processing techniques.

I. 4D cell segmentation/tracking system

Epithelial morphogenesis in the fruit fly Drosophila melanogaster embryo is considered to be an excellent model for collective cell migrations. Drastic cell rearrangements lead drastic structural changes to build elaborate tubular organs such as the tracheal network. We are developing a software pipeline, which automatically recognizes individual cell shapes out of 3D space and tracks them through time. This system extracts cell boundaries and reconstitutes cell shapes as a skeletonized chain of voxels spanning 3D space. This abstract form of cell visualization makes it possible to describe morphometric quantities and kinetics of cells in single-cell resolution (Figure 1). These morphometric quantities allow us to perform comparative studies on shapes and behaviors precisely among several experimental conditions, to gain a better understanding of the genetic programs underlying organogenesis. We are now applying this system to several experimental models to determine the practicality of the system.

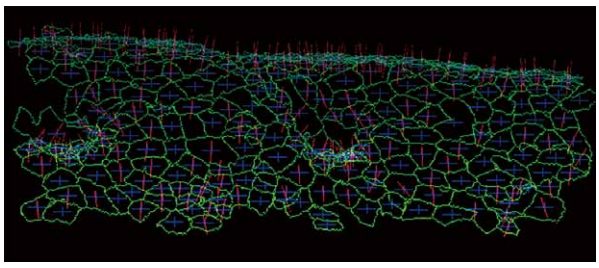

Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of a fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta) are indicated for each cell.

II. Particle tracking for tissue deformation analysis

Besides cell boundary extraction, we also developed a derived algorithm for particle image velocimetry (PIV). This system is designed to measure tissue deformation even though the imaging constraints do not allow identification of individual cells out of images. This implementation detects structural characteristics, such as uneven fluorescence distributed over the specimen and tracks these patterns along a time-series. Despite that the tissue was labeled with non-targeted cytoplasmic GFP, this tracking software successfully outlined developmental dynamics of Xenopus neuroectoderm (Figure 2).

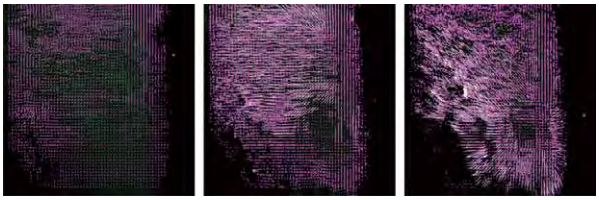

Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signals over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed the microscopy.

III. A GUI application for manual image quantification

Biologically significant image features are not always significant to computational algorithms due to their structural instability. This kind of difficulty requires human eye inspection for feature extractions. A GUI (Graphical User Interface) application we developed can easily visualize 4D imaging data and has made manual feature annotations easy (Figure 3). This application is freely available at our website (https://is.cnsi.jp/).

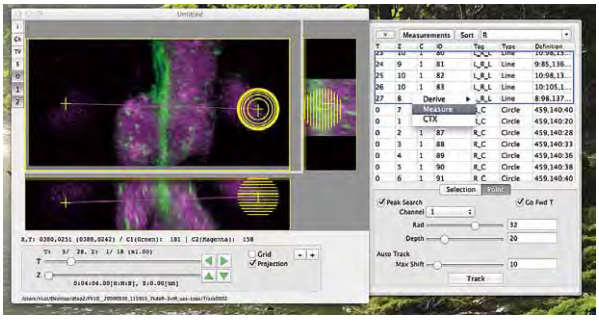

Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

LABORATORY OF BIOLOGICAL DIVERSITY

KIMORI Group

Specially Appointed Assistant Professor:
KIMORI, Yoshitaka

Image processing methods significantly contribute to visualization of biomedical targets acquired from a variety of imaging techniques, including: wide-field optical and electron microscopy, X-ray computed tomography, magnetic resonance imaging and mammography. Quantitative interpretation of the deluge of complicated biomedical images, however, poses many research challenges.

We have developed new computational methods based on mathematical morphology for quantitative image analysis. One of the most important purposes of image processing is to derive meaningful information, which is expressed as structural properties in images. Mathematical morphology is a nonlinear image processing method based on the set theory and is useful for the extraction of structural properties from an image. It can be used as a fundamental tool to analyze biomedical images and provides an objective, accurate alternative to manual image analysis.

Novel image analysis method based on mathematical morphology: quantifying morphological features of actin filaments in plant cells

Image processing is a crucial step in the quantification of biomedical structures from images. As such, it is fundamental to a wide range of biomedical imaging fields. Image processing derives structural features, which are then numerically quantified by image analysis. We can better evaluate complex shapes and detect subtle morphological changes in organisms by quantifying the shape properties. Therefore, we have developed a shape analysis method based on morphological image processing, and have applied it to image analysis of actin cytoskeletal filaments in root-hair cells of *Arabidopsis thaliana*. Actin cytoskeletal filaments have critical roles in various cellular processes in plant cells. To understand actin-dependent organelle motility, we analyzed the organization of actin filaments in the cells.

We measured three shape features of the actin filaments in wild-type and mutant (*root hair defective 3* (*rhd3*) mutant) plants. One feature i.e. thickness (*T*) was extracted from grayscale images; the others i.e. multi-orientation index (*MOI*) and complexity (*C*) were extracted from binary images. *T* of an actin filament was measured by a pattern spectrum which provides a distribution of filament thickness. *MOI* was measured by applying a series of opening operations to obtain the orientation distribution of the filament. *C* was computed from the fractal dimension of the filament network pattern. As the *MOI* and *C* quantify the complexity properties of the filament patterns in two-dimensional space, finally, these binary-based features were combined into a single feature called the binarized filament pattern feature (*BFPF*).

The *T* of the wild-type and mutant were 1.88 μm and 2.33 μm, respectively, and those were statistically different ($p < 0.001$). Also, *MOI* of the wild-type and *rhd3* mutant images were statistically different ($p < 0.01$), too. Finally, we calculated the *C*. The mean fractal dimension of the actin filament was significantly larger in the wild-type than in the *rhd3* mutant ($p < 0.001$). Furthermore, the mean *BFPF* (obtained by combining *MOI* and *C*) were again significantly differed between the two groups ($p < 0.001$). These results are summarized in a scatter plot of *BFPF* (*x*-axis) versus *T* (*y*-axis) (Left panel of Figure 1). First, we note that actin filaments are thicker in the *rhd3* mutant than in the wild-type. Second, both *MOI* and *C* are significantly larger in the wild-type filaments than in the *rhd3* mutant filaments. Overall, the filament patterns in two-dimensional space are more complex in the wild-type than in the *rhd3* mutant. The *rhd3* mutant data in the scatter plot are divisible into two classes (Class 1 and Class 2), distinguished by linear discriminant analysis (LDA). The right panel of Figure 1 shows three images selected from each class.

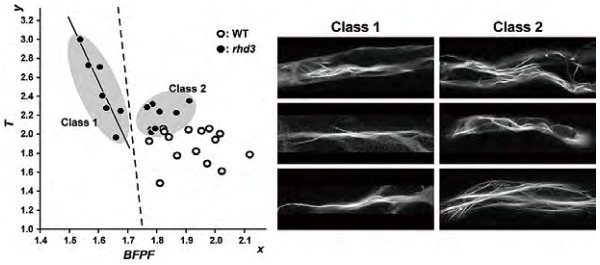

Figure 1. Left: Scatter plot of *BFPF* versus *T* for the actin filament of wild-type and the *rhd3* mutant. Data of the *rhd3* mutant is divided into two classes. Dashed line is classification boundary obtained by LDA. Solid line is boundary obtained by linear regression of the data in Class 1. Right: Actin filaments of the *rhd3* images belonging to each class.

Publication List:

〔Original paper〕

● Ohya, Y., Kimori, Y., Okada, H., and Ohnuki, S. (2015). Single-cell phenomics in budding yeast. Mol. Biol. Cell. *26*, 3920-3925.

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

● Kimori Y., Hikino K., Nishimura M. and Mano S. Quantifying Morphological Features of Actin Cytoskeletal Filaments in Plant Cells Based on Mathematical Morphology. J. Theor. Biol. 2015 Nov 10.
● Yasuda, T., Kimori, Y., Nagata, K., Igarashi, K., Watanabe-Asaka, T., Oda, S., and Mitani, H. Irradiation-injured brain tissues can self-renew in the absence of the pivotal tumor suppressor p53 in the medaka (*Oryzias latipes*) embryo. J. Radiat. Res. 2015 Sep 25.

DIVISION OF MOLECULAR ENVIRONMENTAL ENDOCRINOLOGY †

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Synthetic chemicals found in the environment have the capacity to disrupt the development and function of the endocrine system in both wildlife and humans. This has drawn public concern since many of these chemicals may bind to estrogen receptors (ERs) and evoke estrogenic effects. Early evidence that exposure to estrogenic chemicals during development could pose a threat to human health came from studies of a synthetic hormone, diethylstilbestrol (DES), which was used to prevent premature birth and spontaneous abortion. Laboratory experiments showed that exposure to sex hormones during critical windows of perinatal life caused the immune and nervous systems, bone, muscle, and the liver of animals to be affected. Although many of these chemicals can bind to ERs in wildlife and humans, the molecular basis for the action of environmental estrogens remains poorly understood. Thus, understanding the molecular mechanisms through which environmental estrogens and sex hormones act during critical developmental windows is essential.

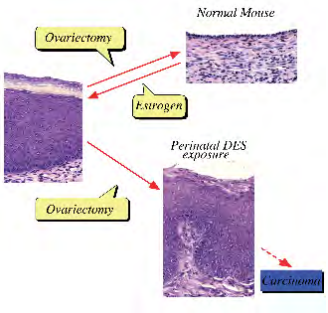

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

I. Developmental origin of adult disease: Perinatal estrogen exposure induces persistent changes in reproductive tracts

The emerging paradigm of the "embryonic/fetal origins of adult disease" provides a powerful new framework for considering the effects of endocrine disrupters on human and animal health. In 1971, prenatal DES exposure was found to result in various abnormalities of the reproductive tract in women. This syndrome was named the DES syndrome. Similar abnormalities have been demonstrated in experimental animals exposed perinatally to estrogens. Developmental estrogen exposure in mice, for example, induces persistent proliferation of vaginal epithelial cells (Figure 1). We found that the persistent changes in the vagina in mice exposed neonatally to estrogens result from the persistent activation of erbBs and ERα, and sustained expression of EGF-like growth factors (Figure 2). Currently, we are analyzing the methylation status in the mouse vagina using a microarray (MeDIP-chip). We found several differentially methylated or demethylated DNA profiles in neonatally DES-exposed mouse vaginae and controls. We thus consider that neonatal DES exposure affects DNA methylation profiles, resulting in persistent abnormalities in mouse reproductive organs. We also found that ERα is indispensable for normal vaginal epithelial cell differentiation in mice.

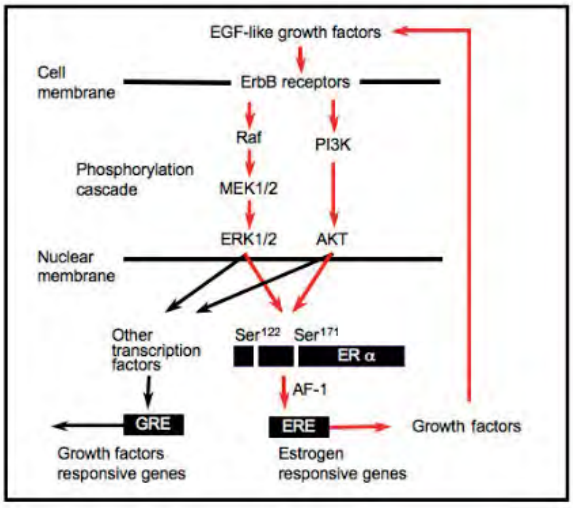

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

II. Estrogen receptors of birds, reptiles, amphibians, and fishes

Steroid and xenobiotic receptors (SXR) have been cloned from various animal species (fish, amphibians, reptiles, birds, and mammals) by our group and we have demonstrated species-specific differences in their responses to various environmental and endogenous chemicals (receptor gene zoo). Thus, simple predictions of chemical effects based on data from a few established model species are not sufficient to develop real world risk assessments. ER and ER-like genes have been cloned from various animal species including rockshell, *Amphioxus*, lamprey, catshark, whale shark, lungfish, sturgeon, gar, *polypterus*, arowana, roach, stickleback, mosquitofish, mangrove *Rivulus*, Japanese giant salamander, Tokyo salamander, newt, axolotl, toad, *Silurana tropicalis*, American alligator, Nile crocodile, freshwater turtle, Japanese rat snake, Okinawa habu, and vultures. Functional studies showed that the *Amphioxus* ER sequence does not bind estrogen but *Amphioxus* steroid

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2015. The former title is indicated by an asterisk (*).
†: This laboratory was closed on 31 March, 2016.

receptor and lamprey ER exhibited ligand-dependent transactivation, proving that invertebrate and primitive vertebrates, such as the Agnatha, have a functional ER. We found that medaka ER subtypes have their specific functions, and medaka, zebrafish and stickleback ERs are more sensitive to estrogen/estrogen-like chemical exposures than other fishes by reporter gene assay. Thus, these approaches are efficient to evaluate the relationship between species and their sensitivities to chemicals.

III. Evolutionary history and functional characterization of androgen receptor genes in jawed vertebrates

Vertebrates show diverse sexual characteristics which are regulated by androgens. To elucidate the evolutionary history and functional diversification of androgen receptor (AR) genes in vertebrates, we cloned the AR cDNAs from a shark, basal ray-finned fishes (Actinopterygii), namely bichir and sturgeon (Acipenseriformes), and teleosts including a basal teleost, arowana (Osteoglossiformes). Molecular phylogenetic analysis revealed that a gene duplication event gave rise to two different teleost ARs (α and β) and likely occurred in the actinopterygian lineage leading to teleosts after the divergence of Acipenseriformes but before the split of Osteoglossiformes. Functional analysis revealed that the shark AR activates the target gene via the androgen response element by classical androgens. The teleost ARα showed unique intracellular localization with a significantly higher transactivation capacity than that of teleost ARβ. These results indicate that the most ancient type of AR, as activated by the classic androgens as ligands, emerged before the Chondrichthyes-Osteichthyes split and the *AR* gene was duplicated during a teleost-specific gene duplication event (Figure 3).

IV. Papillary process formation in medaka

Androgens play key roles in the morphological specification of male type sex characteristics and reproductive organs, whereas little is known about the developmental mechanisms. Medaka show a prominent masculine sexual character, papillary processes in the anal fin, which has been induced in females by exogenous androgen exposure. We have identified androgen-dependent expressions of *Bmp7* and *Lef1* are required for the bone nodule outgrowth leading to the formation of the papillary process in the postal region of the anal fin. We have also developed a testing method for screening of chemicals having androgen and anti-androgenic activity using the anal fin in juvenile medaka.

V. Environmental sex differentiation in Daphnids and American alligators

Daphnia magna has been used extensively to evaluate the organism- and population-based responses of toxicity or reproductive toxicity tests. These tests, however, provide no information about the mode of action of the tested compounds. Therefore, we applied an ecotoxicogenomic assessment of *D. magna*. We established a *Daphnia* EST database and developed an oligonucleotide-based DNA microarray

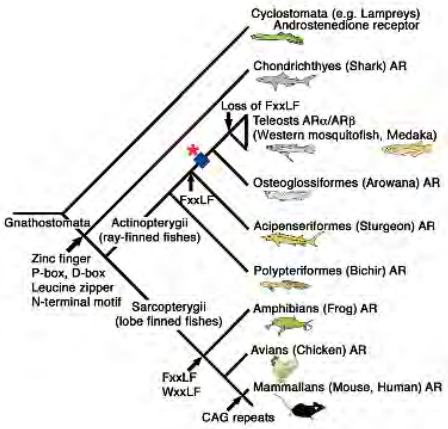

Figure 3. Evolutionary relationships of androgen receptor sequences.

with high reproducibility and demonstrated the usefulness of the array for the classification of toxic chemicals as well as for the molecular understanding of chemical toxicity in a common freshwater organism. *D. magna* and *D. pulex* reproduce asexually (parthenogenesis) when they are in an optimal environment for food, photoperiod and population density. Once environmental conditions become suboptimal, they alter their reproductive strategy from asexual to sexual reproduction (Figure 4). Chemicals are able to affect the sex determination of daphnids and we found that juvenile hormone (JH) agonists (insect growth regulators), for example, induce the production of male offspring. The molecular basis of environmental sex determination is largely unknown in daphnids. To understand the molecular mechanisms of this phenomenon, we isolated sex determination-related genes. Also, we have developed a method to inject genes into *D. magna* and *D. pulex* embryos which will allow us to study gain- and loss-of function analyses in more detail in these species. Using these techniques, we demonstrated that DSX1 (double sex 1), one of the DM-domain genes, is essential for male differentiation in *D. magna*. We have developed an RNAi method and a TALEN method using *D. pulex*. To further explore the signaling cascade of sexual differentiation in *D. magna*, a gene expression profile of JH-responsive genes is essential. We are identifying JH-responsive genes in the ovary of *D. magna* and *D. pulex* exposed to JH agonist and methyl farnesoate (JH identified in decapods) at the critical timing of JH-induced sex determination in *D. magna* and *D. pulex*. We have identified a JH receptor (heterodimer of methoprene-tolerant and steroid receptor co-activator) in daphnids and the function of ecdysone in the molting and ovulation in *D. magna*.

