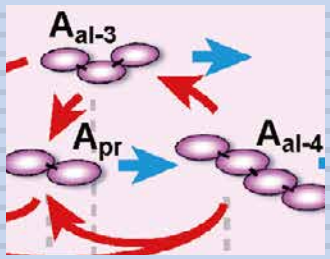
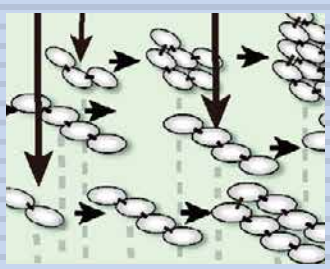
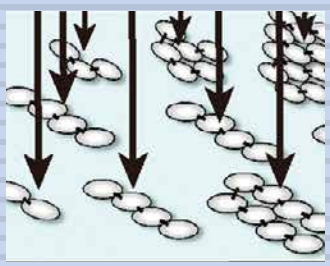
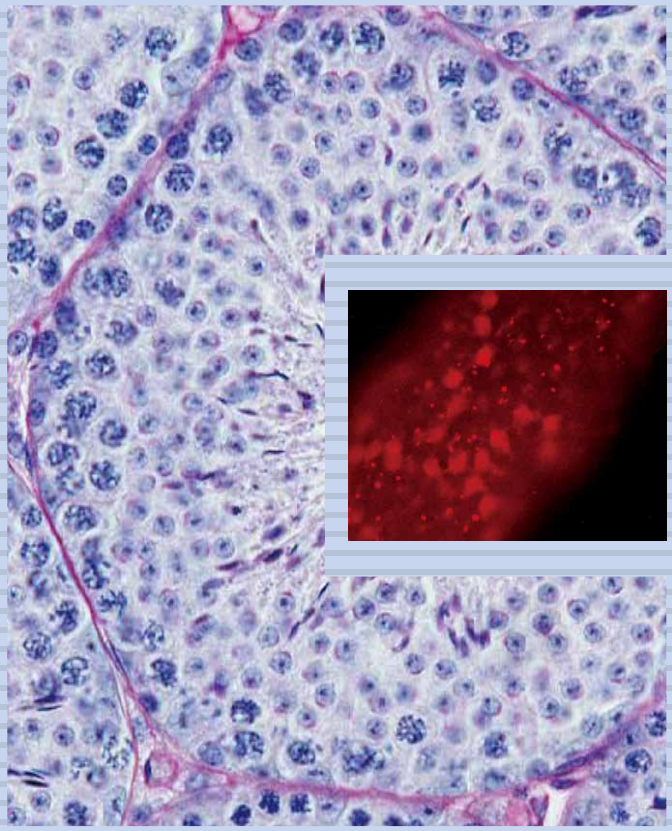
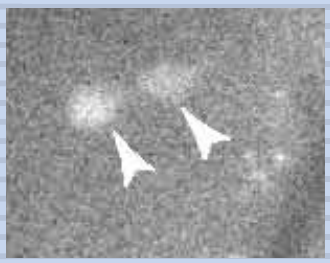
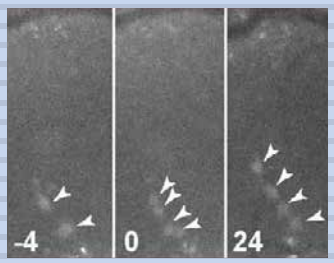


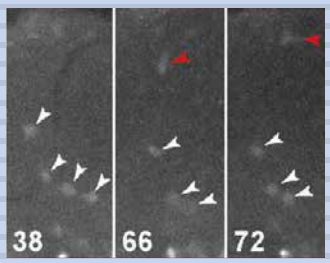












National Institute for Basic Biology

2014 ANNUAL REPORT

CONTENTS

The cover items are related to a study dissecting the nature and behavior of mouse spermatogenic stem cells in the testis (Hara *et al*., Cell Stem Cell, 2014). This study observed the *in vivo* fate behavior of the undifferentiated population of spermatogonia through intravital live-imaging and pulse-labeling studies, combined with biophysical modeling. The results showed that both singly isolated spermatogonia –which have been considered the only stem cell type– and syncytial spermatogonia –which have been considered committed for differentiation– comprise a single stem cell pool, in which cells stochastically interconvert between these morphologically different states. See page 30 of this report for details.

It is my great pleasure to introduce to you all the 2014 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 a serious burden to the scientific community to achieve full compliance in research activities, excluding any misconduct, and recover the reliance of the society to science. These circumstances have set a strong headwind against basic sciences, through which 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 in the society.

Please find in this booklet a summary of the research, collaborative, educational, and international activities of NIBB in 2014. I would like to note that we welcomed several new colleagues in 2014, including one specially appointed professor, one associate professor, two specially appointed associate professors, one assistant professor, one specially appointed assistant professor, and five NIBB research fellows, while four colleagues transferred to other institutes.

Finally I would like to congratulate Specially Appointed Associate Prof. Tsuyoshi Shimmura for winning the Encouragement Award from the Japanese Society of Animal Science. I would also like to congratulate young colleagues for winning awards from academic societies and SOKENDAI, 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
July 27, 2015

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 (NAO), 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).

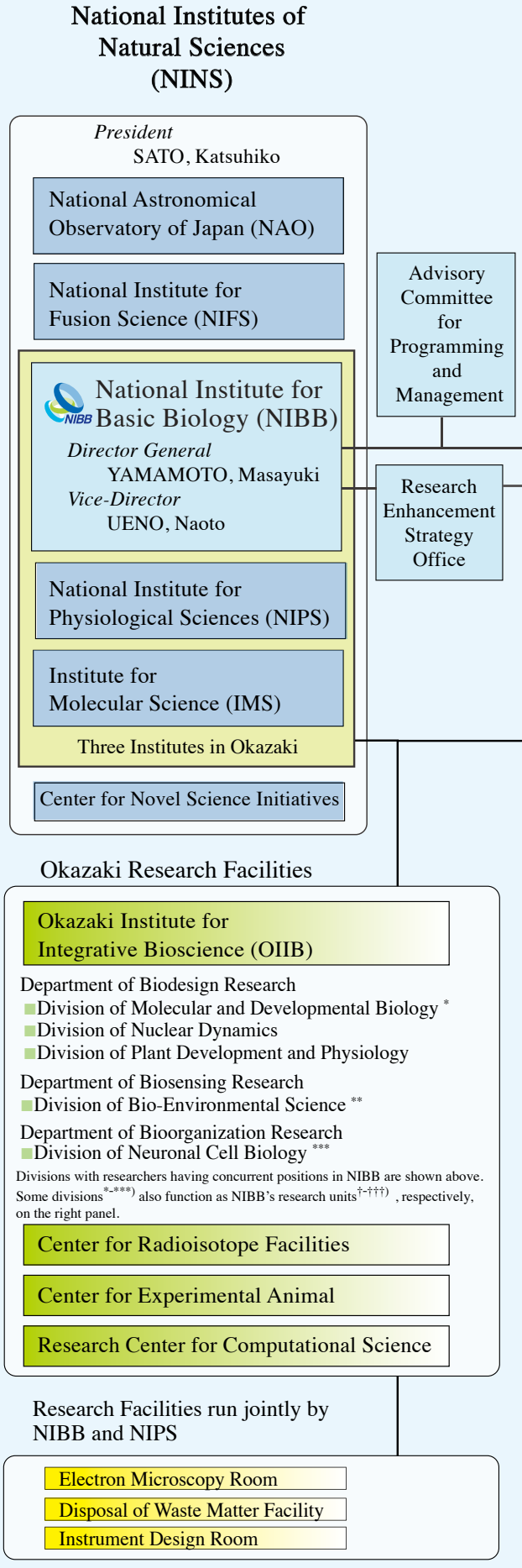
Policy, Decision Making, and Administration

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. 88) 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, 2015

Research Units

- **Cell Biology**
  - Laboratory of Cell Responses
  - Laboratory of Neuronal Cell Biology†††
  - Laboratory of Cell Sociology
  - 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 Brain Circuits
  - Laboratory of Neurophysiology
- **Evolutionary Biology and Biodiversity**
  - Division of Evolutionary Biology
  - Division of Symbiotic Systems
  - Laboratory of Morphodiversity
  - Laboratory of Bioresources
  - Laboratory of Biological Diversity
- **Environmental Biology**
  - Division of Molecular Environmental Endocrinology ††
  - 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

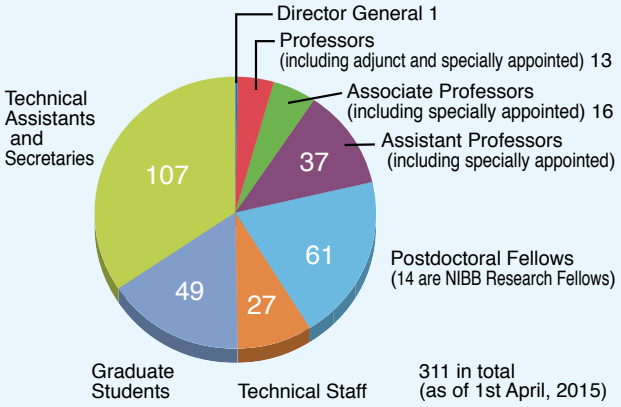
- **Okazaki Administration Office**

Research and Research Support

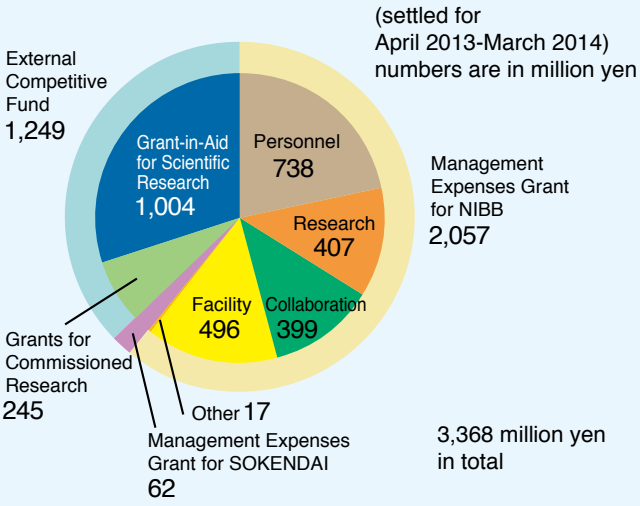
NIBB's research programs are conducted in research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research 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 bioresource preservation technology have been 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. The Center for Radioisotope Facilities is one of the latter and is 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 its 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, 2015)

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
	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	MATSUZAKI, Masanori	Professor, National Institute for Basic Biology
	MINAGAWA, Jun*	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 2015
\# 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. Projects for the development of bioresource preservation technology are solicited by the IBBP center. For these projects, research expenses in addition to travel expenses are provided

year	2011	2012	2013	2014
Priority collaborative research projects	6	5	2	1
Collaborative research projects for model organisms/ technology development	2	3	4	2
Individual collaborative research projects	88	89	89	87
NIBB workshops	6	6	4	3
Collaborative experiments using the large spectrograph	9	14	15	12
Collaborative experiments using the DSLM	8	5	9	10
Collaborative experiments using the next generation DNA sequencer	45	47	41	37
Facility Use (Training Course Facility)	0	2	1	0
Collaborative research projects for bioresource preservation technology development			9	10
total	164	171	174	162

Collaborative Research Projects by Year

NIBB Core Research Facilities

The NIBB Core Research Facilities were launched in 2010 to support research in NIBB and also to 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. 74).

The Functional Genomics Facility maintains a wide 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. 95). 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.

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.84).

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. The 10th NIBB-EMBL symposium "Quantitative Bioimaging" was held in March, 2013.

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 (p.92).

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 62nd conference "Force in Development" was held in November, 2014 (p.91).

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 (p. 93). 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.