Sex determination mechanisms can be broadly categorized

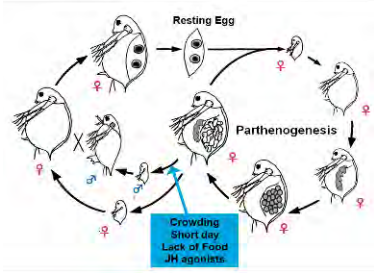

Figure 4. Life cycle of *Daphnia*.

by either a genotypic or environmentally driven mechanism. Temperature-dependent sex determination (TSD), an environmental sex determination mechanism most commonly observed among vertebrates, has been observed especially among reptiles from 1966. However, the temperature-dependent triggering mechanism of TSD and the subsequent differentiation cascade has long remained unknown. We have isolated and cloned the thermosensitive cation channel, TRP vanilloid subtype 4 (TRPV4) as a male-cascade trigger for the American alligator, *Alligator mississippiensis*, in response to high environmental temperature, and demonstrated its thermal activation at temperatures proximate to TSD-related temperatures in the alligator. Furthermore, using pharmacological exposure to manipulate TRPV4 channel activity, we have demonstrated that TRPV4 channel activity has a direct relationship with male differentiation gene expression, suggesting that AmTRPV4 is involved in the male differentiation cascade, and proposed a novel mechanism for the sex determination pathway.

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DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

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Plants and algae have a large capacity to acclimate themselves to changing environments. We are interested in these acclimation processes, in particular, how efficiently yet safely they harness sunlight for photosynthesis under fluctuating light conditions. Using a model green alga, we are studying the molecular mechanisms underlying photoacclimation of the photosynthetic machinery. We are also applying the knowledge obtained in the studies of a model green alga to various phytoplankton, including *Symbiodinium* in corals and sea anemones in tropical oceans, to explore how environmentally important photosynthetic organisms thrive in their ecological niche.

I. Acclimation of photosynthesis

Using a green unicellular alga *C. reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes such as state transitions, non-photochemical quenching, photoinhibition, and cyclic electron flow by means of biochemistry, molecular genetics, various physiological analyses, and optical spectroscopy.

1-1 State transitions

Photosynthetic organisms respond to changes in light quality by regulating the absorption capacity of their PSs. These short-term acclimations use redox-controlled, reversible phosphorylation of LHCIIs to regulate the relative absorption cross-section of the two photosystems, commonly referred to as state transitions. Each of the two charge-separation devices—PSI and PSII—in the thylakoid membranes has a distinct pigment system with unique absorption characteristics. Thus, an imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate with time. Because the two photosystems are functionally connected in series under normal conditions, plants and algae must constantly balance their excitation levels to ensure the optimal efficiency of electron flow. State transitions occur under such conditions to balance the light-harvesting capacities of the two photosystems, thereby minimizing the unequal distribution of light energy. State 1 occurs when PSI is preferentially excited and the light-harvesting capacities of PSII and PSI are increased and decreased, respectively, to adjust the excitation imbalance; this state is indicated by a higher chlorophyll fluorescence yield at room temperature. Conversely, State 2 occurs when PSII is preferentially excited and the light-harvesting capacities of PSII and PSI are decreased and increased, respectively, to readjust the excitation imbalance; this state can be monitored as a lower chlorophyll fluorescence yield at room temperature (Figure 1).

In *C. reinhardtii* WT cells, the modulation of CEF normally occurs in parallel with state transitions. When the light-induced reduction of Cyt *bf* was probed in State 1- and State

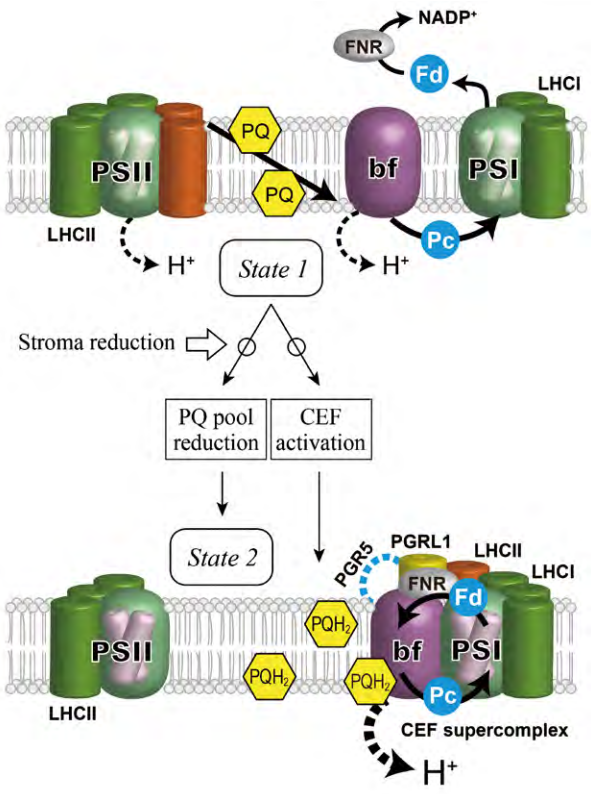

Figure 1. Schematic representation of the regulation of electron flow and state transitions in *C. reinhardtii*. Upper: when PSI is preferentially excited, the stroma of chloroplast and the PQ pool are oxidized. Under these conditions, LHCIIs are bound to PSII (State 1). The photosynthetic electron flow proceeds in linear electron flow (LEF) mode, generating NADPH as well as a proton gradient across the thylakoid membrane that is used for ATP production. Middle: when the stroma is reduced, first, cyclic electron flow (CEF) is activated by the association of Cyt *bf* and FNR with PSI to form a super-supercomplex (CEF supercomplex). PGRL1 and possibly PGR5 are also associated with the CEF supercomplex; second, PQ pool is reduced and migration of the mobile LHCIIs (*orange*) from PSII to PSI occurs; Lower: the cells are in State 2, and the photosynthetic electron flow proceeds in CEF mode.

2-adapted cells, a differential sensitivity to the addition of the PSII inhibitor DCMU was observed. In the presence of DCMU, the reduction of Cyt *bf* was suppressed in State 1 but not in State 2. An identical sensitivity to an inhibitor of Cyt *bf*, DBMIB, was observed in both State 1 and State 2, suggesting that PSII-independent Cyt *bf* reduction occurs only in State 2. However, state transitions and CEF/LEF switching are not mechanically linked in *C. reinhardtii* but the two phenomena are rather coincidental. Under anaerobic conditions, the independent knockdown of three thylakoid membrane proteins, PGRL1, CAS, and ANR1, resulted in decreased CEF activity, but the ability to undergo state transitions was unaffected. This was further supported by the low CEF activity in a State 2-locked mutant of *C. reinhardtii*. Although the lateral migration of mobile LHCIIs occurred in the ptox2 mutant, which was State 2-locked due to a lack of plastid terminal oxidase 2, the effects on $P700^+$ re-reduction were negligible. CEF was thus proposed to be correlated with a reduced state of the stroma.

1-2 Non-photochemical quenching

Absorption of light in excess of the capacity for photosynthetic electron transport is damaging to photosynthetic organisms. Several mechanisms exist to avoid photodamage, which are collectively referred to as non-photochemical quenching (NPQ). This term comprises at least two major processes: state transitions (qT), the change in the relative antenna sizes of PSII and PSI as described in the previous section, and energy-dependent quenching of excess energy (qE), the increased thermal dissipation triggered by lumen acidification. Recently, we isolated the PSII-LHCII supercomplex from both WT *C. reinhardtii* and the *npq4* mutant, which is qE-deficient and lacks the ancient light-harvesting protein LHCSR. LHCSR3 was present in the PSII-LHCII supercomplex from the high light-grown WT but not in the supercomplex from the low light-grown WT or the *npq4* mutant. The purified PSII-LHCII supercomplex containing LHCSR3 showed a normal fluorescence lifetime at a neutral pH (7.5) by single-photon counting analysis but exhibited a significantly shorter lifetime (energy-quenching) at pH 5.5, which mimics the acidified lumen of the thylakoid membranes in high light-exposed chloroplasts. The switching from light-harvesting mode to energy-dissipating mode observed in the LHCSR3-containing PSII-LHCII supercomplex was inhibited by DCCD, a protein-modifying agent specific to protonatable amino acid residues. We conclude that the PSII-LHCII-LHCSR3 supercomplex formed in high light-grown *C. reinhardtii* cells is capable of energy dissipation upon protonation of LHCSR3.

1-3 Photodamage to photosystem II

Light, the driving force of photosynthesis, damages photosynthetic machinery, primarily photosystem II (PSII), and it results in photoinhibition. A new photodamage model, the two-step photodamage model, suggests that photodamage to PSII initially occurs at the oxygen evolving complex (OEC) because of light energy absorbed by manganese and that the PSII reaction center is subsequently damaged from light energy absorbed by photosynthetic pigments due to the limitation of electrons to the PSII reaction center. However, it was uncertain whether this model is applicable to photodamage to PSII under visible light as manganese absorbs visible light only weakly. In our study, we demonstrated using PSII membrane fragments isolated from spinach leaves that visible light damages OEC prior to photodamage to the PSII reaction center. This finding supports that the two-step photodamage model is applicable to photodamage to PSII by visible light.

1-4 Cyclic electron flow in plants

CEF around PS I is difficult to quantify. In our study, a new method was introduced to measure CEF in wild-type and *pgr5* and *ndh* mutants of *Arabidopsis*. We obtained the linear electron flux ($LEFO_2$) through both photosystems and the total electron flux through PS I (ETR1) in *Arabidopsis* in CO_2-enriched air. $\Delta Flux = ETR1 - LEFO_2$ is an upper estimate of CEF, which consists of two components, an antimycin A-sensitive, PGR5 (proton gradient regulation 5 protein)-dependent component and an insensitive component facilitated by a chloroplastic nicotinamide adenine dinucleotide dehydrogenase-like complex (NDH). Our results demonstrated that (1) 40% of the absorbed light was partitioned to PS I; (2) at high irradiance a substantial antimycin A-sensitive CEF occurred in the wild type and the *ndh* mutant; (3) at low irradiance a sizable antimycin A-sensitive CEF occurred in the wild type but not in the *ndh* mutant, suggesting an enhancing effect of NDH in low light; and (4) in the *pgr5* mutant, the wild type, and *ndh* mutant treated with antimycin A, a residual ΔFlux existed at high irradiance, attributable to charge recombination and/or pseudo-cyclic electron flow. Therefore, in low-light-acclimated plants exposed to high light, ΔFlux has contributions from various paths of electron flow through PS I.

II. Ecophysiology of micro algae

Our new projects are the study of photoacclimation of dinoflagellates that can live in a symbiotic relationship with cnidarians, and the study of oil-producing *Chlamydomonas*. We are particularly interested in a dinoflagellate *Symbiodinium* living with corals and sea anemones (Figure 3), and the oil-producing *Chlamydomonas* grown under natural pond-like environments. We are trying to elucidate how their photosynthetic machinery acclimates to variable light and temperature conditions. Furthermore, our interest has been expanded to higher plants.

2-1 Diversification of light harvesting complex gene family via intra- and intergenic duplications in the coral symbiotic alga *Symbiodinium*

The unicellular dinoflagellate alga *Symbiodinium* is known to be an endosymbiont of cnidarian animals including corals and sea anemone, and provide carbohydrates generated through photosynthesis to host animals. Although *Symbiodinium* possesses a unique light-harvesting complex (LHC) gene family called chlorophyll *a*-chlorophyll c_2-peridinin protein complex (acpPC), the genome-level gene diversity and evolutionary trajectories have not been investigated. We show phylogenetic analysis revealing that

many of the acpPC/LHCs were encoded in highly duplicated genes with the multi-subunit polyprotein structures in the nuclear genome of *Symbiodinium minutum*. This provided an extended list of the LHC gene family in a single organism, including 82 loci encoding polyproteins composed of 164 LHC subunits recovered in the phylogenetic tree. In *S. minutum*, 5 phylogenetic groups of the Lhcf-type gene family, which is exclusively conserved in algae harboring secondary plastids of red algal origin, and 5 groups of the Lhcr-type, of which members are known to be associated with PSI in red algae and secondary plastids of red algal origin were identified. Notably, members classified to a phylogenetic group of the Lhcf-type (group F1) are highly duplicated, which can explain the presence of an unusually large number of LHC genes in this species. While some gene units were homologous to other units within single loci of the polyprotein genes, intergenic homologies between separate loci were conspicuous in other cases, implying that gene unit 'shuffling' by gene conversion and/or genome rearrangement might have been a driving force for the diversification. These results suggest that, through vigorous intra- and intergenic gene duplication events, the genomic framework of the photosynthesis has been forged in the coral symbiont dinoflagellate algae.

Figure 2. Fluorescence image of the tiny sea anemone *Aiptasia*, a model system for studies of dinoflagellate (*Symbiodinium*)-cnidarian symbiosis. Each red dot is a cell of *Symbiodinium*.

2-2 Novel characteristics of photodamage to PSII in a high-light-sensitive *Symbiodinium* phylotype

Symbiodinium is genetically diverse, and acquiring suitable *Symbiodinium* phylotypes is crucial for the host to survive in specific environments, such as high-light conditions. The sensitivity of *Symbiodinium* to high light differs among *Symbiodinium* phylotypes, but the mechanism that controls light sensitivity has not yet been fully resolved. In the present study using high-light-tolerant and -sensitive *Symbiodinium* phylotypes, we examined what determines sensitivity to high light. In growth experiments under different light intensities, *Symbiodinium* CS-164 (clade B1) and CCMP2459 (clade B2) were identified as high-light-tolerant and -sensitive phylotypes, respectively. Measurements of the maximum quantum yield of photosystem II (PSII) and the maximum photosynthetic oxygen production rate after high-light exposure demonstrated that CCMP2459 is more sensitive to photoinhibition of PSII than CS-164, and tends to lose maximum photosynthetic activity faster. Measurement of photodamage to PSII under light of different wavelength ranges demonstrated that PSII in both *Symbiodinium* phylotypes was significantly more sensitive to photodamage under shorter wavelength regions of light spectra (<470 nm). Importantly, PSII in CCMP2459, but not CS-164, was also sensitive to photodamage under the regions of light spectra around 470-550 and 630-710 nm, where photosynthetic antenna proteins of *Symbiodinium* have light absorption peaks. This finding indicates that the high-light-sensitive CCMP2459 has an extra component of photodamage to PSII, resulting in higher sensitivity to high light. Our results demonstrate that sensitivity of PSII to photodamage differs among *Symbiodinium* phylotypes and this determines their sensitivity to high light.

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DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

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Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. For example, animals restrict breeding to specific seasons to maximize survival of their offspring in temperate zones. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. We use comparative approaches to understand these mechanisms. Japanese quail and medaka provide excellent models to study these mechanisms because of their rapid and robust seasonal responses. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation.

I. Mechanism of seasonal testicular regression

Birds have evolved highly sophisticated mechanisms of seasonal regulation, and their testicular mass can change a hundred-fold within a few weeks. Recent studies revealed that seasonal gonadal development is regulated by central thyroid hormone (TH) activation within the hypothalamus depending on the photoperiodic changes. By contrast, the mechanisms underlying seasonal testicular regression remain unclear. We examined the effects of short day and low temperature on testicular regression in quail. Low temperature stimulus accelerated short day (SD)-induced testicular regression by shutting-down the hypothalamus-pituitary-gonadal (HPG) axis and inducing meiotic arrest and germ cell apoptosis. Induction of triiodothyronine (T_3) coincided with the climax of testicular regression. Temporal gene-expression analysis over the course of apoptosis revealed suppression of LH response genes and activation of T_3 response genes involved in amphibian metamorphosis within the testis. Daily i.p. administration of T_3 mimicked the effects of low temperature stimulus on germ-cell apoptosis and testicular mass. We concluded that birds utilize low temperature-induced circulating T_3 not only for adaptive thermoregulation but also to trigger apoptosis in order to accelerate seasonal testicular regression.