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. 80).

Strains of Japanese morning glory maintained in the center

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, a Facebook page, and a Twitter account. 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. 72). The application of the adaptive optics to microscopy is under way in collaboration with the National Astronomical Observatory. 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 8th Forum held in March 2014 was immediately followed by the Meeting for the Future Plan of Bio-Imaging Network and served an important role in preparing for formation of a scientific community network in the field of imaging science (p. 94). A training course in bioimage analysis was also held in 2014 (p. 95).

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. The OBC10 is being planned with a new framework on the energy production in chroloplasts.

Cultivation of Future Researchers

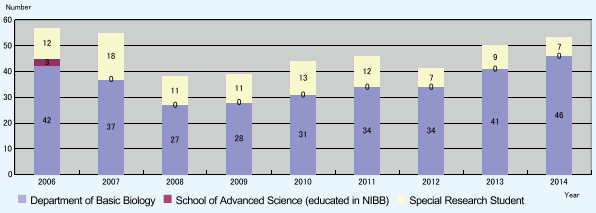
NIBB constitutes the Department of Basic Biology in the School of Life Science of the SOKENDAI (Graduate University for Advanced Studies). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree.

Graduate students enrolled in other universities and institutions can apply to be special research students eligible to conduct research for fixed periods of time 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.

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. 96).



Graduate students educated by NIBB

Personnel changes in 2014*

Newly assigned in NIBB

Name	Position	Research Unit	Date
TAKAJI, Masafumi	NIBB Research Fellow	Division of Brain Biology	February 1
MIYANARI, Yusuke	Specially Appointed Associate Professor	Laboratory of Nuclear Dynamics (Orion Project)	March 1
NISHIMURA, Mikio	Specially Appointed Professor	Research Enhancement Strategy Office	April 1
YAMASHITA, Akira	Specially Appointed Associate Professor	Laboratory of Cell Responses	April 1
OKADA, Kazunori	NIBB Research Fellow	Division of Molecular and Developmental Biology	April 1
NOMURA, Kengo	NIBB Research Fellow	Division of Molecular Neurobiology	April 1
MASAMIZU, Yoshito	Assistant Professor	Division of Brain Circuits	June 1
TATEMATSU, Kiyoshi	Specially Appointed Assistant Professor	Research Enhancement Strategy Office	July 1
TAKAHASHI, Shunichi	Associate Professor	Division of Environmental Photobiology	October 1
EBINA, Teppei	NIBB Research Fellow	Division of Brain Circuits	November 1
SHI, Dongbo	NIBB Research Fellow	Division of Embryology	December 1

Newly affiliated in other universities and institutes

Name	New Affiliation	Position	Date
MATSUBAYASHI, Yoshikatsu	Nagoya University	Professor	April 1
SHINOHARA, Hidefumi	Nagoya University	Assistant Professor	April 1
YAMADA, Kenji	Kyoto University	Assistant Professor	April 1
WAKE, Hiroaki	National Institute for Physiological Sciences	Associate Professor	April 1

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

Awardees in 2014

Name	Position	Award
SHIMMURA, Tsuyoshi	Specially Appointed Assistant Professor	The Japanese Society of Animal Science, Encouragement Award
TOYOTA, Kenji	SOKENDAI Graduate Student	The Students' Most Valuable Oral Presentation Award, Chubu Branch Meeting, The Zoological Society of Japan
SAKAE, Yudai	SOKENDAI Graduate Student	The Students' Valuable Poster Presentation Award, Chubu Branch Meeting, The Zoological Society of Japan
FUKUSHIMA, Kenji	SOKENDAI Graduate Student	SOKENDAI President's Award
TOYOTA, Kenji	SOKENDAI Graduate Student	SOKENDAI President's Award
FUJIMORI, Chika	Postdoctoral Fellow	The Young Researchers' Most Valuable Oral Presentation Award, The Japan Society for Comparative Endocrinology

DIVISION OF CELL MECHANISMS †

Professor
NISHIMURA, Mikio

Assistant Professor:	*MANO, Shoji*
	YAMADA, Kenji
Technical Staff:	*KONDO, Maki*
NIBB Research Fellow:	*GOTO-YAMADA, Shino*
Postdoctoral Fellow:	*KANAI, Masatake*
	WATANABE, Etsuko
	KAMIGAKI, Akane
	NITO, Kazumasa
SOKENDAI Graduate Student:	*SHIBATA, Michitaro*
Technical Assistant:	*SAITO, Miyuki*
	HIKINO, Kazumi
	YOSHINORI, Yumi
	YAMAGUCHI, Chinami
	NAKAI, Atsushi
Secretary:	*UEDA, Chizuru*

Since plants spread their roots in the ground, they must survive in a given environment. To adapt to the environment, they positively utilize environmental changes in their life cycle as important signals that are necessary for their survival. Plant cells can induce, degenerate and differentiate their organelles to adapt to environmental changes. The flexibility of plant organelles is the basis of the strategy for environmental adaptation in plants.

The aim of this division is to clarify the molecular mechanisms underlying the induction, differentiation, and interaction of organelles, and to understand the integrated function of individual plants through organelle dynamics. The Scientific Research on Innovative Areas “Environmental sensing of plants: Signal perception, processing and cellular responses” was started to clarify the molecular mechanisms underlying organelle differentiation and interaction.

I. Reversible transformation of plant peroxisomes

Dramatic metabolic changes that underlie the shift from heterotrophic to autotrophic growth occur in the greening of seed germination. Accompanying these metabolic changes are the functional transformations of many constitutive organelles. Etioplasts differentiate into chloroplasts while mitochondria acquire the ability to oxidize glycine. Glyoxysomes, which are peroxisomes engaged in the degradation of reserve oil stored in the oil body via β-oxidation and the glyoxylate cycle, are transformed into leaf peroxisomes that function in several crucial steps of photorespiration. After the functional transition of glyoxysomes to leaf peroxisomes during the greening of pumpkin cotyledons, the reverse transition of leaf peroxisomes to glyoxysomes occurs during senescence. Gene expression, alternative splicing, protein translocation and protein degradation control the functional transformation between glyoxysomes and leaf peroxisomes.

II. Transcriptomics, proteomics and phenomics of plant peroxisomes

Enzymes localized in plant peroxisomes are synthesized in the cytosol and function after their post-translational transport into peroxisomes. Almost all of the peroxisomal matrix proteins contain one of two targeting signals (PTS1 and PTS2) within the molecules. PTS1 is a unique tripeptide sequence found in the carboxyl terminus of the mature proteins. In contrast, PTS2 is involved in a cleavable amino terminal presequence of peroxisomal proteins that are synthesized as a precursor protein with larger molecular mass.

We identified 256 gene candidates of PTS1- and PTS2-containing proteins and another 30 genes of non-PTS-containing proteins from the *Arabidopsis* genome. Custom-made DNA microarrays covering all these genes were used to investigate expression profiles of the peroxisomal genes in various organs. They revealed that peroxisomes in root cells play a pivotal role in polyamine catabolism. We also made a two-dimensional protein map of glyoxysomes and leaf peroxisomes isolated from *Arabidopsis* and soybean. Peptide MS fingerprinting analyses allowed us to identify novel peroxisomal membrane proteins, i.e. voltage-dependent anion-selective channel and adenine nucleotide carrier 1 (PNC1). We also found that peroxisomal membrane ATP-binding cassette transporter promotes seed germination by inducing pectin degradation under the control of abscisic acid signaling. The overall results provide us with new insights into plant peroxisomal functions.

Bioinformatic analysis of the *Arabidopsis* genome predicted the presence of 15 kinds of genes, called *PEX* genes, for peroxisomal biogenesis factors. We demonstrated that PEX5 and PEX7 form a cytosolic receptor complex and recognize PTS1- and PTS2-containing proteins, respectively. PEX14 is a peroxisomal membrane docking protein that captures the receptor-cargo complex. We also comprehensively investigated whether or not these predicted *PEX* genes function in peroxisome biogenesis by generating knock-down mutants that suppress *PEX* gene expression by RNA-interference. Phenotypes of these mutants allowed us to identify the functional *PEX* genes, which can be classified into two groups: *PEX* genes regulating for peroxisomal protein import and *PEX* genes regulating for peroxisomal morphology. We continue to investigate the detailed molecular functions of other *PEX* genes. Of these, we recently proposed that function of PEX7 is maintained by a quality control mechanism involving RabE1c.

III. Identification of novel components essential for peroxisome biogenesis

To better understand peroxisome biogenesis and functions, we isolated a number of *Arabidopsis* mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants) based on them having a different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes, numbers and distribution can be visualized with GFP.

Up to date, we reported the function of gene-products of *APEM1*, *APEM2*, *APEM3*, *APEM4* and *APEM9*. Recently,

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

we found that *APEM10* encodes Lon protease 2, which has roles in chaperon and proteinase, and that modulates peroxisome degradation processed by autophagy. Taken together with the analyses using *peup1*, *peup2* and *peup4* mutants, which were defective in Autophagy-related 2, (ATG2), ATG18a and ATG7, respectively, we were able to update the model for functional transformation of peroxisomes (Figure 1).

We are currently characterizing other *apem* and *peup* mutants. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, functions and maintenance, and to address the mechanism at the molecular level.

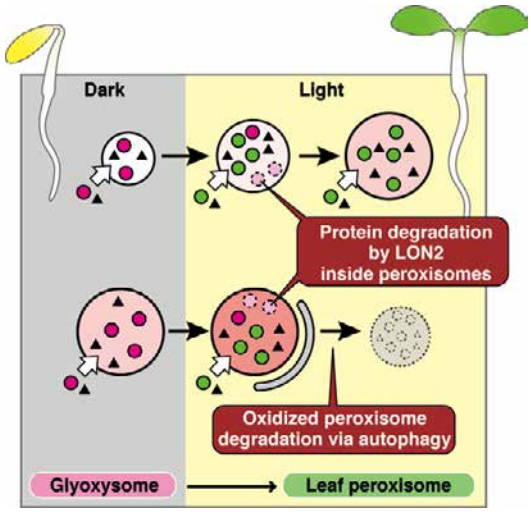


Figure 1. A model for the coordinated regulation of functional transformation of peroxisomes and peroxisome quality control by LON2/APEM10 and autophagy during functional transition.

IV. ER derived organelles for protein storing and defense strategy

Plant cells develop various types of endoplasmic reticulum (ER)-derived structures with specific functions. ER bodies are ER-derived compartments observed in *Arabidopsis*. They are rod-shaped structures surrounded by ribosomes, and are widely distributed in the epidermal cells of whole seedlings (Figure 2). Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid treatment. ER bodies accumulate β-glucosidase PYK10. When plant cells are damaged, PYK10 forms large protein aggregates. The aggregate formation increases glucosidase activity. These findings suggest that ER bodies function in the defense against pathogens and herbivores, possibly producing toxic products. *Arabidopsis nai1* mutants have no ER bodies in the entire plant and do not accumulate PYK10. *NAI1* encodes a transcription factor and regulates the expression of *PYK10* and *NAI2*. The *Arabidopsis nai2* mutant has no ER bodies and reduced accumulation of PYK10. *NAI2* encodes a unique protein that localizes to the ER body. Membrane protein of ER body 1 (MEB1) and MEB2 are integral membrane proteins of the ER body and have iron/manganese transport activity. These results suggest that the ER body has specific membrane proteins that are involved in defense against metal stress as well as pathogens and herbivores. We are now investigating ER body formation and function using ER body deficient mutants, and heterologously expressing NAI2 in onion and tobacco cells.

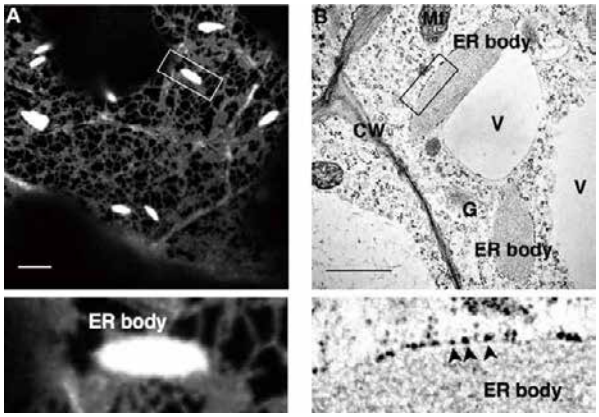


Figure 2. ER bodies in *Arabidopsis thaliana*. A confocal micrograph (A) and an electron micrograph (B) of cotyledon and root epidermal cells, respectively. Arrowheads indicate ribosomes on the surface of the ER body membranes. ER-localized GFP labels ER bodies as well as the typical ER network, and electron microscopy identifies ribosomes at the cytosolic surface of ER bodies, both of which indicate the luminal continuity between ER and ER bodies. Enlarged images of the squared regions are shown below. CW, cell wall; V, vacuole; Mt, mitochondrion; G, Golgi body; Bars,10μm (A) and 1μm (B).

V. Roles of molecular chaperones on cell differentiation

Molecular chaperones are cellular proteins that function in the folding and assembly of certain other polypeptides into oligomeric structures. We have found that HSP90 inhibitor induces genes with heat shock response element (HSE) motifs in their promoters, suggesting that heat shock transcription factor (HSF) is involved in the response. *Arabidopsis* HSFs interacted with HSP90. Thus, it appears that in the absence of heat shock, HSP90 actively suppresses HSF function. During heat shock, HSP90 is transiently inactivated, which leads to HSF activation. These data indicate that HSP90 regulates correct gene expression for heat acclimatization in plants. We also observed that HSP90 is involved in hormone responses in *Arabidopsis*. The evolutional and functional characterizations are now being investigated.

VI. Update of The Plant Organelles Database 3 (PODB3) and Plant Organelles World

The Plant Organelles Database 3 (PODB3) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. 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. The electron micrograph database, which was added as new content, provides information on the ultrastructures in plant cells. The perceptive organelles database shows organelle dynamics in response to environmental stimuli. The organelles movie database contains time-lapse images and 3D structure

rotations. The organellome database is a compilation of static image data of various tissues of several plant species at different developmental stages. The functional analysis database is a collection of protocols for plant organelle research. Through these databases, users can easily grasp plant organelle dynamics. Plant Organelles World, which is based on PODB3 is an educational tool to engage members of the non-scientific community to explore plant biology. We hope that PODB3 and Plant Organelles World are of help to researchers and the general public.

Figure 3. The graphical user interface of the PODB3 (http://podb.nibb.ac.jp/Orgenellome).

Publication List

〔Original papers〕

- Goto-Yamada, S., Mano, S., Nakamori, C., Kondo, M., Yamawaki, R., Kato, A., and Nishimura, M. (2014). Chaperone and protease functions of LON protease 2 modulate the peroxisomal transition and degradation with autophagy. Plant Cell Physiol. *55*, 482-496.
- Mano, S., Nakamura, T., Kondo, M., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. (2014). The Plant Organelles Database 3 (PODB3) update 2014: integrating electron micrographs and new options for plant organelle research. Plant Cell Physiol. *55*, e1.
- Shibata, M., Oikawa, K., Mano, S., and Nishimura, M. (2014). Measurement of the number of peroxisomes. Bio-Protoc. *4*, e1284.
- Yoshimoto, K., Shibata, M., Kondo, M., Oikawa, K., Sato, M., Toyooka, K. Shirasu, K. Nishimura, M., and Ohsumi, Y. (2014). Organ-specific quality control of plant peroxisomes is mediated by autophagy. J. Cell Sci. *127*, 1161-1168.

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

- Motomura, K., Le, Q.T., Hamada, T., Kutsuna, N., Mano, S., Nishimura, M., and Watanabe, Y. Diffuse DCP2 accumulates in DCP1 foci under heat stress in *Arabidopsis thaliana*. Plant Cell Physiol. 2014 Oct 22.

〔Review articles〕

- Goto-Yamada, S., Mano, S., and Nishimura, M. (2014). The role of peroxisomes in plant reproductive processes. In Sexual reproduction in animals and plants. – Edited by Sawada, H., Inoue, N., and Iwano, M. Springer Japan, pp. 419-429.
- Goto-Yamada, S., Mano, S., Oikawa, K., Shibata, M., and Nishimura, M. (2014). Interaction between chaperone and protease functions of LON2, and autophagy during the functional transition of peroxisomes. Plant Signal. Behav. *9*, e28838.
- Nakano, R.T., Yamada, K., Bednarek, P., Nishimura, M., and Hara-Nishimura, I. (2014). ER bodies in plants of the Brassicales order: Biogenesis and association with innate immunity. Front. Plant Sci. *5*, 73, doi: 10.3389/fpls.2014.00073.
- Shibata, M., Oikawa, K., Yoshimoto, K., Goto-Yamada, S., Mano, S., Yamada, K., Kondo, M., Hayashi, M., Sakamoto, W., Ohsumi Y., and Nishimura, M. (2014). Plant autophagy is responsible for peroxisomal transition and plays an important role in the maintenance of peroxisomal quality. Autophagy *10*, 936-937.

DIVISION OF INTERCELLULAR SIGNALING BIOLOGY †

Professor
MATSUBAYASHI, Yoshikatsu

Assistant Professor:	*SHINOHARA, Hidefumi*
NIBB Research Fellow:	*TABATA, Ryo*
Postdoctoral Fellow:	*KAKITA, Mitsuru*
	OKAMOTO, Satoru
Technical Assistant:	*OGAWA-OHNISHI, Mari*
	SUMIDA, Kumiko
	YASUE, Naoko
Secretary:	*OKUBO, Masayo*

Cell-to-cell signaling mediated by secreted signals and membrane-localized receptors is one of the critical mechanisms by which growth and development of multicellular organisms are cooperatively regulated. Signal molecules that specifically bind receptors are generally referred to as ligands. Because membrane-localized receptors act as master switches of complex intracellular signaling, identification of the ligand-receptor pair is one of the central issues of post-genome research. We are working to clarify the mechanisms by which plant development is regulated through identification of novel ligands such as small peptides and their specific receptors using *Arabidopsis* genome information, biochemical analysis and phenotypic observation.

I. Secreted peptide signals

Following complete sequencing of the *Arabidopsis* genome, a number of genes encoding small secreted peptides have been identified by *in silico* database analysis. Based on our own analysis, we identified 979 putative secreted peptide genes with an open reading frame (ORF) size between 50 and 150 amino acids in the *Arabidopsis* genome. These 979 ORFs include many functionally uncharacterized peptides. Although estimation of the total number of biologically relevant secreted peptide signals is difficult at present, the presence of many "orphan receptors" among receptor-like kinases in *Arabidopsis* suggest that a substantial number of intercellular signals remain to be identified.

One structurally characteristic group of peptide signals is "post-translationally modified small peptides". These peptides are characterized by the small size of mature peptides (less than 20 amino acids) and the presence of post-translational modifications. In these peptide signals, peptide chain length and post-translational modifications are generally very important for their receptor binding activity and physiological functions.

1-1 C-terminally encoded peptide (CEP)

C-terminally encoded peptide (CEP) is a 15-amino-acid peptide involved in mediating long-distance nitrogen (N)-demand signaling. The CEP family was identified by *in silico* screening for a family of secreted peptides that share short, conserved domains near the C-terminus, a feature that is common to several posttranslationally modified small peptide signals in plants. CEP1 is secreted as a 15-amino acid peptide originating from a C-terminal conserved domain (the CEP domain) through posttranslational proline hydroxylation and proteolytic processing. A total of 15 *CEP* family genes (*CEP1* through *CEP15*) have been found in the *Arabidopsis* genome.

When external N availability is lowered, CEP expression is promptly upregulated in the portion of the root system directly experiencing N starvation. CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by CEP receptors leads to the production of a putative shoot-derived descending signal that upregulates nitrate transporter genes in the roots. This mechanism supports N acquisition when nitrate is unevenly distributed within the soil (Figure 1). CEP family peptides induced on one side of the roots by local N starvation mediate upregulation of nitrate transporter genes in the distant part of the roots exposed to N-rich conditions to compensate for N deficiency.

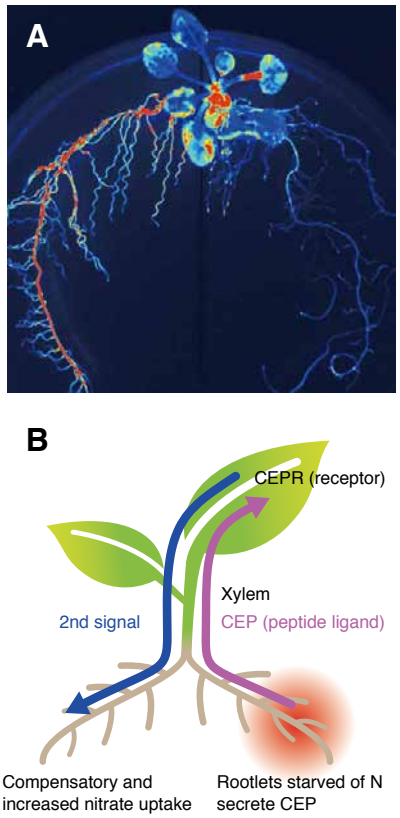


Figure 1. CEP mediates systemic N-demand signaling. (A) Systemic N-demand signaling visualized by *pNRT2.1:LUC*. N starvation on one side (right) of the root system leads to an upregulation of nitrate uptake on the other side of the root system (left). (B) Mode of action of CEP family peptides in systemic N-demand signaling. N-starvation induces CEP expression in roots. CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by CEPR leads to the production of a putative shoot-derived descending signal that upregulates nitrate transporter genes in the roots.

1-2 Other novel peptide signal candidates

The common feature of known small post-translationally modified peptide signals is that they are encoded by multiple paralogous genes whose primary products are approximately 70- to 110-amino-acid cysteine-poor secreted polypeptides

†: This laboratory was closed on 31 March, 2014.

that share short conserved domains near the C-terminus. We have identified several novel polypeptide families that fulfill the above criteria by *in silico* screening and determined their mature structures by analyzing apoplastic peptide fractions by nano LC-MS/MS. Functional analysis of these peptides is now going on.

II. Receptors for secreted peptide signals

The receptors or putative receptors for peptide signals identified to date belong to the receptor kinase (RK) or receptor-like protein (RLP) families. Among RKs, the largest subfamily is the leucine-rich repeat RK (LRR-RK) family, which consists of 216 members in *Arabidopsis*. The majority of receptors for small post-translationally modified peptide signals belong to this family. Especially, an increasing number of LRR X and LRR XI members are now being confirmed as receptors for several endogenous small peptide ligands, suggesting that these subgroups are an attractive target for binding analysis with novel peptide signals.

Although both genetic and biochemical methods have been used to identify ligand-receptor pairs in plants, genetic redundancy often interferes with the former approach, and the low levels at which ligand and receptor molecules are often present in tissues can make the latter approach very difficult. As described above, an increasing number of ligand candidates are being identified. If individual receptor kinases could be functionally overexpressed in certain cells at sufficiently high levels and sufficiently high quality for biochemical binding analysis, such a receptor library would facilitate identification of ligand-receptor pairs in plants. To this end, we established a functional and efficient expression system of plant receptor kinases in tobacco BY-2 cells and prepared an expression library of all the potential receptor candidates. This approach was successfully used for identification of CEP receptors.