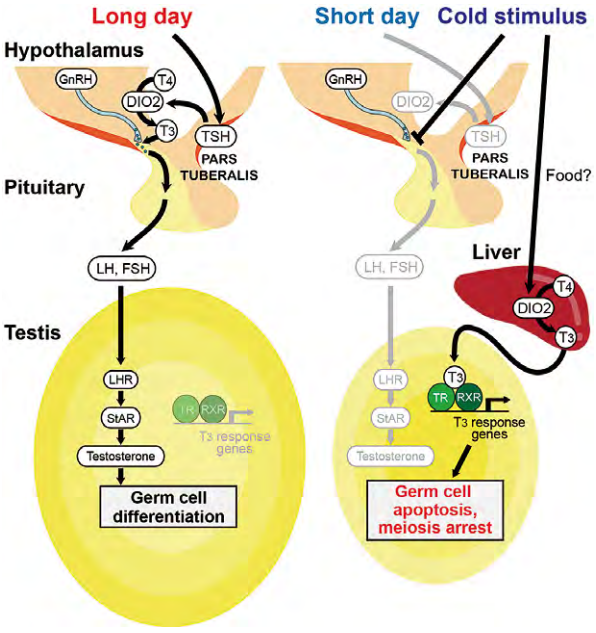

Figure 1. Mechanisms regulating seasonal testicular development and regression in birds.

II. Ontogeny of the saccus vasculosus, a seasonal sensor in fish

Thyroid-stimulating hormone (thyrotropin: TSH) secreted from the pars distalis (PD) of the pituitary gland stimulates the thyroid gland. In contrast, TSH secreted from the pars tuberalis (PT) of the pituitary gland regulates seasonal reproduction in birds and mammals. The ontogeny of thyrotrophs and the regulatory mechanisms of TSH are markedly different between the PD and PT. Interestingly, fish do not have an anatomically distinct PT, and the saccus vasculosus (SV) of fish is suggested to act as a seasonal sensor. Thus, it is possible that the SV is analogous to the PT. We examined the ontogeny of the pituitary gland and SV using rainbow trout. A histological analysis demonstrated the development of the pituitary anlage followed by that of the SV. Transcription factors *Lhx3* and *Pit-1*, which are required for the development of PD thyrotrophs, clearly labelled the pituitary anlage. The common glycoprotein α subunit (*CGA*) and TSH β subunit (*TSHB*) genes were also detected in the pituitary anlage. In contrast, none of these genes were detected in the SV anlage. We then performed a microarray analysis and identified *Parvalbumin* (*Pvalb*) as a marker for SV development. Because *Pvalb* expression was not detected in the pituitary anlage, no relationship was observed between the development of the SV and pituitary gland. In contrast to embryos, *Lhx3*, *Pit-1*, *CGA*, and *TSHB* were all expressed in the adult SV. These results suggest that the morphological differentiation of SV occurs during the embryonic stage, but that the functional differentiation into a seasonal sensor occurs in a later developmental stage.

III. Forward genetic analysis of seasonal time measurement

It is well established that the circadian clock is somehow involved in seasonal time measurement. However, it remains unknown how the circadian clock measures day length. Additionally, it is not known how animals adapt to seasonal changes in temperature. It has been reported that medaka populations that were caught at higher latitudes have more sophisticated responses to day length and temperature. For example, medaka fish caught in Hokkaido have a critical

day length (i.e., duration of photoperiod required to cause a response) of 13 h, while those caught in Okinawa have an 11.5 h critical day length. To uncover the underlying mechanism of seasonal time measurement, we are currently performing a forward genetic analysis in medaka populations collected from various latitudes all over Japan.

3-1 Variation in seasonal responses with latitude in medaka fish

To perform a forward genetic analysis, we have collected thousands of medaka fish from all over Japan. We have examined the effects of changing day length to determine the critical day lengths that will cause seasonal responses in the gonad and we found differences in critical day length between medaka from higher latitudes and lower latitudes (Figure 2).

3-2 Quantitative trait loci (QTL) analysis of critical day length

To identify the genes regulating critical day length, quantitative trait loci (QTL) analysis was conducted using more than 700 F2 medaka derived from crosses between Northern and Southern populations. As a result, we identified significant QTLs. We are now performing genome re-sequencing using various medaka strains that show different critical photoperiods.

IV. Transcriptome analysis of seasonality in medaka fish

Homeotherms such as birds and mammals do not show clear seasonal responses to changing temperature. In contrast, poikilothermal animals also use changing temperature as a calendar. Medaka provides an excellent model to uncover this mechanism. To elucidate the signal transduction pathway regulating seasonal reproduction in medaka fish, we have examined transcriptome analysis using microarrays. We identified hundreds of genes that respond to day length and temperature changes.

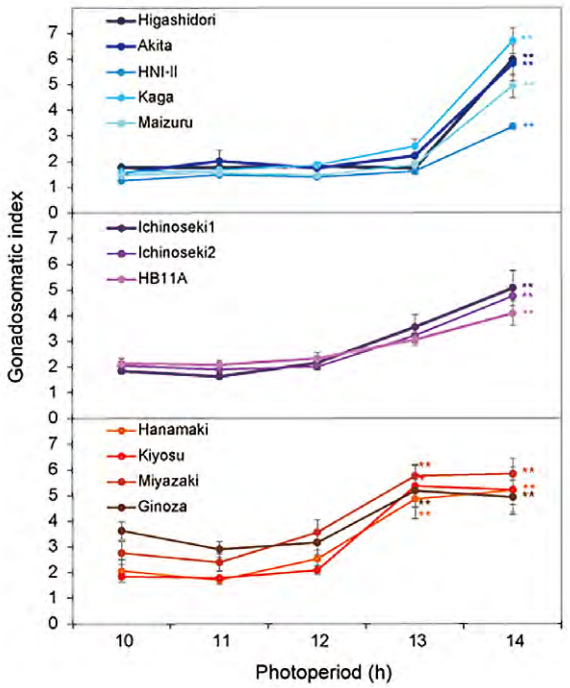

Figure 2. Different critical day length between medaka from higher latitudes and lower latitudes.

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- Ikegami, K., Atsumi, Y., Yorinaga, E., Ono, H., Murayama, I., Nakane, Y., Ota, W., Arai, N., Tega, A., Iigo, M., Darras, V.M., Tsutsui, K., Hayashi, Y., Yoshida, S., and Yoshimura, T. (2015). Low temperature-induced circulating triiodothyronine accelerates seasonal testicular regression. Endocrinology *156*, 647-659.
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LABORATORY OF GENOME INFORMATICS

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The accumulation of biological data has recently been accelerated by various high-throughput "omics" technologies including genomics, transcriptomics, and proteomics. The field of genome informatics is aimed at utilizing this data, or finding the principles behind the data, for understanding complex living systems by integrating the data with current biological knowledge using various computational techniques. In this laboratory we focus on developing computational methods and tools for comparative genome analysis, which is a useful approach for finding functional or evolutionary clues to interpreting the genomic information of various species. The current focus of our research is on the comparative analysis of microbial genomes, the number of which has been increasing rapidly. Since different types of information about biological functions and evolutionary processes can be extracted from comparisons of genomes at different evolutionary distances, we are developing methods to conduct comparative analyses not only of distantly related but also of closely related genomes.

I. Microbial genome database for comparative analysis

The microbial genome database for comparative analysis (MBGD; URL http://mbgd.genome.ad.jp/) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes using the DomClust program combined with the DomRefine program (see Section II below). By means of these programs, MBGD not only provides comprehensive orthologous groups among the latest genomic data, but also allows users to create their own ortholog groups on the fly using a specified set of organisms. MBGD also has pre-calculated ortholog tables for each major taxonomic group, and provides several views to display the entire picture of each ortholog table. For some closely related taxa, MBGD provides the conserved synteny information calculated using the CoreAligner program (see Section III below). In addition, MBGD provides MyMBGD mode, which allows users to add their own genomes to MBGD. Moreover, MBGD now stores recently accumulating draft genome data, and allows users to incorporate them into a user specific ortholog database through the MyMBGD functionality.

To cope with the explosive growth of microbial genome data owing to next generation sequencing technology, we need to improve the database construction procedure continuously. Since recently tens or even hundreds of genome sequences of the same species are available for many bacteria, we are trying to establish an efficient protocol to maintain all-against-all similarity data by separating intra-species comparisons and inter-species comparisons.

II. Orthologous gene classification among multiple genomes at the domain level

As a core technology of our comparative genomics tools, we developed a rapid automated method of ortholog grouping, named DomClust, which is effective enough to allow the comparison of many genomes simultaneously. The method classifies genes based on a hierarchical clustering algorithm using all-against-all similarity data as an input, but it also detects domain fusion or fission events and splits clusters into domains if required. As a result, the procedure can split genes into the domains minimally required for ortholog grouping.

Although DomClust can rapidly construct orthologous groups at the domain level, its classification quality has room for improvement since it is based only on pairwise sequence alignment. We developed a procedure to refine the DomClust classification based on multiple sequence alignments. The method is based on a scoring scheme, the domain-specific sum-of-pairs (DSP) score, which evaluates domain-level classification using the sum total of domain-level alignment scores. We developed a refinement pipeline to improve domain-level clustering, DomRefine, by optimizing the DSP score. DomRefine is now used to construct the standard ortholog table covering all the representative genomes stored in MBGD.

Domain-level classification is a unique feature of our ortholog classification system in MBGD. Now, we are analyzing the database and trying to characterize domain fusion events that occurred during prokaryotic and eukaryotic evolution.

III. Identification of the core structure conserved among taxonomically related microbial genomes

Horizontal gene transfers (HGT) have played a significant role in prokaryotic genome evolution, and the genes constituting a prokaryotic genome appear to be divided into two classes: core and accessory. The core gene set comprises intrinsic genes encoding the proteins of basic cellular functions, whereas the accessory gene set comprises HGT-acquired genes encoding proteins which function under particular conditions. We consider the core structure of related genomes as a set of sufficiently long segments in which gene orders are conserved among multiple genomes so that they are likely to have been inherited mainly through vertical transfer, and developed a method named CoreAligner to find such structures. We systematically applied the method to various bacterial taxa to define their core gene sets.

IV. Development of a workbench for comparative genomics

We have developed a comparative genomics workbench named RECOG (Research Environment for COmparative Genomics), which aims to extend the current MBGD system by incorporating more advanced analysis functionalities. The central function of RECOG is to display and manipulate a large-scale ortholog table. The ortholog table viewer is a spreadsheet like viewer that can display the entire ortholog table, containing more than a thousand genomes. In RECOG,

several basic operations on the ortholog table are defined, which are classified into categories such as filtering, sorting, and coloring, and various comparative analyses can be done by combining these basic operations. In addition, RECOG allows the user to input arbitrary gene properties and compare these properties among orthologs in various genomes.

We continue to develop the system and apply it to various genome comparison studies under collaborative research projects. In particular, we are trying to apply the RECOG system to comparative analyses of transcriptomic data and even metagenomic data.

V. Ortholog data representation using the Semantic Web technology to integrate various microbial databases

Orthology is a key to integrate knowledge about various organisms through comparative analysis. We have constructed an ortholog database using Semantic Web technology, aiming at the integration of numerous genomic data and various types of biological information. To formalize the structure of the ortholog information in the Semantic Web, we have constructed the Ortholog Ontology (OrthO). On the basis of OrthO, we described the ortholog information from MBGD in the form of Resource Description Framework (RDF) and made it available through the SPARQL endpoint. On the basis of this framework, we are trying to integrate various kinds of microbial data using the ortholog information as a hub, as part of the MicrobeDB.jp project developed under the National Bioscience Database Center.

In addition, to facilitate the utilization of the RDF databases distributed worldwide, we developed a command-line tool, named SPANG, that simplifies querying distributed RDF stores using the SPARQL query language.

VI. Characterization of the gene repertoire of *H. pylori* pan-genome

Genomes of bacterial species can show great variation in their gene content, and thus systematic analysis of the entire gene repertoire, termed the "pan-genome", is important for understanding bacterial intra-species diversity. We analyzed the pan-genome identified among 30 strains of the human gastric pathogen *Helicobacter pylori* isolated from various phylogeographical groups. For this purpose, we developed a method (FindMobile) to define mobility of genes against the reference coordinate determined by the core alignment created by CoreAligner, and classified each non-core gene into mobility classes. We also identified co-occurring gene clusters using phylogenetic pattern clustering combined with neighboring gene clustering implemented in the RECOG system (Figure 1). On the basis of these analyses, we identified several gene clusters conserved among *H. pylori* strains that were characterized as mobile or non-mobile. This work is in collaboration with Prof. Kobayashi, Univ. Tokyo.

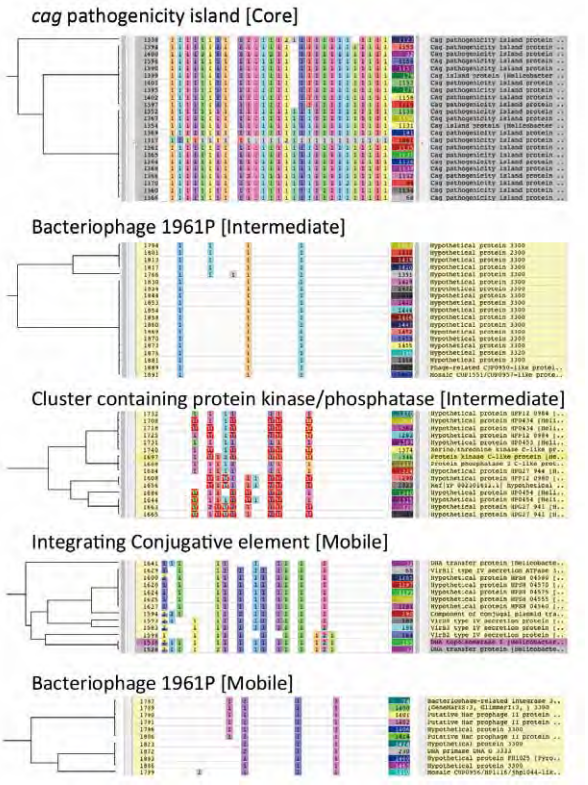


Figure 1. The five largest co-occurring gene clusters identified among 30 strains of *H. pylori* using the RECOG system

Publication List:

〔Original papers〕

- Abe, R., Toyota, K., Miyakawa, H., Watanabe, H., Oka, T., Miyagawa, S., Nishide, H., Uchiyama, I., Tollefsen, K.E., Iguchi, T., and Tatarazako, N. (2015). Diofenolan induces male offspring production through binding to the juvenile hormone receptor in *Daphnia magna*. Aquat. Toxicol. *159*, 44-61.
- Chiba, H., Nishide, H., and Uchiyama, I. (2015). Construction of an ortholog database using the Semantic Web technology for integrative analysis of genomic data. PLoS One *10*, e0122802.
- Fernández-Breis, J.T., Legaz-García, M.C., Chiba, H., and Uchiyama, I. (2015). Towards the semantic standardization of orthology content. Proc. Semant. Web Appl. Tool. Life Sci. 74-83.
- Hayashi, S., Kawaguchi, A., Uchiyama, I., Kawasumi-Kita, A., Kobayashi, T., Nishide, H., Tsutsumi, R., Tsuru, K., Inoue, T., Ogino, H., Agata, K., Tamura, K., and Yokoyama, H. (2015). Epigenetic modification maintains intrinsic limb-cell identity in *Xenopus* limb bud regeneration. Dev. Biol. *406,* 271-282.
- Kawai, M., Uchiyama, I., Takami, H., and Inagaki, F. (2015). Low frequency of endospore-specific genes in subseafloor sedimentary metagenomes. Environ. Microbiol. Rep. *7*, 341-350.
- Takami, H., Arai, W., Takemoto, K., Uchiyama, I., and Taniguchi, T. (2015). Functional classification of uncultured "*Candidatus* Caldiarchaeum subterraneum" using the Maple system. PLoS One *10*, e0132994.
- Uchiyama, I., Mihara, M., Nishide, H., and Chiba, H. (2015). MBGD update 2015: microbial genome database for flexible ortholog analysis utilizing a diverse set of genomic data. Nucleic Acids Res. *43*, D270-D276.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

NIBB Research Fellow: *TANIGUCHI, Atsushi*
Technical Assistant: *ISHIBASHI, Tomoko*

Our laboratory is currently pursuing two paths of scientific inquiry that are of interest to developmental biology. One goal is to clarify the mechanism of left-right (L-R) determination. The other is to develop imaging technologies for biological analyses.