Publication List

〔Original paper〕

- Bidadi, H., Matsuoka, K., Sage-Ono, K., Fukushima, J., Pitaksaringkarn, W., Asahina, M., Yamaguchi, S., Sawa, S., Fukuda, H., Matsubayashi, Y., Ono, M., and Satoh, S. (2014). CLE6 expression recovers gibberellin deficiency to promote shoot growth in *Arabidopsis*. Plant J. *78*, 241-252.
- Tabata, R., Sumida, K., Yoshii, T., Ohyama, K., Shinohara, H., and Matsubayashi, Y. (2014). Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Science *346*, 343-346.

〔Review article〕

- Matsubayashi, Y. (2014). Posttranslationally modified small-peptide signals in plants. Annu. Rev. Plant Biol. *65*, 385–413.

LABORATORY OF CELL RESPONSES

Director General
YAMAMOTO, Masayuki

Specially Appointed Associate Professor
YAMASHITA, Akira

Postdoctoral Fellow: *SHICHINO, Yuichi*
Visiting Scientist: *OTSUBO, Yoko*
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, TORC1 and TORC2. TORC1 contains Tor2 as its catalytic subunit and is essential to suppress sexual differentiation in the presence of nitrogen. However, the critical effectors of TORC1 that function in the suppression of sexual differentiation have remained largely unknown.

We demonstrated that TORC1 could phosphorylate an RNA-binding protein, Mei2, *in vitro*. Mei2 is the master regulator that switches the cell cycle from mitotic to meiotic. Mei2 is also known to be involved in earlier steps of sexual differentiation. We found that non-phosphorylatable Mei2, in which the nine phosphorylation sites were changed to alanine, became more stable than the wild-type in the presence of nitrogen. We further showed that Mei2 was polyubiquitylated *in vivo* in a TORC1-dependent manner.

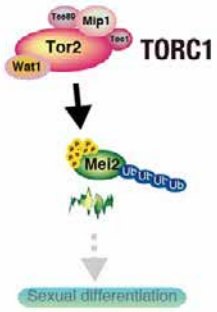


Figure 1. A schematic of the suppression of sexual differentiation by TORC1 through phosphorylation of Mei2.

From these observations we propose that TORC1 contributes to the suppression of sexual differentiation in the presence of rich nutrition through phosphorylation and destabilization of Mei2 (Figure 1) (Otsubo et al., 2014).

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

Expression of hundreds of genes is 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 meiosis-specific nuclear body in fission yeast, called Mei2 dot, promotes the progression of meiosis by sequestering and inhibiting Mmi1 protein, a crucial factor involved in the selective elimination of meiosis-specific transcripts (Figure 2). The Mei2 dot is composed of the RNA-binding protein Mei2 and a long non-coding RNA species termed meiRNA. We have shown previously that Mei2 physically interacts with meiRNA and forms the dot structure at the chromosomal *sme2* locus, which encodes meiRNA, depending on the transcription of meiRNA (Figure 2). We have further shown that meiRNA carries numerous copies of the DSR motif, which is recognized specifically by Mmi1, and that meiRNA is indeed a target eliminated by Mmi1. Thus, the molecular mechanisms underlying the specific localization of meiRNA to its genetic locus and the importance of this localization in the inactivation of Mmi1 provide interesting questions.

We found that Mmi1 is essential to anchor meiRNA to its coding locus *sme2*. Meanwhile, overexpression of meiRNA promoted accumulation of Mmi1 to the *sme2* locus and reduced active Mmi1 in the cell. These findings indicate that Mmi1 facilitates the retention of meiRNA at its genetic locus as it is transcribed, and that the anchored meiRNA then attracts Mmi1 and inhibits the function of this protein (Shichino et al., 2014).

III. Meiosis-specific cell cycle regulation

In both mitosis and meiosis, the accumulation and activation of M-phase promoting factor (MPF), composed of Cdk and B-type cyclin (Cdc2 and Cdc13 in *S. pombe*, respectively) is required before nuclear division. When cells exit from M phase to enter the next phase of the cell cycle, the MPF level must be reduced. In meiosis, how is the MPF

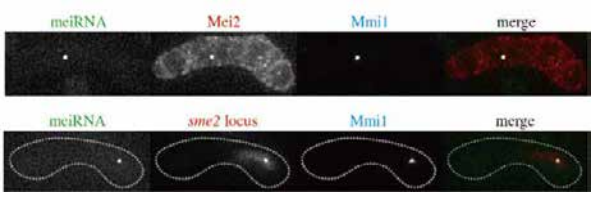


Figure 2. Co-localization of the Mei2 dot components. The upper panels display localization of meiRNA, Mei2 and Mmi1, and the lower panels display localization of meiRNA, the *sme2* locus and Mmi1. The cellular contour is shown in a dotted line.

level regulated between the first meiotic division (meiosis I) and the second meiotic division (meiosis II), during which two sequential nuclear divisions take place? We are interested in the molecular mechanisms enabling the meiotic cell cycle. We have shown previously that a meiosis-specific inhibitor of the anaphase-promoting complex/cyclosome (APC/C), namely Mes1, protects Cdc13 from complete destruction at anaphase of meiosis I. This secures the retention of a sufficient level of MPF activity to conduct meiosis II.

We isolated mutants defective in *spo5*, which encodes a meiosis-specific RNA-binding protein, in a new screening to identify mutants deficient in the progression of meiosis II. We found that *spo5* mutant cells lost Cdc13 prematurely prior to meiosis II (Figure 3). The defect in meiosis II in the *spo5* mutants was recovered by increasing the CDK activity. Furthermore, accumulation of *cdc13* transcripts during meiosis II was lower in *spo5* mutant cells than in wild-type cells. These findings indicate that Spo5 is a novel factor that contributes to the regulation of the expression of Cdc13 during meiosis (Arata *et al*. 2014).

Publication List

〔Original papers〕

- Aoi, Y., Kawashima, S.A., Simanis, V., Yamamoto, M., and Sato, M. (2014). Optimization of the analogue-sensitive Cdc2/Cdk1 mutant by in vivo selection eliminates physiological limitations to its use in cell cycle analysis. Open Biol. 4, 140063.
- Arata, M., Sato, M., Yamashita, A., and Yamamoto, M. (2014). The RNA-binding protein Spo5 promotes meiosis II by regulating cyclin Cdc13 in fission yeast. Genes Cells 19, 225-238.
- Hirai, H., Arai, K., Kariyazono, R., Yamamoto, M., and Sato, M. (2014). The kinetochore protein Kis1/Eic1/Mis19 ensures the integrity of mitotic spindles through maintenance of kinetochore factors Mis6/CENP-I and CENP-A. PLoS One 9, e111905.
- Okada, N., Toda, T., Yamamoto, M., and Sato, M. (2014). CDK-dependent phosphorylation of Alp7-Alp14 (TACC-TOG) promotes its nuclear accumulation and spindle microtubule assembly. Mol. Biol. Cell 25, 1969-1982.
- Otsubo, Y., Yamashita, A., Ohno, H., and Yamamoto, M. (2014). S. pombe TORC1 activates the ubiquitin-proteasomal degradation of the meiotic regulator Mei2 in cooperation with Pat1 kinase. J Cell Sci. 127, 2639-2646.
- Shichino, Y., Yamashita, A., and Yamamoto, M. (2014). Meiotic long non-coding meiRNA accumulates as a dot at its genetic locus facilitated by Mmi1 and plays as a decoy to lure Mmi1. Open Biol. 4, 140022.
- Togashi, N., Yamashita, A., Sato, M., and Yamamoto, M. (2014). Functional significance of nuclear export and mRNA binding of meiotic regulator Spo5 in fission yeast. BMC Microbiol. 14, 188.

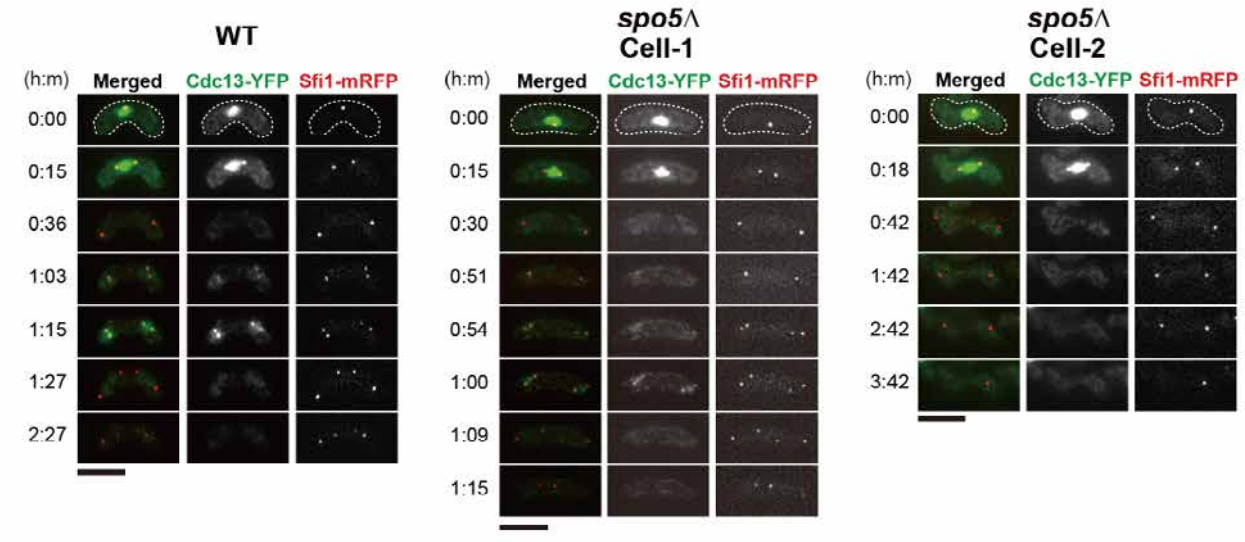


Figure 3. Cdc13 disappears prematurely in the *spo5* mutants. Cdc13 and a spindle pole body marker Sfi1 were monitored in wild-type and *spo5* mutant cells in the course of meiosis. In wild-type cells, Cdc13 signal diminished at anaphase of meiosis I but reappeared at the beginning of meiosis II. In *spo5* mutant cells, Cdc13 signal reappeared only faintly (Cell-1) or did not reappear (Cell-2).

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 mechanism of localized protein synthesis using mice in order to better understand its relation to the formation of synapses and neural networks, memory, learning, and behavior.

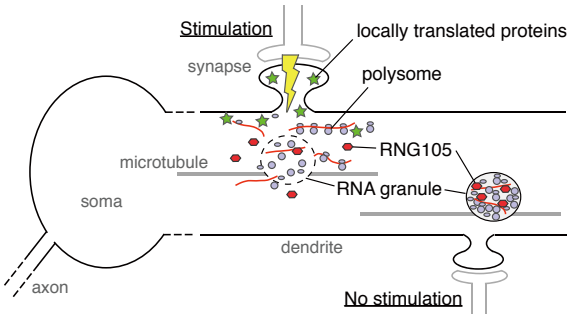


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Dendritic mRNA localization and learning and memory in RNG105 conditional knockout mice

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 (Shiina *et al*., J. Neurosci. 30, 12816-12830, 2010). 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).

To investigate the role of RNG105 in the adult mouse brain, we have generated RNG105 conditional knockout (cKO) mice in which the *rng105* gene was disrupted in the brain after birth. Using RNG105 cKO adult mice, we performed comprehensive analysis of dendritic localization of mRNAs. Brain slices were dissected to isolate somatic and dendritic layers of hippocampal CA1 neurons, and from each layer, RNA was purified and analyzed by deep sequencing. Comparison of mRNAs from dendritic and somatic layers identified mRNAs concentrated in dendrites and mRNAs concentrated in the soma (Figure 2). The difference in the mRNA composition between dendrites and soma was relatively small in RNG105 cKO mice compared to control mice (Figure 2), which suggested that the dendritic mRNA composition in RNG105 cKO neurons changed toward the somatic type, i.e., dendritic localization of specific mRNAs was reduced in RNG105 cKO mice. Gene ontology analysis revealed that dendritically enriched mRNAs included small G protein regulators and translation regulators, which were reduced in dendrites of RNG105 cKO mice. We are going to investigate whether proteins encoded by these mRNAs are involved in dendritic synapse function and neuronal network formation.

We further analyzed behavior of RNG105 cKO mice. Passive avoidance test is one of the learning and memory tests, in which mice receive an electric foot shock in a room and thereafter they remember the situation and do not enter the room for more than several days. In this test, RNG105 cKO mice did not enter the room at 5 minutes after the foot shock, but entered the room after 24 hours. We performed another learning and memory test, Morris water maze. In this test, mice learn the location of a hidden platform that allows the mice to escape from water. After several days of training, control mice learned the platform location and escape on the platform faster than before the training. However, the training did not shorten the latency of RNG105 cKO mice to

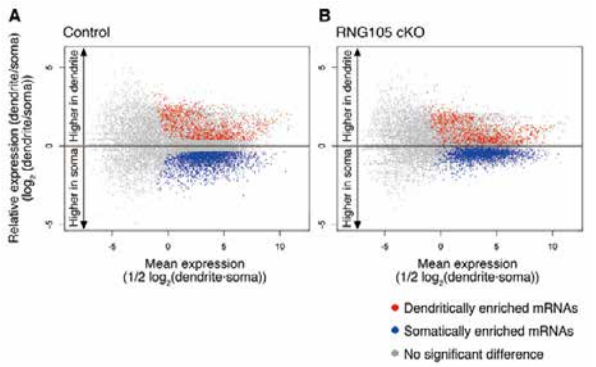


Figure 2. Comparison of mRNA composition between dendrite and soma of hippocampal CA1 neurons. (A) RNAs from triplicate samples were analyzed with Cufflinks. The graph shows MA plot between dendrite RNAs and soma RNAs from control neurons. The x-axis shows average $\log_2$ expression of dendrite RNAs and soma RNAs and the y-axis shows $\log_2$ fold change of dendrite RNAs vs. soma RNAs. Gray, all RNAs; red, mRNAs significantly concentrated in dendrites; blue, mRNAs significantly concentrated in soma. (B) MA plot between dendrite RNAs and soma RNAs from RNG105 cKO neurons. The same mRNAs are colored the same as in A.

find the platform. These results suggested that RNG105 cKO mice had deficits in learning and memory.

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). We have obtained RNG140 knockout mice and examined RNG140 mRNA expression by qRT-PCR. RNG140 mRNA was most highly expressed in the brain and significantly reduced in the brain of RNG140 knockout mice. We are going to investigate the role of RNG140 in higher brain functions in adult mice.

II. RNA granule assembly and disassembly modulated by NFAR2 and NF45

We used proteomic analyses to identify proteins associated with RNG105. Among the identified proteins, we focused on nuclear factor associated with dsRNA 2 (NFAR2) and its binding partner, nuclear factor 45 (NF45). NFAR2 co-localized with and enhanced the assembly of RNG105-containing RNA granules, whereas NF45 induced the disassembly of RNA granules (Figure 3).

NFAR2 has a GQSY domain that is structurally and functionally similar to the low complexity (LC) sequence domain of FUS/TLS, which is known to drive RNA granule assembly. We have found that the GQSY domain of NFAR2 has the ability to interact with RNG105-containing messenger ribonucleoprotein (mRNP) complexes and enhances the assembly of RNG105-containing RNA granules.

Another domain of NFAR2, the DZF domain, was not necessary for the interaction with the RNG105 mRNP complexes, but was involved in positive and negative regulation of RNA granule assembly by being phosphorylated by PKR, a master kinase inducing RNA granule assembly, and by association with NF45, respectively (Figure 3).

Analysis of translation activity at the single cell level by ribopuromycilation assay revealed that NFAR2 repressed translation, but the NFAR2-dependent repression was de-repressed by NF45.

Our results suggest a model in which NFAR2 functions as a connector of RNG105 mRNP complexes through its multivalent domains, i.e., the GQSY domain and the DZF domain, in the assembly of RNA granules that is linked with translation repression. The connector function may be enhanced by phosphorylation by PKR, whereas it is blocked by NF45 binding, which disassembles RNA granules and de-represses translation. We are going to elucidate the roles of NFAR2 and NF45 in neurons, including their relation to neurodegeneration, because defective regulation of RNA granule assembly by LC sequence domain-containing proteins such as FUS/TLS and TDP-43 has been recently suggested to be associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).

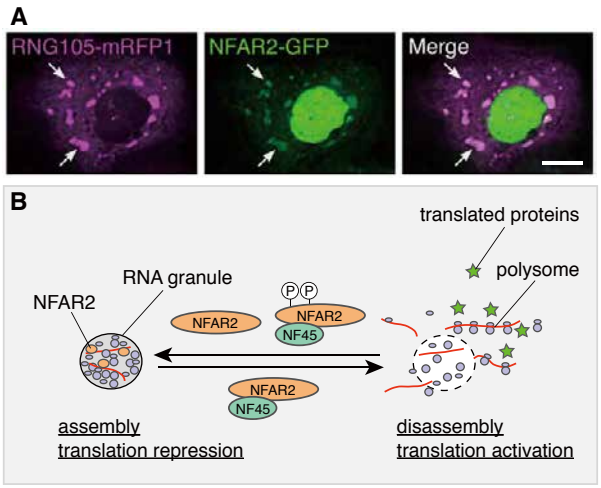


Figure 3. Effects of NFAR2 and NF45 on the assembly of RNA granules and translation activity. (A) A6 cells were co-transfected with RNG105-monomeric red fluorescent protein 1 (mRFP1) and NFAR2-GFP. NFAR2 was predominantly localized to the nucleus, and also co-localized to and enlarged RNG105-containg RNA granules (arrows). Scale bar, 10 μm. (B) A model of RNA granule assembly and translation activity modulated by NFAR2 and NF45. NFAR2 enhances RNA granule assembly and represses translation. NF45 binds to NFAR2, disassembles RNA granules and de-represses translation. Phosphorylation of NFAR2 by PKR reduces the effect of NF45.

Publication List

〔Original paper〕

● Shiina, N., and Nakayama, Kei. (2014). RNA granule assembly and disassembly modulated by nuclear factor associated with double-stranded RNA 2 and nuclear factor 45. J. Biol. Chem. *289*, 21163-21180.

LABORATORY OF CELL SOCIOLOGY

Assistant Professor
HAMADA, Yoshio

Technical Assistant: *GONDA, Naoko*

Mammalian eggs implant onto the maternal uterine wall after hatching at the blastula stage, due to a lack of enough nutrients to support development until birth. In order to transfer nutrients and oxygen into the fetus, and metabolic wastes into the mother, the placenta has evolved to establish close connections between the maternal and fetal vasculature. There is amazing diversity from one animal to another in the features, such as gross structures, microscopic arrangements and developmental processes, but the function of this organ is invariable.

Primates and rodents have the ancestral type of placenta, classified as heomochorial, 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 that are derived from the trophoblast cell lineage. In mice, maternal blood coming out from the spiral arteries invaded by trophoblast cells that replace the endothelium passes across the fetal layers of the placenta and then enters into the labyrinth where fetal blood vessels form and feto-maternal exchange occurs. Maternal and fetal vascular circuits in the established mouse placenta are shown schematically in Figure 1.

Notch2 null mutation induces lethality around embryonic day 11 because of impaired maternal vasculature formation. However, both cellular and molecular events in the process of vasculature formation are little known, especially as to how the maternal blood bed expands among the tightly packed trophoblast mass. Our histological studies showed that a stream of maternal blood along the interface between the labyrinthine trophoblast and spongiotrophoblast was slightly opened in the mutant placenta. Expression of the Notch2 gene is observed in spongiotrophoblast and Giant cells, but in neither the labyrinthine trophoblast nor in the multi-nuclear Syncytiotrophoblast that is derived by cell fusion of the mono-nuclear labyrinthine trophoblast (Figure 2). Thus, we conclude that cellular and molecular events to form the maternal blood bed occur in the spongiotrophoblast and Giant cell layers.

We have assumed that the cellular event that expands the maternal blood bed is the deletion of spongiotrophoblast cells in a significant number from the developing placenta. Thus, we are trying to detect trophoblast cell death or detachment with various methods.

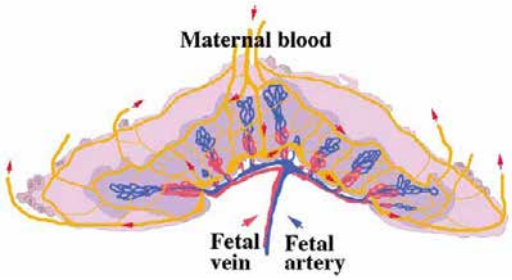


Figure 1. Schematic maternal and fetal vascular circuits in mouse placenta. Maternal blood flows in streams drawn in yellow in the direction shown by small red arrows. Fetal blood flowing in the artery (blue line) reaches the border of the spongio- and labyrinth layers and returns to the fetal vein (red line).

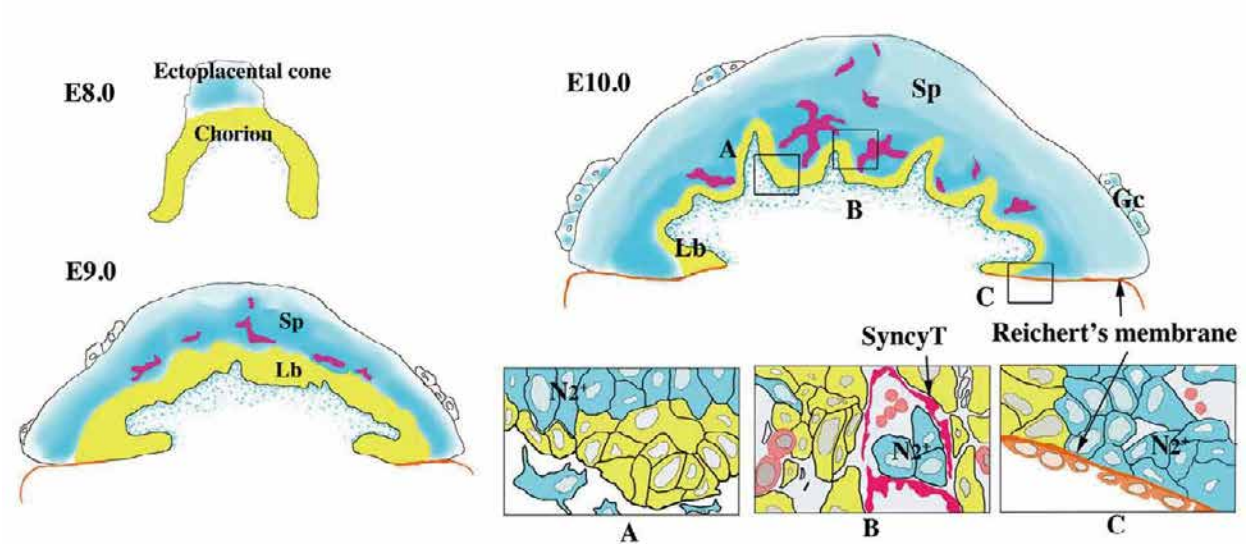


Figure 2. Expression of Notch2 gene in developing mouse placenta. The Notch2 gene is expressed in the ectoplacental cone at E8.0, but not in the chorion. At E9.0, the ectoplacental cone and chorion differentiate into spongiotrophoblast (Sp) and labyrinthine trophoblast (Lb), respectively. The expression is detected in Sp. At E10.0, differentiation of syncytiotrophoblast (SynT) and vasculogenesis of fetal blood are obvious in the placenta. Notch2 expression occurs in Sp, Giant cell layer (Gc), and mesenchymal cells derived from allantois. However, it is not detected in SynT and Lb far away from maternal blood or separated by SynT.