I. Initial step for left-right asymmetry

In mammalian development, the initial left-right asymmetry first appears as cilia-driven leftward fluid flow on the ventral surface of the node, and the flow provides the initial cue for asymmetric gene expressions that actually determine asymmetric morphogenesis. The mechanisms that convert the flow to asymmetric gene expression, i.e. the flow sensing mechanism, remains controversial, with several models being proposed, and involvement of Ca^{2+} being suggested.

We pursued this question by measuring Ca^{2+} dynamics in the node and found that the node cells cause apparently stochastic elevation of Ca^{2+}, and its spatiotemporal distribution is equal at the left and right sides but becomes more frequent at the left after late headfold stage, when the flow-induced commitment occurs. This asymmetry was disrupted in dynein mutant *iv*/*iv* and *pkd2*$^{-/-}$ mutants, in accordance to their left-right phenotypes.

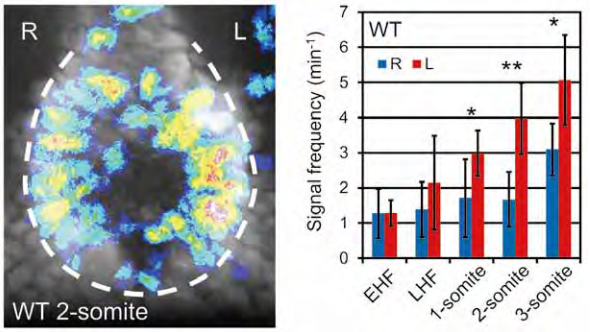


Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

Based on these findings, we are now examining the validity of the two major proposed models of flow sensing, mechanosensing and chemosensing, and a third novel mechanism.

II. Development of light-sheet microscopy

Light-sheet microscopy has many advantages for live imaging including low photobleaching and phototoxicity, high penetration depth, and fast image acquisition. This method also has peculiar disadvantages, however. Specifically scattering of excitation light within the specimen leading to illumination of areas besides the focal plane, and deterioration of contrast. A solution to these problems is combining Light-sheet microscopy with two-photon excitation (TPE), but this results in a narrow field of view, because generation of TPE images requires very high photon density, i.e. focusing with a high numerical aperture (NA) lens.

We utilized a new fiber laser with high peak power to overcome this problem, and enabled observation of larger specimens using a hybrid TPE light-sheet microscope.

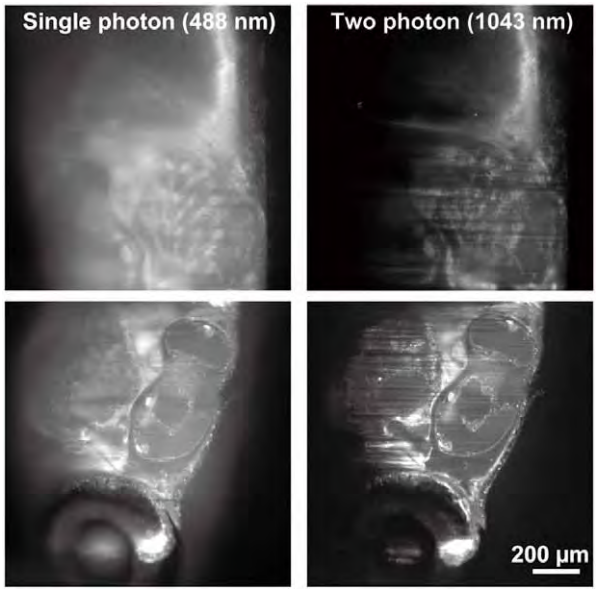


Figure 2. Two-photon light-sheet microscopy. Left: Fluorescent images of a medaka taken by a conventional (one-photon) light-sheet microscope. Right: Images of the same area taken by our two-photon light-sheet microscope.

Publication List:

〔Original paper〕

● Nakai, Y., Ozeki, M., Hiraiwa, T., Tanimoto, R., Funahashi, A., Hiroi, N., Taniguchi, A., Nonaka, S., Boilot, V., Shrestha, R., Clark, J., Tamura, N., Draviam, V.M., and Oku, H. (2015). High-speed microscopy with an electrically tunable lens to image the dynamics of in vivo molecular complexes. Rev. Sci. Instrum. *86*, 013707.

LABORATORY OF NUCLEAR DYNAMICS

Specially Appointed Associate Professor
MIYANARI, Yusuke

Postdoctoral Fellow:	*KURIHARA, Misuzu*
SOKENDAI Graduate Student:	*ISHII, Satoko*
Visiting Graduate Studen:	*KAKIZUKA, Taishi*
Technical Assistant:	*SANBO, Chiaki*
	TAGAWA, Ayako
Secretary:	*HACHISUKA, Midori*

A fundamental question in biology is to understand the mechanisms underlying cell-fate decision. Genomic reprogramming after mammalian fertilization reverts terminally differentiated gametes into toti- or pluri-potent states to start a new developmental program. Cell lineage allocation in the reprogramming process is accompanied by drastic changes in the pattern of gene expression, epigenetic configurations, and nuclear organization. We aim to reveal the roles of chromatin dynamics in cell lineage-allocation by deciphering the molecular mechanisms underlying remodeling of nuclear organization and their effects on developmental gene expression, using mouse embryos and embryonic stem (ES) cells as model systems.

Epigenetic reprogramming in early mouse embryos.

Genomic reprogramming reverts fully differentiated cells to a totipotent state to start a new developmental program. In the early mouse embryo, terminally differentiated gametes are reprogrammed after fertilization thereby acquiring a totipotent state. Upon the fourth cleavage, 8-cell stage embryos, which have undergone the process of compaction, will give rise to the morula (Figure 1). The outer cells of the morula will differentiate into the epithelial trophectoderm (TE) of the blastocyst. The inner cells of the morula will become the inner cell mass (ICM) of the blastocyst. The ICM subsequently leads to the formation of two lineages, epiblast (EPI) and primitive endoderm (PE), with the former representing pluripotency as it gives rise to the embryo itself. The reprogramming event is accompanied by epigenetic modifications and changes in chromatin structures throughout the embryo, which are essential for regulation of gene expression involved in differentiation, and reprogramming of the EPI.

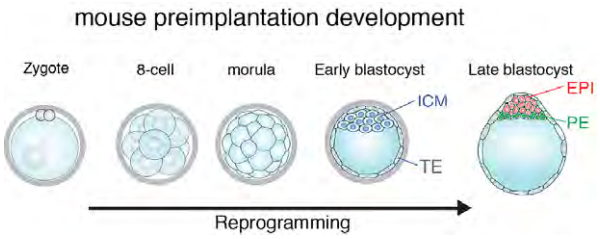


Figure 1. Lineage allocation in mouse preimplantation development

Remodeling of nuclear architecture in development

Chromatin is organized in a non-random fashion within three-dimensional nuclear space. During developmental processes, nuclear architecture is dramatically reconstructed, resulting in establishment of cell-type specific nuclear organization. Defects in structural components of the nucleus are responsible for developmental aberrations and several human diseases. Remodeling of nuclear architecture leads to spatial arrangement of genes, which could affect genome functions including gene expression. However, regulatory mechanisms underlying nuclear reorganization during cell-fate decision remains largely unknown.

Chromatin structure

Spatiotemporal organization of genomic DNA within the nucleus is suggested as an emerging key player to regulate gene expression. The developmental program accompanies nuclear remodeling, resulting in construction of cell-type specific nuclear architecture. Firstly, chromosomes are confined in discrete nuclear spaces, "chromosome territories" (Figure 2). Within them, further levels of 3D organization, "topologically associating domains" (TADs), are observed. TADs can be defined as linear units of chromatin containing several gene loci, and fold as discrete 3D structures in which gene loci frequently interact with each other. Recent works have revealed that folding of "local" chromatin structures such as enhancer-promoter looping is associated with genome functions. Despite the drastic changes of these hierarchical chromatin structures, their role in cell-fate decision remains largely unexplored.

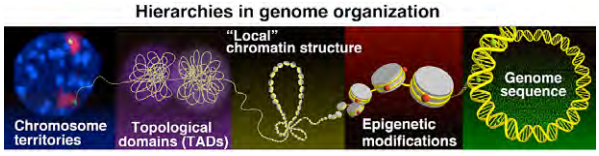


Figure 2. Hierarchical chromatin structure

Approach

We have developed a powerful imaging technology termed TALE-mediated Genome Visualization (TGV), which allows us to track specific genomic sequences in living cells (Miyanari Y, Nature Structural & Molecular Biology, 2013). Importantly, this technique is versatile and can be extended to allow many robust applications, which will be integrated into our study to manipulate several genome functions. Based on new technological development, we aim to understand biological roles of chromatin dynamics in cell-fate decision.

LABORATORY OF PLANT DEVELOPMENT AND PHYSIOLOGY

Specially Appointed Associate Professor
KAWADE, Kensuke

Adjunct Professor (BIO-NEXT Project, OIIB): *TSUKAYA, Hirokazu*
Technical Assistant: *YAMAGUCHI, Chinami*
Secretary: *HACHISUKA, Midori*

There has been growing evidence that metabolic regulation has specific impacts on plant development. The picture emerging depicts the metabolism as a dynamic system that controls and/or supports developmental progression. Despite these advances, the metabolic regulation behind developmental process remains largely unclear. We aim to uncover as-yet-unknown relationships between developmental and metabolic processes in plants and their biological meaning by elucidating molecular mechanisms for the system. To address this, we primary use molecular genetics and metabolomics approaches using *Arabidopsis thaliana* as a model, in conjunction with standard molecular biology and biochemistry techniques.

I. Exploring as-yet-unknown relationships between development and metabolism

To explore as-yet-unknown relationships between developmental and metabolic processes, we carried out phenome screening of *Arabidopsis thaliana* enzyme mutants. We examined more than 11 traits including leaf size, primary root length, seed color, *etc*. A large number of mutants grew normally compared to wild type, probably due to gene functional redundancy. However, we found that one mutant had a shorter primary root than wild type. Further detailed morphological analysis revealed that this could be attributed to a delay of germination and seedling establishment. Irregular patterning of the cotyledon is also observed in the mutant (Figure 1) although the penetrance was low. Based on these observations, together with the expression profile, we now hypothesize that the enzyme works during seed development to control seed quality, and presumably embryo patterning. To know the *in vivo* substrate of this enzyme and which metabolic pathway is involved in this developmental process, metabolomics analysis will be conducted.

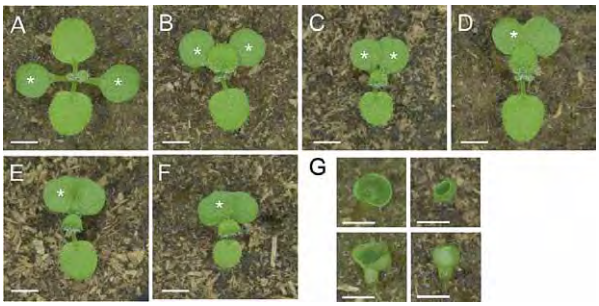


Figure 1. Irregular patterning of cotyledons in the enzyme mutant. Wild type has two symmetrically-arranged cotyledons (A). In contrast, our enzyme mutant shows asymmetrically-arranged (B and C), fused (D-E) and cup-shaped cotyledons (G). Asterisks indicate cotyledons. Bar = 2 mm.

II. Characterizing an interaction between development and metabolism

In addition to the phenome screening using enzyme mutants, as another topic, we are now examining coordinated regulation of plant growth and amino acid metabolism. We previously found that one of key factor for plant development regulates the expression of amino acid metabolism-related genes. Moreover, we uncovered that the mutant for this gene shows hypersensitivity specifically to leucine. Growth arrest is observed when this mutant is grown on culture media containing higher concentrations of leucine. Other amino acids such as histidine did not inhibit the mutant's growth, indicating that there is a specific interaction between the responsible gene and leucine metabolism. To directly know whether the gene indeed regulates leucine metabolism, we have established an amino acid profiling system with high sensitivity and accuracy using liquid chromatography-mass spectrometry (LC-MS) in collaboration with the Functional Genomics Facility in NIBB, and are now examining the amino acid prolife in the mutant. Although the gene is well studied as a key component for plant development, our data shed light on a novel aspect of the gene's function, which could be a key to deciphering how amino acid metabolism is interwired with vigorous plant growth.

Publication List:

〔Original paper〕

● Nakayama, H., Kawade, K., Tsukaya, H., and Kimura, S. (2015). Detection of the cell proliferation zone in leaves by using EdU. Bio-protocol *5*, 18.

〔Review articles〕

● Hisanaga, T., Kawade, K., and Tsukaya, H. (2015). Compensation: a key to clarifying the organ-level regulation of lateral organ size in plants. J. Exp. Botany *66*, 1055-1063.
● Kawade, K., and Tanimoto, H. (2015). Mobility of signaling molecules: The key to deciphering plant organogenesis. J. Plant Res. *128*, 17-25.

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 is 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 studies that utilize mass spectrometers and DNA sequencers. We also act as a bridge between experimental biology and bioinformatics.

We recently largely renovated the building of the Functional Genomics Facility. For example, the Visitors Lab and the Visitors Office were newly designed so that we can promote collaboration projects. Indeed, in 2015, more than 200 researchers visited to use our new facility and developed active collaborations.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing HiSeq2500, HiSeq1500, and MiSeq (Illumina), and PacBio RS II (PacificBio Sciences), the Functional Genomics Facility is committed to joint research aiming to explore otherwise inaccessible new fields in basic biology.

Figure 1. Next-generation sequencer

During 2015 we carried out 44 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.

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2015, we analyzed approximately 620 samples with mass spectrometers and 14 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 (excerpts)

- 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. Triple TOF LC/MS/MS 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 on Cooperation:

〔Original papers〕

- Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., Baumann, A., and Blenau, W. (2015). Cockroach GABAB receptor subtypes: molecular characterization, pharmacological properties and tissue distribution. Neuropharmacology *88*, 134–144.
- Bourguignon, T., Lo, N., Cameron, S.L., Sobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T., and Evans, T.A. (2015). The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. *32*, 406–421.
- Habu, Y., Ando, T., Ito, S., Nagaki, K., Kishimoto, N., Shigenobu, S., *et al*. (2015). Epigenomic modification in rice controls meiotic recombination and segregation distortion. Mol. Breeding *35*, 103.
- Hojo, M.K., Ishii, K., Sakura, M., Yamaguchi, K., Shigenobu, S., and Ozaki, M. (2015). Antennal RNA-sequencing analysis reveals evolutionary aspects of chemosensory proteins in the carpenter ant, *Camponotus japonicus*. Sci. Rep. *5*, 13541.
- Kinoshita, A., ten Hove, C.A., Tabata, R., Yamada, M., Shimizu, N., Ishida, T., Yamaguchi, K., Shigenobu, S., Takebayashi, Y., Iuchi, S., *et al*. (2015). A plant U-box protein, PUB4, regulates asymmetric cell division and cell proliferation in the root meristem. Development *142*, 444–453.
- Numa, H., Yamaguchi, K., Shigenobu, S., and Habu, Y. (2015). Gene body CG and CHG methylation and suppression of centromeric CHH methylation are mediated by DECREASE IN DNA METHYLATION1 in Rice. Mol. Plant *8*, 1560-1562.
- Shikanai, Y., Yamagami, M., Shigenobu, S., Yamaguchi, K., Kamiya, T., and Fujiwara, T. (2015). *Arabidopsis thaliana* PRL1 is involved in low-calcium tolerance. Soil Sci. Plant Nutr. *61*, 951-956.
- Shimizu, N., Ishida, T., Yamada, M., Shigenobu, S., Tabata, R., Kinoshita, A., Yamaguchi, K., Hasebe, M., Mitsumasu, K., and Sawa, S. (2015). BAM 1 and RECEPTOR-LIKE PROTEIN KINASE 2 constitute a signaling pathway and modulate CLE peptide-triggered growth inhibition in *Arabidopsis* root. New Phytol. *208*, 1104-1113.
- Toyokura, K., Yamaguchi, K., Shigenobu, S., Fukaki, H., Tatematsu, K., and Okada, K. (2015). Mutations in plastidial 5-aminolevulinic acid biosynthesis genes suppress pleiotropic defect in shoot development of mitochondrial GABA shunt mutant in Arabidopsis. Plant Cell Physiol. *56*, 1229-1238.
- Toyota, K., Miyakawa, H., Yamaguchi, K., Shigenobu, S., Ogino, Y., Tatarazako, N., Miyagawa, S., and Iguchi, T. (2015). NMDA receptor activation upstream of methyl farnesoate signaling for short day-induced male offspring production in the water flea, *Daphnia pulex*. BMC Genomics *16*, 186.
- Wakae, K., Aoyama, S., Wang, Z., Kitamura, K., Liu, G., Monjurul, A.M., Koura, M., Imayasu, M., Sakamoto, N., Nakamura, M., Shigenobu, S., *et al*. (2015). Detection of hypermutated human papillomavirus type 16 genome by next-generation sequencing. Virology *485*, 460–466.
- Wu, T., Kamiya, T., Yumoto, H., Sotta, N., Katsushi, Y., Shigenobu, S., Matsubayashi, Y., and Fujiwara, T. (2015). An *Arabidopsis thaliana* copper-sensitive mutant suggests a role of phytosulfokine in ethylene production. J. Exp. Bot. *66*, 3657-3667.