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
	HARA, Yusuke
SOKENDAI Graduate Student:	*MIYAGI, Asuka*
	HAYASHI, Kentaro
	TOMINAGA, Hitoshi
Visiting Undergraduate:	*TOMINAGA, Hitoshi**
Technical Assistant:	*YAMAMOTO, Takamasa*
	MURAKAMI, Michiyo
	WATANABE, Mika
	YASUE, Naoko
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. The roles of PCP core components in mouse development

In epithelia, the roles of planar cell polarity (PCP) have been extensively studied, whereas in non-epithelia, they have yet to be fully understood. We are exploring the roles of PCP in the mesenchymal tissues by using mouse genetics. Recently, we generated a hypomorphic allele of mouse *Prickle1*, one of the core PCP factors. We previously reported (Tao, H. *et al*. Proc. Natl. Acad. Sci., USA, 2009) *Prickle1* null/null mice die around E6.0 in gestation due to the failure of gastrulation. In contrast, the *Prickle1* hypomorphic mutant mice we generated by a partial deletion of the gene survived to P0. Interestingly, the mutant mice had shortened noses. Detailed analyses at the cell level suggested that Prickle1 governs convergent extension of nasal cartilage cells which is required for the lengthening of the nose. Wnt5, a ligand that activates the PCP pathway was found to be expressed forming a concentration gradient of the transcripts from the distal tip of developing nose. As ubiquitous overexpression of Wnt5 caused short noses, the concentration gradient of Wnt5 may be important for proper nose elongation. Our findings further suggests that PCP signaling employed multiple times at different places during development may be one of the universal mechanisms of organ morphogenesis, especially for organ elongation. We hope that in combination with comparative genomic analysis, these PCP mutant mice will serve as good models that can explain morphological variations in mammals.

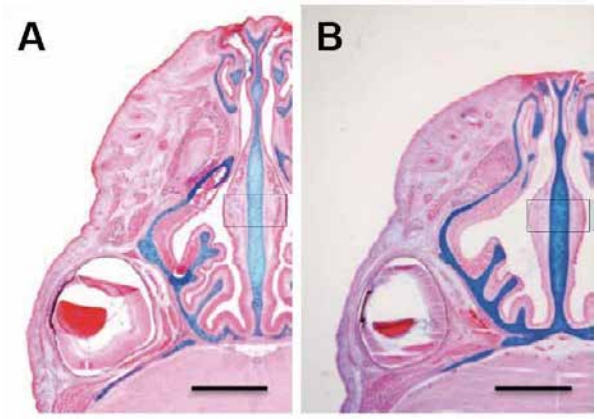


Figure 1. Nose phenotype of mouse *Prickle1* mutant.
The hypomorph allele (B) shows shortened nose cartilage stained with alcian blue due to insufficient convergent extension compared to that of wild type (A).

II. Regulation of cell adhesion by the ubiquitin system during gastrulation

During gastrulation, dorsal mesoderm cells migrate toward the midline and align along the antero-posterior (A-P) axis to form the notochord. In this process, cells repeatedly undergo cell adhesion and detachment, and thus, cell-to-cell interaction must be tightly regulated. We found that one of the cadherin superfamily cell adhesion molecules, paraxial protocadherin (PAPC), plays an essential role in this process. The PAPC transcript first appears in the dorsal marginal zone at the early gastrula stage and is subsequently restricted to the paraxial mesoderm in *Xenopus* and zebrafish. Using *Xenopus* embryos, we demonstrated that PAPC is also regulated at the protein level and is degraded and excluded from the plasma membrane in the axial mesoderm by the late gastrula stage. This regulation requires phosphorylation-dependent poly-ubiquitination. PAPC is phosphorylated by GSK3 in the evolutionarily conserved cytoplasmic domain, and this in turn is necessary for poly-ubiquitination by an E3 ubiquitin ligase β-TrCP. We also show that precise control of PAPC by phosphorylation/ubiquitination is essential for normal *Xenopus* gastrulation cell movements. Taken together, our findings unveil a novel mechanism of regulation of a cell adhesion protein and show that this system plays a crucial role in vertebrate embryogenesis. (Kai, M. *et al*., PLoS One *in press*).

III. Cellular behavior during neural tube closure

During the formation of the neural tube, an anlage of the central nervous system, neural progenitor cells emerge in a broad region on the dorsal side and gradually change their

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

positions toward the midline. However, what kind of cellular and molecular dynamics occur during this process is not clear, probably because analyses at the single cell level with high spatial and temporal resolutions have not been conducted. We analyzed zebrafish neurulation at single cell resolution, and found that progenitor cells showed non-muscle myosin II-dependent asynchronous and periodic movements. We also found that actomyosin cytoskeleton showed an isotropic, periodic remodeling in the cell cortex, while it showed directionaly constant movements in peripheral cellular protrusions. Careful examination revealed that F-actin remodeling was temporarily correlated with a deformation of cell morphology, suggesting that the periodic actomyosin contractility contributes to the convergence movements. We further found that components of the planar cell polarity (PCP) pathway were required for the periodic F-actin remodeling, suggesting that the PCP/noncanonical Wnt pathway controls the periodicity of the actomyosin. Our findings unveil a repertoire of cellular movements based on periodic actomyosin contractility that contributes to the convergence movements of multicellular organisms.

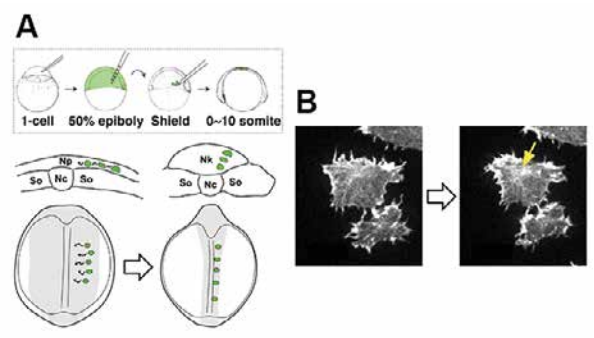


Figure 2. Live-cell imaging analyses at single cell resolution.
(A) A small number of presumptive neural cells expressing fluorescent proteins were transplanted to non-labeled host embryos. Then, cellular movements in the neural plate during convergence were observed at short time intervals. Np, neural plate; Nk, neural keel; So, somite; Nc, notochord. (B) Cortical F-actin was distributed as a meshwork-like structure and periodically concentrated. The time interval of the periodicity corresponds to that of the convergence movements.

IV. A novel plasma membrane structure capturing centrosome determines the orientation of cell division

The orientation of the mitotic spindle has been proposed to control cell fate choices, tissue morphogenesis and architecture, thus playing an important role in shaping embryonic forms. It is already reported that most cells divide along the A-P axis at the last division in ascidain epidermis.

With live imaging observation, we found a novel membrane structure invaginating along A-P polarity toward the centrosome in the epidermal last cell division cycle. Live-imaging observation showed the invagination toward the centrosome. Observation using Serial block face Scanning Electron Microscopy (SBF-SEM) confirmed this invagination reached the centrosome. The result of UV laser ablation indicated that mechanical tension, which was generated between the centrosome and plasma membrane, might form this invagination. With cell cycle progression, the length of invagination became short and the centrosome was pulled to the posterior side. Thus, we hypothesize that these membrane invaginations are involved in spindle orientation along the A-P axis. We would like to propose a novel template of spindle orientation with membrane invagination capturing the centrosome.

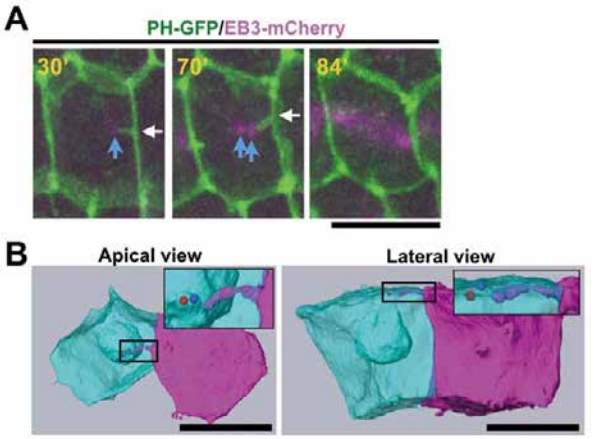


Figure 3. Novel membrane invagination structure in ascidian epidermal cell.
(A) Live imaging of membrane invagination toward microtubule organizing center (MTOC), seemingly, centrosome. PH-GFP (green), plasma membrane probe; EB3-mCherry (magenta), MTOC probe; numbers (orange), recording time in minutes. Anterior membrane invaginates (white arrow) toward MTOC (blue arrow) (30'), centrosomes are aligned (70') and eventually spindle is formed (84') along the A-P axis. (B) 3D images reconstructed from SBF-SEM data. Centrosomes are indicated as red and blue balls. The black-lined squares are enlarged in upper right panels. All bars show 10 μm.

V. Notochord and evolution of chordates

Recently, much more attention has been paid to cephalochordate amphioxus to answer an important question of metazoan evolution, namely the origin and evolution of chordates. The phylum Chordata consists of three subphyla, Cephalochrdata, Urochordata and Vertebrata. A long debate on whether cephalocordates or urochordates are an early divergence among chordates has recently reached a consensus in which cephalochordates are believed to be more ancestral, leaving urochordates and vertebrates as a sister group. While the amphioxus develop well-organized somites, their neural tube lacks a brain-like structure and the notochord contains myofibrils. In order to characterize the three organs of adult amphioxus, we examined their differential gene expression profiles by RNA-seq analysis. The analysis of differential expression profiles of genes highly expressed in the notochord, somite muscle, and neural tube, revealed a molecular affinity between the notochord and the somite muscle, as previous studies suggested. In addition, we could identify 335 genes that are preferentially expressed in the notochord, 411 genes in the somite muscle, and 514 in the neural tube. These genes play roles in the formation, maintenance and function of the three organs. The repertoires of genes provide a molecular basis for further studies of amphioxus.

VI. Subfunctionalization of duplicated MyoD genes in polyploid *Xenopus laevis*

Polyploid organisms offer a model for understanding the evolutionary effects of gene duplication. The African clawed frog *Xenopus laevis* has experienced recent allopolyploidization, which gave rise to the pseudotetraploid genome. We found that the duplicated *MyoD* genes (*XlMyoDM* and *XlMyoDZ*) are under subfunctionalization as indicated by their differential expression patterns. The combined expression pattern of *XlMyoDM* and *XlMyoDZ* corresponds to that of the unduplicated *Xenopus tropicalis XtMyoD*. To test the possibility that the ancestral function of MyoD is subdivided between the duplicated copies, we asked whether *XlMyoDM* and *XlMyoDZ* regulate different sets of target genes. An overexpression experiment of either *XlMyoD* gene followed by RT-qPCR of a selected MyoD target genes showed that both *XlMyoDM* and *XlMyoDZ* genes possess comparable activity of inducing the target genes we examined. To find the differential gene regulation by *XlMyoDM* and *XlMyoDZ* genome-wide, the overexpressed samples were subjected to a transcriptome analysis. By comparing *XlMyoDM*, *XlMyoDZ* and *XtMyoD* target genes, we will answer the important questions whether either *XlMyoD* gene acquired novel functions or lost its original functions during evolution.

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 cellular 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 can be bleached in the laboratory by raising culture temperature.
(left) Symbiotic Aiptasia cultured at 25℃. (right) Aposymbiosis induced by culturing at 30-34℃.