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

- Sato, K., Tanaka, T., Shigenobu, S., Motoi, Y., Wu, J., and Itoh, T. Improvement of barley genome annotations by deciphering the Haruna Nijo genome. DNA Res. 2015 Nov 29.

● *Research activity by S. Shigenobu*

Specially Appointed Associate Professor:	*SHIGENOBU, Shuji*
NIBB Research Fellow:	*MAEDA, Taro*
	OGAWA, Kota
Postdoctoral Fellow:	*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.

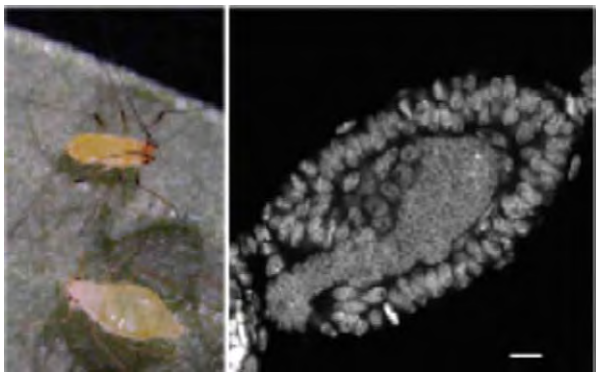


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

Publication List:

〔Original papers〕

- Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., Baumann, A., and Blenau, W. (2015). Cockroach GABAB receptor subtypes: molecular characterization, pharmacological properties and tissue distribution. Neuropharmacol. *88*, 134–144.
- Bourguignon, T., Lo, N., Cameron, S.L., Sobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T., and Evans, T.A. (2015). The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. *32*, 406–421.
- Hojo, M.K., Ishii, K., Sakura, M., Yamaguchi, K., Shigenobu, S., and Ozaki, M. (2015). Antennal RNA-sequencing analysis reveals evolutionary aspects of chemosensory proteins in the carpenter ant, *Camponotus japonicus*. Sci. Rep. *5*, 13541.

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). 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

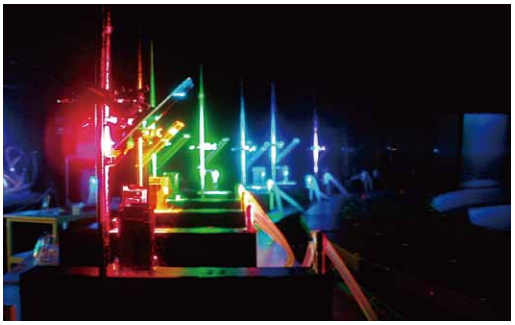


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

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 wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Olympus FV1000, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD camera), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced custom-made laser microscopes with 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 in 2010. In addition, transmission electron microscope service for plant biology has started from 2014.

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 a specimen 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 8 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 10 Individual Collaborative Research projects, including applications for animals and plants.

Workshop and Symposium

In 2015 we held courses on both medaka basic training, which focused on basic techniques for medaka research including imaging, and the 3nd biological image processing training course. 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 9th NIBB Bioimaging Forum focused on physical properties on biology including heat (temperature) and force. In addition, we held symposiums focused on new emerging model animals and amphibians.

Publication List on Cooperation

〔Original papers (Selected)〕

- Kawasumi-Kita, A., Hayashi, T., Kobayashi, T., Nagayama, C., Hayashi, S., Kamei, Y., Morishita, Y., Takeuchi, T., Tamura, K., and Yokoyama, H. (2015). Application of local gene induction by infrared laser-mediated microscope and temperature stimulator to amphibian regeneration study. Dev. Growth Differ. *57*, 601-613.
- Kuboyama, K., Fujikawa, A., Suzuki, R., and Noda, M. (2015). Inactivation of protein tyrosine phosphatase receptor type Z by pleiotrophin promotes remyelination through activation of differentiation of oligodendrocyte precursor cells. J Neurosci. *35*, 12162-12171.
- Nakashima, M., Suzuki, M., Saida, M., Kamei, Y., Hossain, M.B., and Tokumoto, T. (2015). Cell-based assay of nongenomic actions of progestins revealed inhibitory G protein coupling to membrane progestin receptor α (mPRα). Steroids *100*, 21–26.
- Oikawa, K., Matsunaga, S., Shoji Mano, S., Kondo, M., Yamada, K., Hayashi, M., Kagawa, T., Kadota, A., Sakamoto, W., Higashi, S., Watanabe, M., Mitsui, T., Shigemasa, A., Iino, T., Hosokawa, Y., and Nishimura, M. (2015). Physical interaction between peroxisomes and chloroplasts elucidated by in situ laser analysis. Nat. Plants *1*, 15035.
- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2015). Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in Lotus japonicus. Plant Physiol. *167*, 545-557.

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

- Kagawa, N., Honda, A., Zenno, A., Omoto, R., Imanaka, S., Takehana, Y., and Naruse, K. Arginine vasotocin neuronal development and its projection in the adult brain of the medaka. Neurosci. Let. 2015 Dec. 29.

● *Research activity by Y. Kamei*

Specially Appointed Associate Professor: *KAMEI, Yasuhiro*
NIBB Research Fellow: *HATTORI, Masayuki*
Technical Assistant: *CHISADA, Eriko*

Our research group promotes two cutting-edge microscope projects; "observation" and "manipulation" using optical and biological technologies. The aim of our "observation project" is deep-seeing in 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 telescopes on the earth may be disturbed by fluctuations in the atmosphere, AO technology can cancel this disturbance. On the other hand, living materials have particular refractive indexes, therefore, some organelles act as disturbances of the ideal optical path for microscope observation just like the atmosphere does for 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 observation of living organisms in collaboration with Dr. Tamada in NIBB and Dr. Hayano in the National Astronomical Observatory of Japan (NAOJ) and got high-resolution bright field and

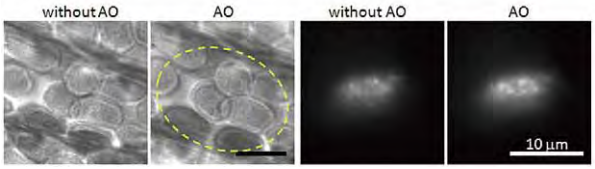


Figure 1. Effects of adaptive optics (AO) to wide-field microscope images (bright field and fluorescence of plant cells).

fluorescent images of living cells. Our results indicated that improvement of optical resolution was restricted to a small area which is called the “isoplanatic patch” (Figure 1).

Second, 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 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), specialized for this purpose (Figure 2). 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 the heat shock response at a desired timing. In 2015, additionally, we confirmed the system was effective in the moss *Marchantia polymorpha* and in the newt *Pleurodeles waltl*.

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. We evaluated time course and spatial heating profiles, and the results presented that temperature of the target area rose rapidly and kept a constant level dependant on IR laser power, additionally, the heated area was adequately as small as a typical cell size.

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 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 the species as expected.

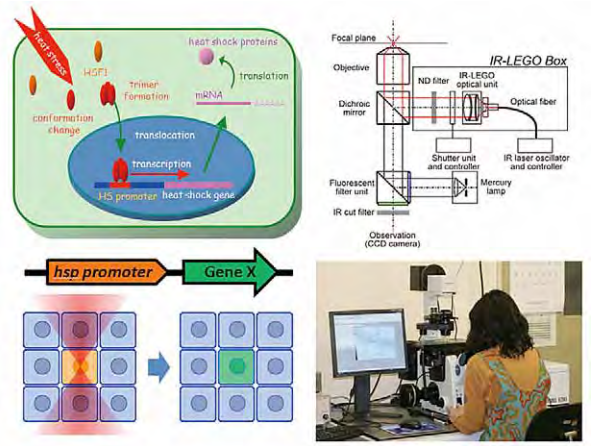


Figure 2. Schematic illustration of local gene induction system and an infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

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 tool for these studies in combination with molecular biological techniques, such as the cre-loxP system. Dr. Shimada in the University of Tokyo wanted to confirm the cell lineage of exo-skeletal tissue such as the scales of medaka fish. She questioned the traditional belief concerning the origin of the exo-skeleton of the body-trunk using transplantation studies. We then started a collaboration to establish a local permanent labeling system in medaka and to make clear the origin of exo-skeletal cells. The system was well working (Figure 3), and the fate tracking results indicated that exo-skeletal tissues were mesodermal in origin, not from neural crest cells, as previously believed (Shimada et al, Nat. Commun, 2013).

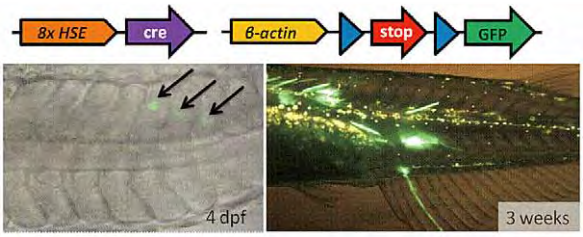


Figure 3. Examples of Cre-loxP mediated long-term GFP marking using IR-LEGO in living medaka individuals for cell linage tracing.

Publication List:

〔Original papers〕

- Kawasumi-Kita, A., Hayashi, T., Kobayashi, T., Nagayama, C., Hayashi, S., Kamei, Y., Morishita, Y., Takeuchi, T., Tamura, K., and Yokoyama, H. (2015). Application of local gene induction by infrared laser-mediated microscope and temperature stimulator to amphibian regeneration study. Dev. Growth Differ. *57*, 601-613.
- Nakashima, M., Suzuki, M., Saida, M., Kamei, Y., Hossain, B., and Tokumoto, T. (2015). Cell-based assay of nongenomic actions of progestins revealed inhibitory G protein coupling to membrane progestin receptor α (mPRα). Steroids *100*, 21-26.
- Yokoi, S., Okuyama, T., Kamei, Y., Naruse, K., Taniguchi, Y., Ansai, S., Kinoshita, M., Young, L. J., Takemori, N., Kubo, T., and Takeuchi, H. (2015). An essential role of the arginine vasotocin system in mate-guarding behaviors in triadic relationships of medaka fish (*Oryzias latipes*). PLoS Genetics *11*, e1005009.

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

- Nishihama, R., Ishida, S., Urawa, H., Kamei, Y., and Kohchi, T. Conditional gene expression/deletion systems for Marchantia polymorpha using its own heat-shock promoter and the cre/loxP-mediated site-specific recombination. Plant Cell Physiol. 2015 Jul. 6.

Data Integration and Analysis Facility

Assistant Professor: *UCHIYAMA, Ikuo*
Technical Staff: *MIWA, Tomoki*
NISHIDE, Hiroyo
NAKAMURA, Takanori
Technical Assistant: *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 as well as providing users' basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the network systems 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 high-performance cluster system (SGI Rackable server C2112-4RP; 40 nodes/800 cores, 96GB memory/node), a shared memory parallel computer (HP ProLiant DL980 G7; 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700; 480TB), 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 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 and collaborative researchers. 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/en).

Figure 1. Biological Information Analysis System

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. 66).

NIBB BioResource Center

Head
FUJIMORI, Toshihiko

To understand the mechanisms of living organisms, studies focusing on each gene in the design of life (the genome) are required. The use of model animals and plants, such as mice, Medaka (*Oryzias latipes*), zebrafish, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, make it possible to produce genetically controlled organisms with markers placed using genetic and cell engineering technology. Such marking allows detailed studies of genes and cell functions. The model organisms mature in a short period of time; therefore, changes in cells, organs, and individuals can be totally and efficiently observed. The NIBB BioResource Center has equipment, facilities, and staff to maintain such organisms safely, efficiently, and appropriately.

Model Animal Research Facility

Associate Professor:	*WATANABE, Eiji*
	TANAKA, Minoru
	NARUSE, Kiyoshi
Technical Staff:	*HAYASHI, Kohji*
	NOGUCHI, Yuji
Technical Assistant:	*TAKAGI, Yukari*
	SUZUKI, Kohta
	SUGINAGA, Tomomi
	FUJIMOTO, Daiji
	MATSUMURA, Kunihiro
	ATSUMI, Miho
	GODA, Misato

Figure 1. Mouse (B6C3F1)

The worldwide genome project has almost been completed and research on basic biology has arrived at a post-genome era in which researchers are focusing on investigating the functions of individual genes. To promote the functional analysis of a gene of interest it is essential to utilize genetically altered model organisms generated using genetic engineering technology, including gene deletion, gene replacement and point mutation.

The NIBB Center for Transgenic Animals and Plants was established in April 1998 to support research using transgenic and gene targeting techniques at NIBB. The NIBB Center for Transgenic Animals and Plants was integrated into the NIBB BioResource center in April 2010, and was renamed "The Model Animal Research Facility".

Technical and supporting staff develop and promote research-supporting activities. A state-of-the-art facility for transgenic animals opened at the end of 2003 in the Yamate area.

The activities of the model animal research facility are as follows:

1. The provision of information, materials, techniques, and animal housing space to researchers.
2. The use of various kinds of instruments to analyze mutant, transgenic, and gene-targeted animals.
3. The development of novel techniques related to transgenic and gene targeting technology.
4. Cryopreservation and storage of transgenic strains.

I. Research support activities (mouse)

In 2001, the NIBB mouse facility (built under specific pathogen free (SPF) conditions) opened in the Myodaiji area and the production, breeding, analysis, cryopreservation and storage of genetically manipulated mouse strains has been conducted there since. The new center facility building in the Yamate area strengthened research activities using genetically altered organisms. The building has five floors and a total floor space of 2,500 m^2 in which we can generate, breed, store and analyze transgenic, gene targeting, and mutant mice under SPF conditions. The mouse housing area was constructed based on a barrier system. This building is also equipped with breeding areas for transgenic small fish, birds, and insects.

In 2015 (from January 1 to December 31), 10,209 fertilized

Figure 2. Equipment for manipulating mice eggs.

eggs (*in vitro* fertilization; 3,585 eggs of 32 lines in which 2,552 eggs of 30 lines were frozen for long-term storage, frozen eggs: 6,624 of 27 lines) and 4,301 mice were brought into the facility in the Yamate area, and 61,456 mice (including pups bred in the facility) were taken out.

A number of strains of genetically altered mice from outside the facility were brought into the mouse housing area by microbiological cleaning using *in vitro* fertilization-embryo transfer techniques, and stored using cryopreservation.

A new mouse facility in the Myodaiji area opened at the beginning of 2005. The facility provides research-supporting activities to researchers in the Myodaiji area. In March 2009, we expanded the facility which includes areas for breeding, behavioral tests, and transgenic studies using various kinds of recombinant viruses. In 2015 (from January 1 to December 31), 71 mice were brought into the facility in the Myodaiji area, and 3,362 mice (including pups bred in the facility) were taken out.

Figure 3. Large sized autoclave in the Myodaiji area.

II. Research support activities (small fish and birds)

The first floor of the center facility building in the Yamate area provides space and facilities to maintain small fish and chick embryos. In the laboratory room for chick embryos, a large incubation chamber is equipped and set at 42 degrees (suitable for chick embryogenesis). The researchers can manipulate chick embryos under optimal conditions, removing biohazard risks. For researchers who employ fish as an experimental model, 480 tanks (1 liter) and 450 tanks (3 liters) are available for medaka and zebrafish, respectively. Water circulates and can be maintained to suit the conditions desired for fish breeding in the aquarium systems. Currently, over three mutant lines and over fifteen transgenic lines of medaka and zebrafish are maintained in our facility. A medaka line that allows gene induction by heat treatment, in combination with a cre/loxP system, has been developed in this facility. In addition to the rooms mentioned above, a room for insects is also available. All the rooms are qualified to meet the criteria for transgenic animals, allowing researchers to generate and maintain these important biological tools.