Publication List

〔Original paper〕

- Yajima, H., Suzuki, M., Ochi, H., Ikeda, K., Sato, S., Yamamura, K., Ogino, H., Ueno, N., and Kawakami, K. (2014). Six1 is a key regulator of the developmental and evolutionary architecture of sensory neurons in craniates. BMC Biol. *12*, 40.

〔Review articles〕

- Hashimoto, M., Morita, H., and Ueno, N. (2014). Molecular and cellular mechanisms of development underlying congenital diseases. Congenit. Anom. (Kyoto) *54*, 1-7.
- Satoh, N., Tagawa, K., Lowe, C.J., Yu, J.K., Kawashima, T, Takahashi, H., Ogasawara, M., Kirschner, M., Hisata, K., Su, Y.H., and Gerhart, J. (2014). On a possible evolutionary link of the stomochord of hemichordates to pharyngeal organs of chordates. Genesis *52*, 925-934.

DIVISION OF DEVELOPMENTAL GENETICS †

Professor
KOBAYASHI, Satoru

Assistant Professor:	*HAYASHI, Yoshiki*
	SATO, Masanao
Technical Staff:	*NODA, Chiyo*
NIBB Research Fellow:	*OTA, Ryoma*
Postdoctoral Fellow:	*FUJISAWA, Chiemi*
	OHARA, Yuya
	ASAOKA, Miho
SOKENDAI Graduate Student:	*SUGIYAMA, Arisa*
	SHINOZUKA, Yuko
	SUGIMORI, Seiko
	MORITA, Shunpei
Technical Assistant:	*SATO, Kaori*
	YAMAMOTO, Manami
	WASHIO, Midori
	KAWAMOTO, Yuri
Secretary:	*HONDA, Satoko*

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 as germline stem cells (GSC) to form eggs and sperm when the organisms are physically matured. Our laboratory aims to find the molecular mechanisms regulating germline segregation, GSC niche function, and metamorphosis in *Drosophila*.

I. Role of maternal Ovo protein in the germline of *Drosophila* embryos

It has been proposed that germline-specific gene expression is initiated by the function of maternal factors that are enriched in the germ plasm. However, such factors have remained elusive. We have done a genome-wide survey of maternal transcripts that are enriched in the germ plasm and encode transcription factors for germline-specific gene expression of *vasa* and/or *nanos*. We finally identified 6 transcripts required for germline-specific gene expression by knockdown experiments using RNA interference (RNAi). Among the 6 transcripts, we focused on *ovo*. The *ovo* gene encodes a DNA-binding, C2H2 Zn-finger protein that is involved in oogenesis and in epidermal development. The *ovo* gene produces at least three alternate isoforms. Ovo-A and Ovo-B function as a negative and a positive transcriptional regulator in the germline, respectively. Ovo-Svb is expressed in the epidermal cells and is required for their differentiation. We found that Ovo-B is the major isoform expressed in primordial germ cells (PGCs) during embryogenesis. To understand its function, we over-expressed the Ovo-A repressor only in the PGCs, and examined their developmental fate. Our data shows that the reduction in maternal Ovo-B activity results in a decrease in the number of primordial germ cells during post-embryonic stages. Thus, maternal Ovo-B has an essential role in germline development in both sexes.

While identifying the downstream genes regulated by Ovo-B in germline, we found that Ovo is required to induce germline-enriched genes, and conversely, it represses somatically-expressed genes in PGCs. Thus, we speculate that maternal Ovo has an important role in germline-fate determination. Collaboration work is now on-going to clarify the function of the *ovo* gene in mouse germline development.

II. The role of HSPGs in germline stem cell niche of *Drosophila*.

Stem cells posses the remarkable capacity to generate daughter cells that retain a stem-cell identity and others that differentiate. Stem cells reside in dedicated cellular microenvironments termed stem-cell niches. These niches dictate stem-cell identity, maintain the stem cell population, and coordinate proper homeostatic production of differentiated cells. The GSC niche in *Drosophila* gonads is a useful model system for studying the stem-cell niche, because the cellular components of this niche have been characterized and the signaling pathways, such as BMPs and JAK/STAT which are essential for GSC maintenance, are known. Ligands for these signaling pathways (niche signals) are secreted from the niche cells, and are received by GSCs to activate the pathway responsible for GSC maintenance. Thus, the GSC niche is defined as the specialized region retaining a sufficient amount of niche signals for GSC maintenance. However, it is not well understood how the distribution of the niche signals is precisely controlled in the GSC niche.

To address this question, we have been investigating the function of Heparan Sulfate Proteoglycans (HSPGs) in the GSC niche. HSPGs are an evolutionally conserved family of sugar modified proteins, which are an essential component of the extracellular matrix. One of the important functions of HSPGs during animal development is to regulate distribution of growth factors in extracellular space by binding to them. Thus, we speculated that HSPGs could retain a sufficient amount of niche signals for GSC maintenance. We found that Glypcan, a membrane-associating type of HSPG, is an essential component of the GSC niche both in female and male gonads. Glypican was highly expressed in niche cells both in ovary and testis, and its mutations caused a significant reduction in GSC number. In the GSC of the mutant ovary, the signaling pathway activated by Dpp (a BMP homologue acting as a niche signal) was impaired. Conversely, ectopic expression of Glypican in female gonads caused an increase in the number of GSCs with Dpp signaling. These results strongly suggest that Glypican defines the female GSC niche by regulating distribution of Dpp.

The question of whether the other HSPGs have functions in the GSC niche remains unclear. Since disrupting biosynthesis of all HSPGs by knockdown of the *NDST* gene caused a more severe GSC-loss phenotype than the Glypican mutant, the other types of HSPGs could have functions in the GSC

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

niche. We found that Syndecan and Perlecan, two evolutionally conserved groups of HSPGs, are essential in the GSC niche. These HSPGs were highly expressed in female GSC niche cells, and reduction of their function in niche cells caused a decrease in GSC number. We further found that, in the ovaries with reduced Perlecan function, ectopic GSC-like cells were also observed. This phenotype has not been observed in Glypican mutants. Thus, we speculated that Perlecan could regulate Dpp distribution in the GSC niche, in a way distinct from Glypican. We have succeeded in visualizing Dpp protein distribution in the female GSC niche. When Glypican was ectopically expressed in female gonads, Dpp distribution was ectopically observed in Syndecan and Perlecan mutant ovaries. Furthermore, we have also succeeded in visualizing the GSC niche signal in male gonads. This enables us to study HSPG function in the male GSC niche.

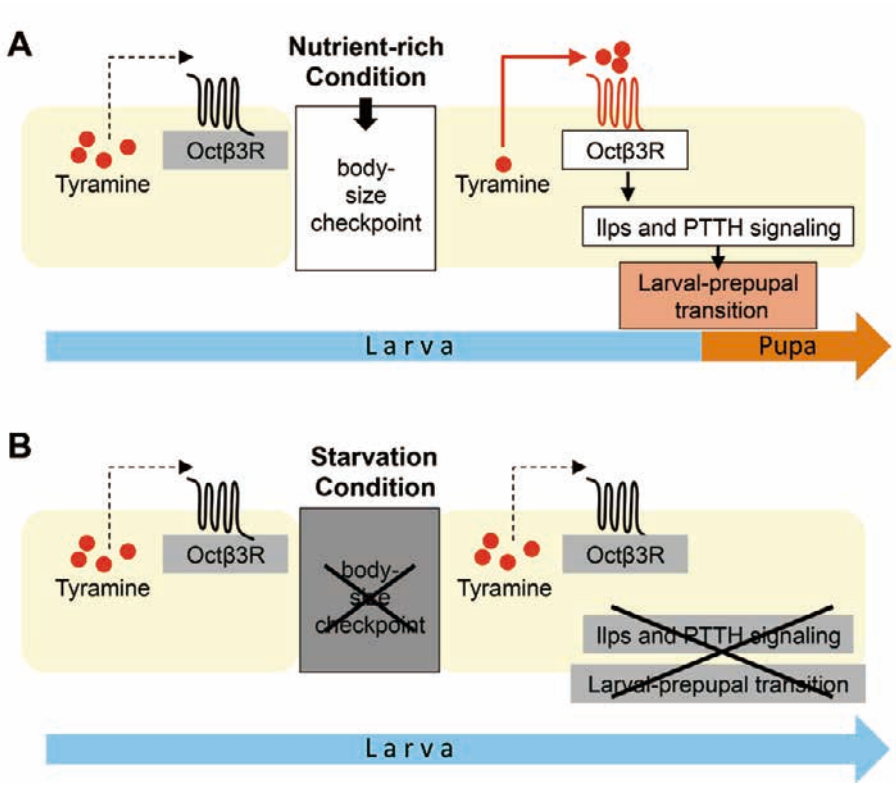


Figure 1. Our model explaining the regulation of metamorphosis by Octβ3R
(A) Before the attainment of body-size checkpoint, tyramine is stored in PG cells, so as not to activate Octβ3R. Once larvae have attained body-size checkpoint under nutrient-rich conditions, tyramine is secreted from the PG to activate Octβ3R signaling in an autocrine manner, leading to the larval–prepupal transition via the Ilps and PTTH signaling pathways. (B) When larvae fail to attain body-size checkpoint under a starvation condition, tyramine remains unsecreted from the PG; consequently, the Octβ3R, Ilps, and PTTH signaling pathways fail to be activated, resulting in arrest at the larval–prepupal transition.

III. The role of β3-octopamine receptor in the developmental transition from larvae to reproductive adults

The developmental transition is a well-known biological process in which the organism alters its body morphology in order to proceed from the juvenile stage to the adult reproductive stage. How this processe is precisely regulated in response to developmental and environmental cues is a longstanding question in biology.

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.

Publication List

〔Original papers〕

● Chanut-Delalande, H., Hashimoto, Y., Pélissier-Monier, A., Spokony, R., Dib, A., Kondo, T., Bohère, J., Niimi, K., Latapie, Y., Inagaki, S., Dubois, L., Valenti, P., Polesello, C., Kobayashi, S., Moussian, B., White, K., Plaza, S., Kageyama, Y., and Payre, F. (2014). Pri peptides are mediators of ecdysone for the temporal control of development.

Nature Cell Biol. 16, 1035-1044.
- Hayashi, M., Sato, M., Iwasaki, Y., Onozawa, T., Katayama, N., Nagasaka, Y., Sadaie, S., Kobayashi, S., and Yoshizaki, G. (2014) Enrichment of spermatogonial stem cells using side population in teleost. Biol. Reprod. 91, 1-8.
- Lim, R., Anand, A., Nishimiya-Fujisawa, C., Kobayashi, S., and Kai, T. (2014). Analysis of Hydra PIWI proteins and piRNAs uncover early evolutionary origins of the piRNA pathway. Dev. Biol. 386, 237-251.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, Sdgc, reveals sex chromosome–dependent differences of medaka germ cells prior to gonad formation. Development 141, 3363-3369.

Division of Molecular and Developmental Biology

Professor
TAKADA, Shinji

Assistant Professor:	*YABE, Taijiro*
	MII, Yusuke
Technical Staff:	*UTSUMI, Hideko*
NIBB Research Fellow:	*OKADA, Kazunori*
Postdoctoral Fellow:	*CHEN, Qiuhong*
	KAMETANI, Yoshiko
	TAKADA, Ritsuko
	FUJIMORI, Sayumi
	WANGLAR, Chimwar
SOKENDAI Graduate Student:	
	SHINOZUKA, Takuma
	TSUCHIYA, Yoshihiro
Visiting Graduate Student:	*WEICHSEL, Alexander*
Technical Assistant:	*TAKASHIRO, Kayoko*
	ITO, Yukiko
	ISHIJIMA, Hannosuke
Secretary:	*UKAI, Sakie*

The body and tissues of the developing embryo are repeatedly divided into sub-regions specified by characteristic gene expression and morphology. The process that gives rise to these sub-regions, according to a defined pattern, is called “pattern formation” or “patterning.” Our laboratories aim to understand the molecular mechanisms underlying pattern formation by several different approaches.