In 2015 (from January 1 to December 31), 5,812 medaka and zebrafish adults and 250 fertilized eggs (including embryos) were brought to the facility and 121,027 medaka and zebrafish (117,021 fertilized eggs and 4,006 adults, including animals bred in the facility) were taken out. In the laboratory for chick embryos there were no fertilized eggs or chicken embryos brought in or taken out this year. These animals were used for research activities in neurobiology and developmental biology.

In 2007 NIBB was approved as a core facility of the National BioResource Project (NBRP) for Medaka by the Japanese Government. We have supported the activities of NBRP Medaka by providing standard strains, mutants, transgenic lines and organizing international practical courses for medaka. In 2014 we began providing the CRISPR/Cas9 genome editing platform as well as the TILLING library screening system to promote the reverse genetic approach. In 2015 we shipped 220 independent medaka strains, 155 cDNA/BAC/Fosmid clones, and 195 samples of hatching enzyme to the scientific community worldwide.

Figure 4. Quarantine room for medaka and zebrafish.

III. Research activities

The associate professors of this center - E. Watanabe, T. Naruse, and M. Tanaka - are the principal investigators of the Laboratory of Neurophysiology, the Laboratory of Bioresources, and the Laboratory of Molecular Genetics for Reproduction, respectively. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. The Laboratory of Bioresources has conducted a genetic and genomic analysis of quantitative traits and Mendelian phenotype variations, as well as evolution of sex determination systems in medaka related species. The Laboratory of Molecular Genetics for Reproduction is studying the molecular mechanisms of reproductive organ development and sex differentiation using mutagenized or transgenic medaka. For details, please refer to the pages of each laboratory (p. 37, 47, and 27).

Model Plant Research Facility

● *Plant Culture Laboratory*

Assistant Professor: *HOSHINO, Atsushi*
TSUGANE, Kazuo
Technical Staff: *MOROOKA, Naoki*
Technical Assistant: *SUZUKI, Keiko*

The Plant Culture Laboratory manages facilities for the cultivation of plants in general and also for the rearing of several animal species that do not fit in other facilities.

The Plant Culture Laboratory equips and manages 62 culture boxes or growth chambers, 4 phytotrons, and 12 rooms with the P1P physical containment level for established and emerging model plants including the thale cress *Arabidopsis thaliana*, several carnivorous plants, the rice *Oryza sativa*, the moss *Physcomitrella patens*, green alga *Chlamydomonas reinhardtii* and several other flowering plants. Most culture space is fully used the whole year by more than 60 researchers from both outside and inside groups.

As well as regular culture conditions, extreme environmental conditions for light and temperature are available for various types of experiments. Three light environmental simulators (max 120,000 lux using xenon lamps) and three chambers (3.4 m^2 each) that can control CO_2 and humidity in addition to temperature and light (max 70,000 lux) conditions are available. A tissue culture rack with dimming LEDs and pulse-width modulation controllers are used for algae culture under precise light control. Autotrophic and heterotrophic culture devices are also available for researchers using cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches. Several analytical instruments including a flow cytometry system and a DUAL-PAM, for DNA content and chlorophyll fluorescent measuring, respectively, are also available.

Next to the institute building of the Myodaiji area, a 386-m^2 experimental farm is maintained for Japanese morning glory and related *Ipomoea* species, several carnivorous plants and other flowering plants necessary to be cultivated outside. Three greenhouses (44, 44, and 45 m^2) with heating are used for the sensitive carnivorous plants. Seven greenhouses (4, 6, 6, 6, 6, 9, and 9 m^2) with air-conditioning are provided for the cultivation of rice *Oryza* sp., *Lotus japonica* and related legume species, as well as mutant lines of the Japanese morning glory. Two greenhouses (9 and 18 m^2) with air-conditioning meet the P1P physical containment level and are available for experiments using transgenic plants. The Plant Culture Laboratory also maintains a 46 m^2 building with storage and workspace. Part of the building is used for rearing of the orchid mantis and the japanese rhinoceros beetle.

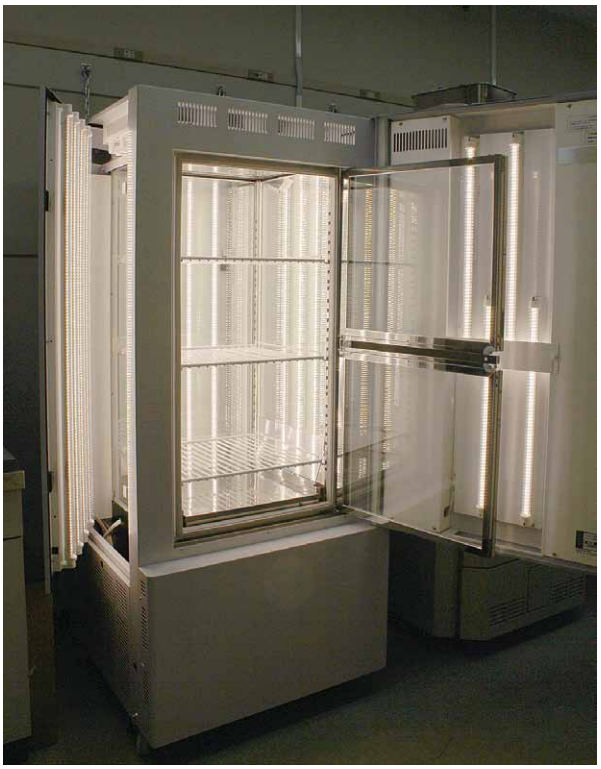


Figure 5. A plant culture box with LED lamps.

● *Morning Glory BioResource Laboratory*

Assistant Professor: *HOSHINO, Atsushi*

The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, and is studied worldwide, especially in plant physiology and genetics. NIBB collects, develops and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning glory, and collaborates with the core organization center, Kyushu University. We collected several mutant lines, and provided 31 DNA clones and 16 *Ipomoea* lines to both local and international biologists this year.

Research activities of the Assistant Professor A. Hoshino are shown on the laboratory page (p. 53).

Cell Biology Research Facility

Assistant Professor: *HAMADA, Yoshio*
Technical Assistant: *SUGINAGA, Tomomi*

The Cell Biology Research Facility provides various equipment for tissue and cell culture. This laboratory is equipped with safety rooms which satisfy the P2 physical containment level, and is routinely used for DNA recombination experiments.

Research activities of Assistant Professor Y. Hamada, the principal investigator of the Laboratory of Cell Sociology, is shown on the laboratory page (p.13).

Figure 6. Equipment for tissue and cell culture.

NIBB Center of the Interuniversity Bio-Backup Project (IBBP Center)

Head
KAWAGUCHI, Masayoshi

Associate Professor
NARUSE, Kiyoshi

Specially Appointed Assistant Professor:
KIMURA, Tetsuaki
TANAKA, Daisuke
Technical Assistant:
AKIMOTO-KATO, Ai
MATSUBAYASHI, Naomi
FUKUMOTO, Tatsuya
Secretary:
HAMATANI, Ayako

In order to realize a life sciences community resilient to natural disasters and calamities, the National Institutes for Natural Sciences (NINS) and Hokkaido University, Tohoku University, University of Tokyo, Nagoya University, Kyoto University, Osaka University, and Kyushu University concluded an agreement on June 1st 2012 to launch a system to 'back up' the biological resources essential to the work being done at universities and research institutions nationwide, called the 'Interuniversity Bio-Backup Project (IBBP)'.

The IBBP Center was established as a centralized backup and storage facility at NIBB, while IBBP member universities set up satellite hubs and work closely with the IBBP center to put in place reciprocal systems for backing up important biological resources that have been developed by researchers residing in the area each university satellite hub is responsible for.

Figure 1. IBBP Center

Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

The IBBP Center includes earthquake proof structures capable of withstanding even very large scale quakes (equipped with emergency backup power generators), cryopreservation facilities equipped with automatic liquid nitrogen feeding systems, deep freezers, and refrigerated storage (mainly for seed stocks), as well as all manner of automated laboratory equipment, cell culture tools, and the latest equipment necessary to back up the genetic resources in a collaborative manner. The specific methods of preservation are freezing of the sperm and eggs of animals, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

University satellite hubs receive preservation requests of biological resources from researchers and report to the Managing Project Committee of IBBP (constituted of faculty of NIBB and satellite institutes), where the relevance of the request is reviewed. When the request is sustained, biological resources to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated) and their particular information will be registered into a database. In the event of a disaster leading to the loss of a researcher's own biological resources, preserved samples will be promptly supplied back to the researcher so they can quickly resume their work.

Through the development of this backup system biological resources that had only been stored at individual research institutes can now be preserved at the state of the art facilities of the IBBP Center, and Japan's research infrastructure has been significantly strengthened.

I. Current status of back up for the biological resources

In 2015, IBBP Center stored 4,238 384-well and 69 69-well plates consisting of 1,634,016 clones as cDNA/BAC clones, 8946 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 80 133mm-straw tubes for sperm and 606 seed samples. In total 1,634,702 samples are stored.

Figure 3. Cryo tube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

II. Collaborative Research Project for the development of new long-term storage technologies and cryo-biological study

As the IBBP Center can only accept biological resources which can be cryopreserved in liquid nitrogen, researchers cannot backup those biological resources for which cryo-preservation methods are not well established, to increase the usability of IBBP, we started a collaborative research project for the development of new long-term storage technologies and cryo-biological study in 2013. This collaborative research includes two subjects 1) Establishment of new storage technology for biological resources for which long-term storage is unavailable. 2) Basic cryobiological research enabling us to improve low temperature storage for biological resources. In 2015 we had ten applications and accepted nine proposals. We are also working to establish a research center for cryo-biological study thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2015 on October 28-29, 2015 at the Okazaki Conference Center, Okazaki, Japan. We had 100 participants from several fields covering physics, chemistry, biology, and technology.

Figure 4. Group photo of Cryopreservation conference 2015

Publication List on Cooperation

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

- Tanaka, D., Ishizaki, K., Kohchi, T., and Yamato, K. T. Cryopreservation of gemmae from the liverwort *Marchantia polymorpha* L. Plant Cell Physiol.2015 Nov. 11.

Research activity by D. Tanaka

Assistant Professor:	*TANAKA, Daisuke*
Technical Assistant:	*AKIMOTO-KATO, Ai*
	FUKUMOTO, Tatsuya

Cryopreservation

Cryopreservation protocols contain components which are usually developed empirically using each biological resource's specific strategy to enhance survival (Benson 2008). The theory of cryopreservation encompasses several interconnected disciplines ranging from the physiological to cryophysical. Water status and cryopreservation, in combination with physiological factors, are the most influential determinants of survival (Figure 1).

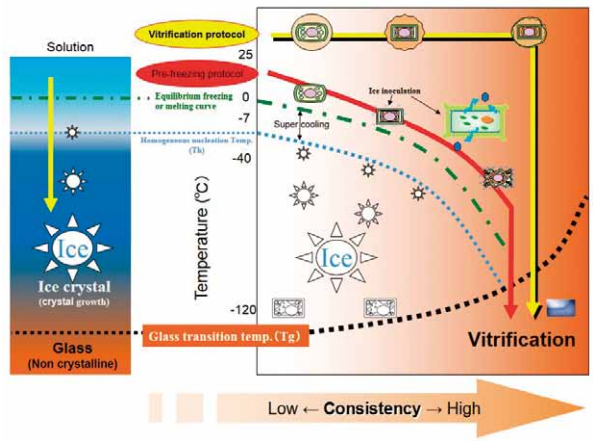


Figure 1. Phase diagram of vitrification of a solution.
Vitrification, a physical process, can be defined as the phase transition of an aqueous solution from a liquid into an amorphous glassy solid, or glass, at the glass transition temperature (Tg), while avoiding ice crystallization.

So far cryopreservation has been considered in terms of the liquid and solid (ice) phases of water. It is also possible to cryopreserve tissues by the process of "vitrification", the solidification of liquids without crystallization (Figure 2). This comprises a "glassy state" as the system is amorphous, lacks organized structure but possesses the mechanical and physical properties of a solid (Taylor *et al*. 2004).

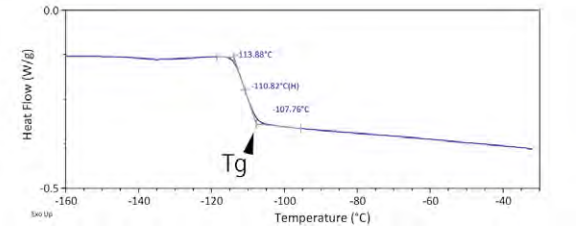


Figure 2. Differential Scanning Calorimetry (DSC) thermogram of Vitrified shoot apices of a Chrysanthemum plant treated with vitrification solution.
Glass transition temperature (Tg):-107

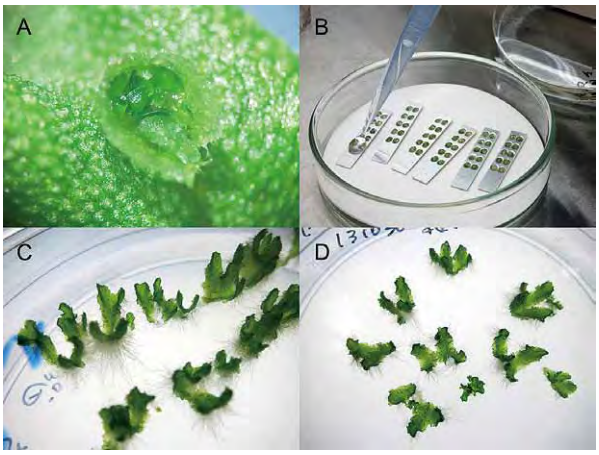


Figure 3. Cryopreservation protocol using aluminum cryo-plate.
A: Preparation of *in vitro* grown gemmae of liverwort. B: Placing pre-cultured gemmae in a cryo-plate's wells. C: Regrowth of cryopreserved livewort line 'Takaragaike-1' 30 days after rewarming. D: Control without cooling in liquid nitrogen).

Vitrification-based protocols are known to be effective for long-term, stable preservation of plant germplasm; this protocol can reduce the cost and manpower for maintaining a large number of germplasm lines and keep many valuable genetic lines for a long term under genetically stable conditions. However, it is still not widely employed as a reliable long-term preservation protocol due to the lack of basic knowledge on cellular and water behavior in tissues when immersed in liquid nitrogen.

In the present study, electron microscopy combined with freeze-substitution was employed to examine the ultrastructure of cells of the gemmae of the liverwort, *Marchantia polymorpha* L., strain Takaragaike-1, which were cooled to the temperature of liquid nitrogen after exposure to various steps of the Cryo-plate protocol (Figure 3).

Publication List

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

● Tanaka, D., Ishizaki, K., Kohchi, T., and Yamato, K. T. Cryopreservation of gemmae from the liverwort *Marchantia polymorpha* L. Plant Cell Physiol.2015 Nov. 11.

Research activity by T. Kimura

Assistant Professor: *KIMURA, Tetsuaki*

Leucophores are similar to xanthophores

Mechanisms generating diverse cell types from multipotent progenitors are crucial for normal development. Neural crest cells (NCCs) are multipotent stem cells that give rise to numerous cell-types, including pigment cells. Medaka has four types of NCC-derived pigment cells (xanthophores, leucophores, melanophores and iridophores), making medaka pigment cell development an excellent model for studying the mechanisms controlling specification of distinct cell types from a multipotent precursor cell. However the genetic basis of chromatophore diversity remains poorly understood.

We reported that *leucophore free-2* (*lf-2*) which affects leucophore and xanthophore differentiation, encodes *pax7a*. Since *lf-2*, a loss-of-function mutant for *pax7a*, causes defects in the formation of xanthophore and leucophore precursor cells, *pax7a* is critical for the development of the chromatophores. This genetic evidence implies that leucophores are similar to xanthophores, although it was previously thought that leucophores were related to iridophores, as these chromatophores have purine-dependent light reflection.

Many leucophores-3 (*ml-3*) mutant embryos exhibit a unique phenotype characterized by excessive formation of leucophores and an absence of xanthophores. We show that *ml-3* encodes *sox5*, which is expressed in premigratory NCCs and differentiating xanthophores. Cell transplantation studies reveal a cell-autonomous role of *sox5* in the xanthophore lineage. *pax7a* is expressed in NCCs and required for both xanthophore and leucophore lineages; we demonstrate that Sox5 functions downstream of Pax7a.

We propose a model in which multipotent NCCs first give rise to *pax7a*-positive bi-potent precursor cells for xanthophores and leucophores; some of these precursor cells then express *sox5*, and as a result of Sox5 action develop into xanthophores (Figure 1). Our findings provide clues for revealing diverse evolutionary mechanisms of pigment cell formation in animals.

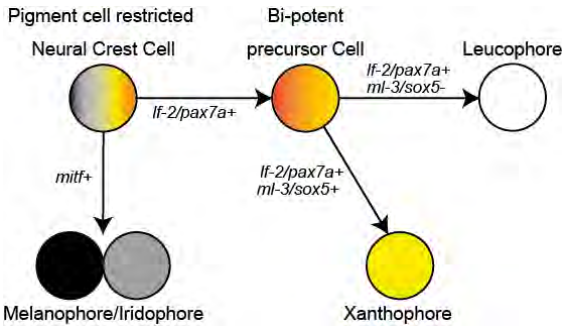


Figure 1. Model for leucophore and xanthophore development from neural crest cell.

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor
KODAMA, Ryuji

Technical Staff:
MATSUDA, Yoshimi
(Radiation Protection Supervisor)
SAWADA, Kaoru
(Radiation Protection Supervisor)
IINUMA, Hideko

Technical Assistant:
KAMIYA, Kiyomi

The technical and supporting staff of the Center for Radioisotope Facilities (CRF) maintain controlled areas in compliance with the law. The CRF is responsible for monitoring the purchase of radioisotopes from the Japan Radioisotope Association (JRIA) and the transfer of radioisotope wastes to JRIA.

Ms. Matsuda, Ms. Iinuma, and Ms. Kamiya maintained the Myodaiji area. Ms. Sawada worked in the Yamate area. Dr. Kodama worked in both areas.

The following are the CRF's notable activities in 2015.

1. An introductory course for radioisotope handling was held after an interval of 6 years due to the repair work of the Common Facilities building I. The content of the course was renewed, allowing participants to experience handling of several types of radiation and radioisotopes (Figure 1A).
2. The drain storage tanks at the Yamate area were cleaned and checked by the maintenance workers after 4 years of use. Both the water level indicator and the water level alarm were exchanged. (Figure 1B)

The number of registrants and the number of users from January 2015 to December 2015 are shown in Table 1.

Users and visitors counted by the access control system of the controlled areas numbered 2,250 during this period. The numbers for each area are shown in Table 2. The annual changes of registrants and the number of totals per fiscal year are shown in Figure 2. The balance of radioisotopes received and used at the CRF is shown in Table 3. The training courses on radioisotope handling were given as in Table 4.

Figure 1. The CRF's notable activities in 2015.
A: The introductory course for radioisotope handling
B: Cleaning of the drain storage tanks at the Yamate area

	Myodaiji area	Yamate area
Registrants	62	78
Users	29	37

Table 1. Numbers of registrants and users at Myodaiji area and Yamate area in 2015.

	Myodaiji area	Yamate area	Total
Users	1,281	665	1,946
Visitors	185	119	304
Total	1,466	784	2,250

Table 2. Users and visitors who entered each controlled area in 2015.

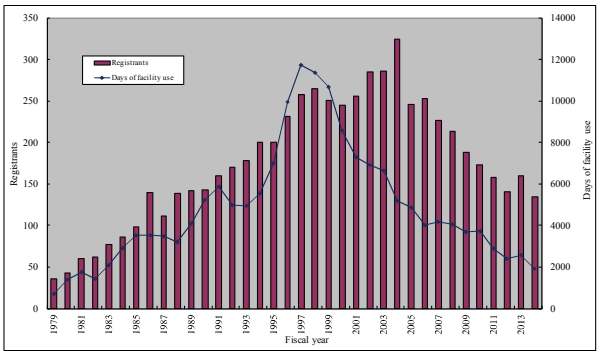


Figure 2. Annual changes of registrants and days of facility use per fiscal year.

	Myodaiji area	Yamate area	Total
^{125}I Received	0	590	590
^{125}I Used	0	165	165
^{35}S Received	74,000	1,110,000	1,184,000
^{35}S Used	14,706	1,343,661	1,358,367
^{32}P Received	216,450	0	216,450
^{32}P Used	200,540	0	200,540
^{14}C Received	37,000	0	37,000
^{14}C Used	18,630	0	18,630
^{3}H Received	40,700	3,718,500	3,759,200
^{3}H Used	28,075	3,715,540	3,743,615

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2015.

Training course	Place	Number of participants
Introductory course for beginners*	Myodaiji	1
Introductory course for beginners*	Yamate	3
Introductory course for experts	Myodaiji	4
Introductory course for experts	Yamate	6
Users training course*	Myodaiji	53
Users training course	Yamate	69

*including English course

Table 4. Training courses for radiation workers in 2015.

Research Enhancement Strategy Office

Director
UENO, Naoto

Vice-Director (Specially Appointed Professor (URA))
NISHIMURA, Mikio

In order to fulfill two goals, to encourage cutting-edge academic research in the field of natural sciences through international joint research, and to contribute to the enhancement of research capabilities of universities etc. in Japan using the world's most advanced research environment for joint utilization and joint research, NINS started in 2013 a research enhancement project with the following four approaches: 1) Support for the promotion of international advanced research, 2) Support for the promotion of joint utilization and joint research in Japan, 3) Dissemination of information and enhancement of public relations in Japan and abroad, 4) Support for researchers, especially young, female or foreign researchers.

The Research Enhancement Strategy Office is aimed at supporting researchers so that NIBB improves its ability as a collaborative research institution, and was restructured in 2013 from the former Strategic Planning Department, the Office of Public Relations, and the Office of International Cooperation which existed from 2005. The Office's activities are mainly carried out by URAs (University Research Administrators) according to the advice of the group adviser chosen from NIBB's professors and in close collaboration with the NINS's Research Enhancement Promotion Headquarters.

Evaluation and Information Group

Associate Professor:	*KODAMA, Ryuji*
Group Adviser:	*YOSHIDA, Shosei*

This group serves as a central office for assisting the Director General in preparing for NIBB's evaluation procedure and in planning long-range strategies for the institute.

The main activities of the group

1) Management of external evaluation processes

NIBB asks non-NIBB members of the Advisory Committee for Programming and Management to answer a questionnaire on research achievements and collaborative research activities of the institute. Several of the members are further invited to participate in a meeting for the evaluation of these activities and for proposing future directions for the institute. This group manages these processes.

2) Editing of the Annual Report (in collaboration with the Public Relations Group)

This group edits the annual report (this print) which summarizes the annual activities of laboratories and facilities and is used in the evaluation procedures of the institute.

3) Assistance in budget requests and long-range planning of the institute

This group also assists the Director General in preparing long-range plans for building the most advanced research facilities, and in budget requests to the government to realize and implement these plans.

4) Assistance in making the plans and reports of the institute

In addition we assist in drafting NIBB's Medium-term Goals and Plans (for a six-year-period), and in instituting Annual Plans to implement them. The department also assists in preparing Business and Performance Reports for the external evaluation on whether we are meeting the goals set both annually and for the medium-term.

Public Relations Group

Specially Appointed Assistant Professor (URA):	*KURATA, Tomoko*
Technical Assistant:	*OTA, Kyoko*
	KAWAGUCHI, Colin
	BAN, Misato
Group Adviser:	*FUJIMORI, Toshihiko*

This group, in order to publicize the activities of NIBB to the widest audience, actively facilitates communication between NIBB and the public, school teachers, and the international community of scientific researchers.

The main activities of the group in 2015

1) Press releases

The group sends news on scientific achievements to newspaper and magazine reporters via leaflets, e-mails and web pages. We also arrange press conferences for some releases.

2) Updating and maintenance of the NIBB web page

3) Editing of publications, production of posters and leaflets

Publication of "NIBB News" (Intra-institutional newsletter, in Japanese), "NIBB English News" (Intra-institutional newsletter, in English). Publication of leaflets introducing the individual researchers of NIBB (in Japanese). Design and distribution of posters of NIBB events.

4) Producing Videos

Creation of videos introducing NIBB and interviews with researchers that are published on the web.

5) Organization of scientific outreach programs

Organizing the Summer Program for university students, and coordinating special classes for middle school students.

International Cooperation Group

Specially Appointed Assistant Professor (URA): *TATEMATSU, Kiyoshi*
Technical Assistant: *TAKAHASHI, Ritsue*
SANJO, Kazuko
NISHIMURA Akiko
Group Advisor: *UENO, Naoto*

NIBB has a mission to continually explore the leading-edge of biology and form research communities that link Japan to the world. For this purpose, NIBB holds scientific meetings including "NIBB Conferences" and "Okazaki Biology Conferences (OBC)", and educational programs such as "NIBB International Practical Courses". Further, NIBB is tightly interacting with the European Molecular Biology Laboratory (EMBL, European member states) and the Temasek Life Sciences Laboratory (TLL, Singapore) on the basis of cooperative agreements, through exchanging people and techniques and jointly holding scientific meetings. NIBB is also conducting the "NIBB International Collaborative Research Initiative" to promote high-level international collaborations between faculty members of NIBB and researchers around the world. NIBB invites leading-edge researchers from abroad as "Guest Professors" to promote academic exchange with NIBB members and to start new international collaborations.

This group supports and coordinates NIBB's activities related to international research collaborations, through organizing the various above-mentioned international scientific meetings and technical courses, coordination of dispatching NIBB's researchers to international conferences, and support of researchers visiting from the institutes mentioned above. This group also supports NIBB internship students visiting from foreign countries, and the dispatching of graduate students of SOKENDAI (the Graduate University for Advanced Studies) to international conferences, which are aimed at nurturing the next generation of researchers in biology. From this year, this group, cooperating with the Okazaki Administration Office, will begin supporting other researchers and students who visit NIBB.

The main activities of the group in 2015

1) Coordination of the International Conferences and the International Practical Course

This group coordinated the following International Conference hosted by NIBB:

The 63rd NIBB Conference "Environment to Bioresponses" Okazaki, Japan, November 30-December 2, 2015 (p. 88)

2) Support of dispatching researchers to international conferences

This group supported sending NIBB researchers to the following events (related to the international cooperative agreements):

EMBO Workshop "Embryonic-Extraembryonic Interfaces Emphasis on molecular control of development in amniotes" Göttingen, Germany, May, 6-9, 2015

3) Support of visiting researchers to NIBB

This group supported visits of foreign researchers related to the following events:

Two Guest Professors coming from Heidelberg University, Germany (February) and Tulane University, USA (November)

4) Support of education related programs

This group supported sending and hosting students participating in the following student-related activities of NIBB:

The 17th EMBL PhD Symposium "Just by Chance? – Randomness & Variability Shaping Biology-" Heidelberg, Germany Octorber, 22-24, 2015 (p. 89)

NIBB Internship Program 2015 (p. 92)

Collaborative Research Group

Specially Appointed Associate Professor (URA): *SHIGENOBU, Shuji*
KAMEI, Yasuhiro
Technical Assistant: *ICHIKAWA, Mariko*
ICHIKAWA, Chiaki
Group Adviser: *YOSHIDA, Shosei*

Specially appointed associate professors of this group belong to the NIBB core research facilities and are responsible for managing collaborative research projects and practical courses taking advantage of their expertise in their field., through which this group explores further promotion of information exchange and collaboration among scientific communities, and also supports the development of new equipment and methods.

In 2015, this group hosted a total of 123 collaboration projects. Through these collaboration projects, 28 research papers were published. A noteworthy achievement was the series study of amphibian regeneration directed by Dr. Yokoyama in Hirosaki University and his colleagues. The NIBB collaborative research group and the NIBB Core Facilities supported their research in bioinformatics and bioimaging technologies as individual collaborative research projects from 2012.

- Kawasumi-Kita, A., Hayashi, T., Kobayashi, T., Nagayama, C., Hayashi, S., Kamei, Y., Morishita, Y., Takeuchi, T., Tamura, K. and Yokoyama, H. (2015). Application of local gene induction by infrared laser-mediated microscope and temperature stimulator to amphibian regeneration study. Dev. Growth Differ. *57*, 601–613.
- Hayashi, S., Ochi, H., Ogino, H., Kawasumi, A., Kamei, Y., Tamura, K. and Yokoyama, H. (2014). Transcriptional regulators in the Hippo signaling pathway control organ growth in Xenopus tadpole tail regeneration. Dev. Biol. *396*, 31-41.
- Hayashi, S., Kawaguchi, A., Uchiyama, I., Kawasumi-Kita, A., Kobayashi, T., Nishide, H., Tsutsumi, R., Tsuru, K., Inoue, T., Ogino, H., Agata, K., Tamura, K. and Yokoyama, H. (2015). Epigenetic modification maintains intrinsic limb-cell identity in Xenopus limb bud regeneration. Dev. Biol. *406*, 271-82.

Gender Equality Promotion Group

Associate Professor: *TSUBOUCHI, Tomomi*
Group Adviser: *TAKADA, Shinji*

This group supports the improvement of the research environment for female researchers in both research and daily life. This includes our research support system for childbirth and childcare, etc., aiming at promoting the employment of female researchers.

In 2015, this group contributed to the management of the research support system covering childbirth and childcare. This group also organized an enlightenment lecture and published a leaflet for gender equality promotion in collaboration with other institutes of NINS.

TECHNICAL DIVISION

Head
KAJIURA-KOBAYASHI, Hiroko

Common Facility Group

Chief: *MIWA, Tomoki*

● ***NIBB Core Research Facilities***

Unit Chief: *KONDO, Maki*
MORI, Tomoko
Subunit Chief: *MAKINO, Yumiko*
YAMAGUCHI, Katsushi
Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
TANIGUCHI- SAIDA, Misako
UCHIKAWA Tamaki
BINO, Takahiro
Technical Assistant: *ICHIKAWA, Chiaki*
NISHIMURA, Noriko
ICHIKAWA, Mariko
ISHIKAWA, Azusa
OKA, Naomi
SHIBATA, Emiko

● ***NIBB Bioresource Center***

Subunit Chief: *HAYASHI, Kohji*
MOROOKA, Naoki
Technical Staff: *NOGUCHI, Yuji*
Technical Assistant: *TAKAGI, Yukari*
SUZUKI, Keiko
SUZUKI, Kohta

● ***Disposal of Waste Matter Facility***

Unit Chief: *MATSUDA, Yoshimi*

● ***Center for Radioisotope Facilities***

Unit Chief: *MATSUDA, Yoshimi*
Subunit Chief: *SAWADA, Kaoru*
Technical Staff: *IINUMA, Hideko*
Technical Assistant: *ITO, Takayo*

Research Support Group

● ***Developmental Biology***

Technical Staff: *TAKAGI, Chiyo*
UTSUMI, Hideko
OKA, Sanae
MIZUGUCHI, Hiroko

● ***Neurobiology***

Unit Chief: *OHSAWA, Sonoko*
Subunit Chief: *TAKEUCHI, Yasushi*

● ***Evolutionary Biology and Biodiversity***

Unit Chief: *FUKADA-TANAKA, Sachiko*
Subunit Chief: *KABEYA, Yukiko*

● ***Environmental Biology***

Unit Chief: *MIZUTANI, Takeshi*
Technical Staff: *NODA, Chiyo*

Reception

Secretary: *TSUZUKI, Shihoko*
KATAOKA, Yukari
UNO, Satoko
MIYATA, Haruko

The Technical Division is a support organization for researchers and research organizations within NIBB. The Technical Division develops and promotes the institute's research activities and maintains the research functions of the institute.

The Technical Division is organized into two groups: the Common Facility Group, which supports and maintains the institute's common research facilities, and the Research Support Group, which assists research activities as described in the reports of individual research divisions.

Technical staff members continually participate, through the Division, in self-improvement and educational activities to increase their capabilities and expertise in technical areas. Generally, technical staff members are attached to specific divisions so that they may contribute their special biological and biophysical knowledge and techniques to various research activities.

The Technical Division hosts an annual meeting for technical engineers who work in various fields of biology at universities and research institutes throughout Japan. At this meeting, the participants present their own activities and discuss technical problems. The proceedings are published after each meeting.

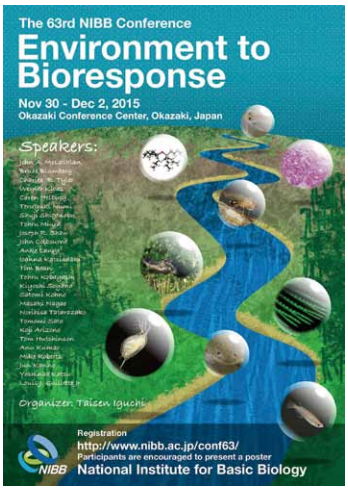
The 63rd NIBB Conference "Environment to Bioresponse"

Organizer: Taisen Iguchi (NIBB, Japan)

November 30 (Mon) - December 2 (Wed), 2015

This symposium, with researchers that have been active in the international forefront, was intended to facilitate deep discussions on the connection between the "environment" and the "organism". In addition to basic biological research on environmental response strategies of organisms, from which comes the molecular basis of evolution leading to the establishment of a variety of species, we discussed various issues regarding the effects of environmental chemicals to humans and wildlife, and the mechanisms of the effects using model species. In addition to mice and humans, a variety of animal species such as beetles, ants, aphids, *Daphnia*, medaka, zebrafish, roach, *Xenopus*, and alligators have been used. We discussed the latest findings concerning the molecular mechanisms of bioresponses such as phenotypic plasticity, metamorphosis, defects of sexual differentiation, and reduction of reproduction, which are caused by environmental factors, hormones, and chemical substances. 15 years ago, I organized The 45th NIBB Conference in order to discuss how to analyze the effects caused by hormone-like substances being released into the environment, on fish, amphibians, reptiles, and mammalians, including humans. The progress made during the 15-years of research was an eye-opener, and our basic understanding for the elucidation of molecular evolution and the function of hormone receptors, the elucidation of sex differentiation mechanisms in various animal species including alligators, *Daphnia*, and aphids, and the decoding of the genome sequence of various animal species has been monumentally advanced. Moreover, the progress of this research has revealed the necessity for promoting research focusing on the epigenetic changes induced by the chemicals and the environment, the mechanism of action of "Obesogen" induced obesity, and the transgenerational and composite effects of these chemicals. This symposium was an opportunity to broadly discuss the impact and bioresponses to environmental factors from a wide range of research fields including perspectives from basic biology and bioinformatics.

(Taisen Iguchi)



Speakers

Bean, Tim P. (Cefas), Blumberg, Bruce (Univ. of California), Colbourne, John (Univ. of Birmingham), Guillette, Louis Jr. (Medical Univ. of South Carolina), Helbing, Caren C. (Univ. of Victoria), Katsiadaki, Ioanna (Cefas), Kloas, Werner (IGB), Kohno, Satomi (Medical Univ. of South Carolina), Lange, Anke (Univ. of Exeter), McLachlan, John A. (Tulane Univ.), Roberts, Mike (Defra), Shaw, Joseph R. (Indiana Univ.), Tyler, Charles R. (Univ. of Exeter), Arizono, Koji (Pref. Univ. of Kumamoto), Horiguchi, Toshihiro (NIES), Iguchi, Taisen (NIBB), Kanno, Jun (NIHS), Katsu, Yoshinao (Hokkaido Univ.), Kobayashi, Tohru (Univ. of Shizuoka), Miura, Toru (Hokkaido Univ.), Nagae, Masaki (Nagasaki Univ.), Niimi, Teruyuki (NIBB), Sato, Tomomi (Yokohama City Univ.), Shigenobu, Shuji (NIBB), Soyano, Kiyoshi (Nagasaki Univ.), Tatarazako, Norihisa (NIES)

The 17th EMBL Ph.D Symposium "Just by Chance? – Randomness & Variability Shaping Biology –"

October 22 (Thu) – 24 (Sat), 2015	EMBL, Heidelberg

Two PhD students from SOKENDAI/NIBB were funded by NIBB to participate in the The 17th EMBL Ph.D Symposium "Just by Chance? – Randomness & Variability Shaping Biology –" (held on 22-24 October). Our students had the chance to give poster presentations at the symposium to introduce their current research. They also attended EMBL's laboratories to exchange experimental information and discuss their research with PhD students, post-docs and PIs.

Comments from students (excerpts)

・Rie Ohashi

Various topics were provided in the Ph.D. Symposium, e.g. developmental biology, evolution, structural dynamics and bioinformatics. I was very interested in the programs because these topics covered "wet" (experimental biology) to "dry" (informatics), which reflects current biology. During the coffee breaks, students were proactive in their communications with each other. I presented my research in a poster session. My theme didn't completely match the theme of the symposium, but many people came to my poster. I was nervous because I had to speak only in English. However, during the poster session, I felt that we were able to understand each other through the scientific topics, much better than through the topics during the coffee break, so it was an enjoyable time.

Afer the symposium I visited the laboratory of Dr. Anne Ephrussi, who aims to reveal the mechanism of local translation in Drosophila embryos. I introduced my research to the lab members in an informal seminar for about 40 min. After that, we discussed my work and their work with a few lab members. I was very glad that they asked many questions. However, it was difficult for me to understand their questions quickly, because their talking speed was very fast for me, so I had to ask people to repeat their questions to understand. We communicated with each other using not only language but also drawing pictures. I reflected on these points a lot during my lab visit, but it was still a very precious time for me. I will make use of this experience in the future.

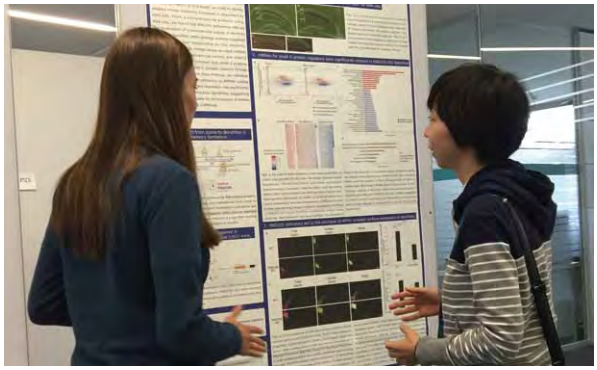
On the last day, I visited Dr. Kota Miura, who is an image analysis coordinator at EMBL. He has been living in Germany for many years. I heard about the various differences between Europe and Japan, including how researchers perceive molecular biology in their research. I was very interested in that, and it was a meaningful experience.

I felt that I have to make more of an effort to survive as a researcher, but I strongly reaffirmed my commitment because of this EMBL visit. Finally, I would like to thank to the staff of NIBB and EMBL for supporting my visit.

・Yousef Kamrani

The aim of the EMBL PhD student symposium was "to explore the importance of randomness and variability in biology"; I realized that applying bioinformatics to biology research should be highlighted and it was a useful hint for me. The symposium attracted mostly early-stage PhD students and high-profile speakers spanning all continents with diverse backgrounds. The symposium program had been formulated with several lectures, short talks and a poster session. At the end of each day, we had a blackboard session and a panel discussion. At the blackboard discussions we could choose our topic of interest and sit down with speakers in a small group to share insights from their most recent research. This time was a great opportunity. Moreover, I was able to strengthen my network of connections with other scientists. After a three day tight schedule, I had a weekend to tour Heidelberg city. I passed through many historic treasures including Heidelberg's Schloss (castle) and the Neckar River's autumn colors absolutely invigorated me.

The second part of my program was a one week training course at the EMBL GeneCore facility headed by Dr. Vladimir Benes. I practiced a protocol provided by Dr. Bernd Klaus -a step by step method- using statistical bioinformatics to analyze high-throughput sequencing data such as DNA-Seq or RNA-Seq. I was supported technically by highly qualified staff in Dr. Benes' group. The atmosphere of his lab was very relaxed and friendly. I am looking forward to having an opportunity again in the near future to visit EMBL.



The 9th NIBB Bio-Imaging Forum "Imaging of Physical Properties"

Organizer: Yasuhiro Kamei, Shigenori Nonaka, Toshihiko Fujimori, Naoto Ueno

January 26 (Mon) - 27 (Tue), 2015

Thirteen lectures (two from NIBB members) on temperature, kinetics, and molecular movement, as well as on how these forces affect biological research, drew a total of 49 participants. This lead to broad multi-disciplinary discussions on the role of physics in living systems. These physical quantities still do not draw a lot of attention in the field of biology, but it was clear that many researchers feel we are closing in on the essence of biological activity from these processes. Many lectures from fields rooted in engineering were given, and for researchers of biological systems this active gathering with members of remarkably different fields lead to fresh exchange and new perspectives. In addition, because it was the first symposium on biological systems in which heat and temperature were the main themes, there was hope from the speakers that more of these kinds of opportunities will become available going forward.

(Yasuhiro Kamei)

The NIBB Genome Informatics Training Course

The NIBB Core Research Facilities organizes a series of training courses on up-to-date research techniques. In 2015 we held two training courses on Genome Informatics. The 3-day programs offer lectures and hands-on tutorials to introduce basic knowledge and skills to deal with large scale genomic data such as DNA sequence data generated by next-generation sequencing (NGS). The programs are specially designed for biologists who are not familiar with bioinformatics.

"Introduction to RNA-seq - from the basics of NGS to de novo analyses"

February 25 (Wed) -27 (Fri), 2015

- Organizer: Dr. Shuji Shigenobu (NIBB Core Research Facilities)
- Lecturers: Dr. Shuji Shigenobu, Dr. Ikuo Uchiyama, Dr. Masanao Sato, Dr. Katsushi Yamaguchi, Ms. Hiroyo Nishide, Dr. Taro Maeda
- Participants: 22 (including 3 from NIBB)
- Program:
  1. UNIX for beginners
  2. NGS basic data formats and NGS basic tools
  3. Introduction to statistics
  4. Introduction to "R"
  5. RNA-seq pipeline: genome-based and transcriptome-based approaches
  6. Multivariate statistics
  7. Exercises

"Introduction to RNA-seq - from the basics of NGS to de novo analyses"

September 9 (Wed) -11 (Fri), 2015

- Organizer: Dr. Shuji Shigenobu (NIBB Core Research Facilities)
- Lecturers: Dr. Shuji Shigenobu, Dr. Ikuo Uchiyama, Dr. Masanao Sato, Dr. Katsushi Yamaguchi, Ms. Hiroyo Nishide
- Participants: 22 (including 3 from NIBB)
- Program:
  (as above)

The 3rd BioImaging Analysis Training Course

The 3rd Bioimaging Training Course was held jointly by the Center for Novel Science Initiatives' Department of Imaging Science, and NIBB. This course was designed for biologists who are relatively new to analyzing datum obtained through advanced microscopy. Therefore the focus of training was learning image processing and analytical techniques through "solving simple problems with image analysis" and "understanding appropriate methods and necessary preparation for consulting experts in technically advanced imaging challenges". There were applications from 83 people for this course, which had a maximum capacity of 20 participants, this clearly suggests the height of the demand for courses on these subjects.

This course's lectures were conducted with the aim of training participants to keep in mind the series of steps leading to fundamental image processing and analysis while obtaining the images to be used (workflows). In addition, we loaned the participants PCs pre-installed with ImageJ, a typical software package for biological image processing and analysis, and images to be used as teaching materials, which were used for practicing the basic operations and settings of image processing. In addition, lectures on how programming of simple "macro-programs" which use these workflows in ImageJ allows automation, which is essential for the large capacity and high-dimensional throughput of microscopic imaging which has become possible in recent years.

At the conclusion of the course each of the students gave commentary and discussed the methods used with examples of actual images from their own research. Every year after the course, participants express feeling "pretty tired, and satisfied" as part of the questionnaire, and certainly there is a true benefit in terms of their image analysis by becoming more familiar with these techniques. In addition, we expect that this course will increase opportunities for joint research relating to biological image analysis. (Kagayaki Kato)

December 7 (Mon) -9 (Wed), 2015

- Organizer: Dr. Kagayaki Kato, Dr. Yoshitaka Kimori, Dr. Yasuhiro Kamei, Dr. Hiroshi Koyama, Dr. Shigenori Nonaka, Dr. Takashi Murata

The NIBB Medaka Basic Training Course

The content of the course was decided through a questionnaire in the Medaka community's mailing list and out of the most requested topics we chose genome editing techniques using CRISPR/Cas9, microinjection techniques, rearing and anesthesia methods, and advanced imaging techniques as feasible subjects for NIBB. We originally planned to accept 8 participants, but as we had more than 20 applicants, we finally accepted 16. We also accepted two observers to learn skills for managing training courses, and asked a graduate student of Kyoto University for a presentation. The two-day course commenced with several lectures and hands on training. The course was tight-scheduled and may have been rather brief, but we believe we informed participants of the research potential of NIBB and of the support we can offer through collaborative programs including techniques needed in individual research projects. The questionnaire after the course revealed that the Medaka rearing methods were most appreciated.

August 6 (Thu) -7 (Fri), 2015

- Organizer: Dr. Yasuhiro Kamei (NIBB Core Research Facilities)
- Lecturers: Dr. Yasuhiro Kamei, Dr. Kiyoshi Naruse, Dr. Yusuke Takehana, Dr. Shigenori Nonaka
- Presentations: Mr. Ansai Satoshi (Kyoto Univ.), Dr. Shigenori Nonaka
- Participants: 16
- Program:
  1. Egg collection and outline of injection techniques
  2. Rearing and observation of embryos
  3. Anesthesia of the embryo
  4. Basics of genome editing techniques
  5. Microinjection
  6. Hatching enzyme treatment and sample preparation for microscopy
  7. Utilization of NMBR Medaka web pages
  8. Microscope handling and observation

The NIBB Internship program

The NIBB Internship program, started in 2009, is a hands-on learning course for overseas students designed to give high-quality experience in real world research and focused education of biology. At the same time, this program aims to internationalize the graduate students of SOKENDAI (Graduate University for Advanced Studies), giving them the opportunity to get to know students and interns with various cultural customs. Another goal of the program is to build connections through providing education to the people who will form the core of international research networks in the future.

To participate in this program, applicants who would like to experience research at NIBB must supply the name of the lab they would like to visit as well as their reasons for choosing it, and a letter of recommendation. Based on this information applicants are chosen to spend set periods of time participating in specific research activities in the lab they applied for. Round trip airfare and housing expenses are provided by the NIBB Internship Program.

In FY 2015 there were 26 applicants, out of which six interns were selected. These interns were from universities located in 4 countries (Turkey, Vientam, Thailand, and Germany) and spent periods ranging from two to twelve weeks experiencing life as a member of a research team. Moreover, one interns from Peru stayed at NIBB on twelve weeks by his own travel grants.

Report from a participant
Pham Van Cuong
VNU University of Science,Vietnam

I continued my journey of science by applying to the NIBB internship program immediately after completing my bachelor's degree. As a newbie in this field, I expected this program would be an opportunity to raise the skills of the scientist inside me: If I face a problem, how should I handle it? How should I solve scientific questions by designing plans or experiments?

Then I chose the laboratory of Assoc. Prof. Kamei, because he is using medaka fish as a model, and a heat-shock-inducible gene-expression system, as my undergraduate laboratory. Also, I wanted to have a chance at IR-LEGO, the technique that uses an infra-red laser to induce expression of the heat shock promoter-driving gene in desired cells. I spent nearly 3 months working in the Kamei-lab, I was supported enthusiastically by members of this laboratory and also the Bioresource Laboratory. Thanks to that help, my experiments (evaluation of heat-shock response and molecular experiments) turned out well and were finished with some expected results. I think the results I obtained were less significant to me than what I learned: techniques, experiment manipulation, and even working habits. And my questions at the beginning were also mostly answered.

To say something about this program, in an unscientific aspect, I feel how lucky I was to be selected for this program: coming to a country I dreamed about as my first time going abroad, being helped by the people surrounding me, and immersing myself in Japanese culture. Thank you for giving me these precious experiences and memories.

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Access

From Tokyo

Take the Tokaido Shinkansen and get off at Toyohashi, change to the Meitetsu Line and get off at Higashi Okazaki Station (approximately 20 minutes from Toyohashi).

From Osaka

Take the Tokaido Shinkansen and get off at Nagoya, change to the Meitetsu Line and get off at Higashi Okazaki Station (approximately 30 minutes from Nagoya).

From Central Japan International Airport

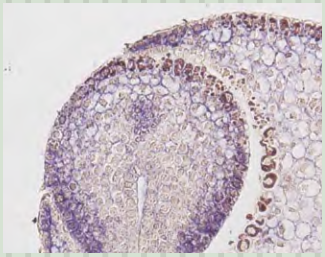
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes from the airport).

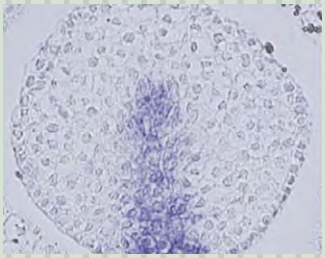
From Higashi Okazaki Station to Each Area

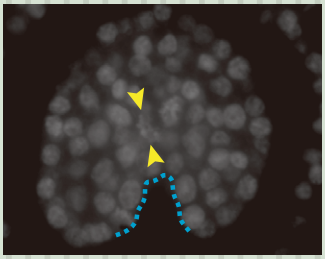
Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

By car

Take the Tomei Expressway to the Okazaki Exit. Turn left at the City Hall S. signal (approximately 10 minutes from the Exit).







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