The most popular model to explain the patterning process is the “morphogen gradient and threshold” theory. Many genetic results indicate that secreted signal proteins such as Wnt, BMP, and Hedgehog function as morphogens in many aspects of the patterning process. In spite of the accumulation of genetic evidence, however, the biochemical characteristics of morphogens, including modification and higher order structure, remain to be elucidated. Thus, one of our major goals is to reveal *the real image* of morphogens and the molecular mechanisms underlying the formation of morphogen gradients, including the secretion and extracellular transport of these morphogens.

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 morphogen gradient and threshold, but by a unique mechanism proceeding periodically. For instance, somites are sequentially generated in an anterior-to-posterior order by converting oscillatory gene expression into repeated 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 mechanisms 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 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, presumably since their secretion and transport are under tight control. To understand the molecular mechanisms underlying morphogenesis mediated by extracellular transmission of Wnt signals, it will be required to reveal manners of diffusion and/or accumulation of Wnt proteins in extracellular space.

For a better understanding of the extracellular transport of Wnt proteins, we started to visualize Wnt proteins in the extracellular space by a two different approaches. One is a live imaging approach with EGFP-tagged Wnt proteins and the other is immnohistochemistry. Our preliminary study indicated that Wnt proteins are not simply diffused during embryogenesis of the mouse. We precisely examined regulatory mechanisms and the biological significance of Wnt protein distribution in mouse embryos. These analyses reveal a novel view of spatial regulation of Wnt signaling.

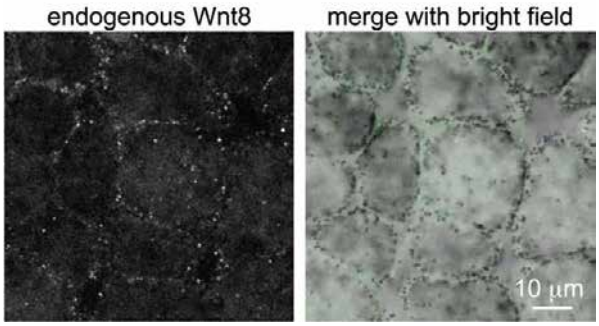


Figure 1. Accumulation of Wnt proteins between cells. Wnt proteins are acculmulated in a punctate pattern between epithelial cells in a *Xenopus* embryo. (Left) Immunostaining of endogenous Wnt8 proteins (Right) The left image is merged with a bright field one.

Extracellular molecules are known to regulate special distribution of Wnt proteins. Heparan sulfate sugar chains with different 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 an enzyme catalyzing N-sulfation of heparan sulfate. Gain and loss of function studies of this enzyme indicated that it is necessary and sufficient for the conversion of N-acetyl HSNSs into N-sulfo HSNSs. Extracellular accumulation of Wnt proteins and the convergent extension movement, which is regulated by Wnt non-canonical signaling, were also affected by perturbation of expression of this enzyme. These results indicated that N-sulfo modification of heparan sulfates are important for extracellular distribution of Wnt proteins and Wnt signaling.

II. Heterogeneity of secreted Wnt proteins secreted from cultured cells

Although the structure of Wnt protein has already been revealed, its higher order structure in extracellular space has not yet been fully understood. One of the proposed forms of Wnt protein in extracellular space binds 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 in these two forms from the same cells.

To address these issues, we systematically examined characteristics of Wnt proteins secreted from several different cell lines. First, we fractionated conditioned media of Wnt3a-expressing cells by density gradient centrifugation with Sodium Bromide. To examine whether Wnt proteins are attached to exosomes, we then collected exsosome-like molecules by ultracentrifugation and fractionated the pellet by sucrose density gradient ultracentrifugation. Our results indicate that secreted forms of Wnt proteins differ among different Wnt subtypes and this diversity is dependent on 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 results 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 mice, the prospective segmentation boundary is periodically established in the anterior PSM at the rostral border of 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 *2*, have shown to be required for the down-regulation of Tbx6 proteins by analysis of 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. However, it is still to be elucidated whether Mesp2 causes the retreat of the Tbx6 protein border through the activation of Ripplys' expression or that Mesp2 and Ripplys regulate this retreat in parallel.

To answer this question, we precisely examined function of mwsp and ripply in the regulation of Tbx6 proteins in zebrafish embryos. By utilizing an antibody against zebrafish Tbx6/Fss, we found that the anterior border of Tbx6 domain coincided with the presumptive intersomitic boundary also in the zebrafish and it shifted dynamically during 1 cycle of segmentation. Consistent with the findings in mice, the *tbx6* mRNA domain was located far anterior to its protein domain, indicating the possibility of posttranscriptional regulation. When both *ripply1/2* were knockeddown, the Tbx6 domain was anteriorly expanded. We further directly demonstrated

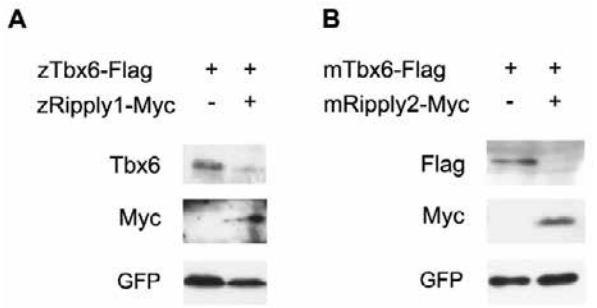


Figure 2. Ripply reduces Tbx6 protein levels: Western blotting with proteins recovered from embryos injected with Flag-tagged zebrafish *tbx6*mRNA and Myc-tagged zebrafish *ripply1* (A) or mouse Ripply2 mRNAs (B). Both zebrafish ripply1 and mouse Ripply2 reduceTbx6 protein levels.

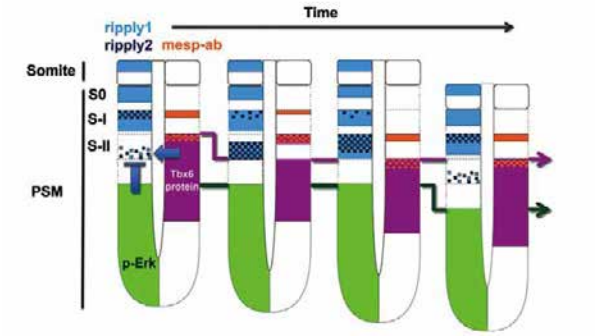


Figure 3. Schematic representation of spatial patterns of Tbx6 and p-Erk domains with *ripply1*, *ripply2*, and *mesp-ab* expressions during a single segmentation cycle. Expression of *ripply1* and *ripply2* is initially activated in the high Tbx6 / low FGF signaling region. These activated Ripplys appear to suppress Tbx6 protein resulting in formation of a new anterior border of the Tbx6 core domain and the upper band.

that Ripply could reduce the expression level of Tbx6 protein depending on physical interaction between Ripply and Tbx6 (Figure 2). Moreover, the onset of *ripply1* and *ripply2* expression occurred after reduction of FGF signaling at the anterior PSM, but this expression initiated much earlier on treatment with SU5402, a chemical inhibitor of FGF signaling. These results strongly suggest that Ripply is a direct regulator of the Tbx6 protein level for the establishment of intersomitic boundaries and mediates a reduction in FGF signaling for the positioning of the presumptive intersomitic boundary in the PSM (Figure 3).

IV. Establishment of gene knock out methodology based on genome editing technologies in the zebrafish

Zebrafish are one of the most suitable model animals for genetic analysis. Until now, chemical mutagenesis-mediated screening provided us a great number of mutant strains, which contribute to studies of morphogenesis of vertebrates. In addition to this well-established genetic approach, recent advances in genome editing technologies have made it possible to create any mutation of a gene of interest.

Previous studies revealed mouse *Mesp2* has essential roles for multiple processes of somitogenesis including determination of position of segment boundaries, generation of somite boundary structure and establishment of rostro-caudal polarity in each somite. Although four *mesp* genes have been identified in the zebrafish genome, their roles in somitogenesis still remained unclear. To address this question we generated mutant fish carrying the frame shift mutation in all *mesp* genes using TALEN mediated mutagenesis and analyzed its phenotype. Unlike the mouse *Mesp2* mutant, zebrafish mesps quadruple knockout embryo exhibited normally segmented somites. We are currently examining several mutants generated by TALEN mediated mutagenesis.

Publication List

〔Original papers〕

- Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y., Yamamoto, S., Yabe, T., Takada, S., Kinoshita, M., Kuroiwa, A., and Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. Proc. Natl. Acd. Sci. USA *111*, 7343-7348.
- Osipovich, A.B., Long, Q., Manduchi, E., Gangula, R., Hipkens, S.B., Schneider, J., Okubo, T., Stoeckert, C.J. Jr., Takada, S., and Magnuson, M.A. (2014). Insm1 promotes endocrine cell differentiation by modulating the expression of a network of genes that includes Neurog3 and Ripply3. Development *141*, 2939-2949.
- Wanglar, C., Takahashi, J., Yabe, T., and Takada, S. (2014). Tbx protein level critical for clock-mediated somite positioning is regulated through interaction between Tbx and Ripply. PLoS One *9*, e107928.

DIVISION OF EMBRYOLOGY

Professor
FUJIMORI, Toshihiko

Assistant Professor:	*TOYOOKA, Yayoi*
	KOYAMA, Hiroshi
Technical Staff:	*OKA, Sanae*
NIBB Research Fellow:	*SATO, Yasufumi*
	SHI, Dongbo
Postdoctoral Fellow:	*KOBAYAKAWA, Satoru*
SOKENDAI Graduate Student:	*KAMEMIZU, Chizuru*
	ITO, Tomoaki
	USAMI, Fumiko
Visiting Graduate Student:	*SHI, Dongbo**
Technical Assistant:	*HIGUCHI, Yoko*
Secretary:	*KATO, Azusa*

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 in 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 live developing embryos, even in mammals. We have established a series of transgenic mouse lines for live imaging, which is a part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CDB. 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

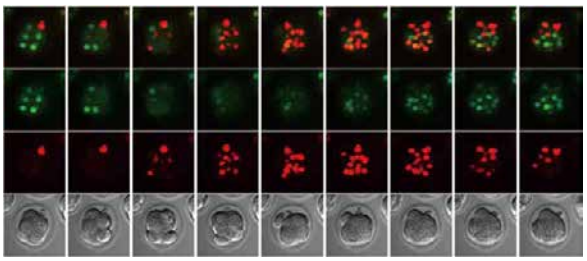


Figure 1. Examples of a morula expressing fucci2, cell cycle markers. The G(1) and S/G(2)/M phases are distinguished by mCherry(red) and mVenus (green) signals respectively.

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 started analysis of 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 CO2 incubators provide better conditions compared with 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 are also trying to observe and reveal aspects of cell shape, morphogenesis, cell lineage, gene expression and cell differentiation by combining these techniques.

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 mice 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.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

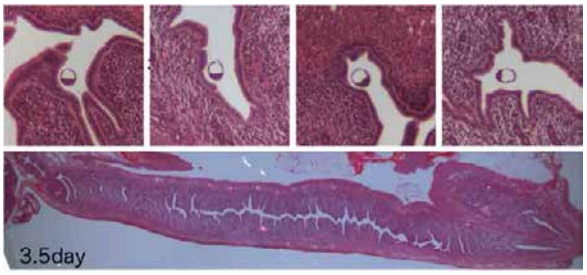


Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

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 performed immunohistochemical analysis of potential PCP regulators in postnatal oviducts. 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. When we closely examined orientation of cilia by electron microscopy they were not orientated in the same direction.

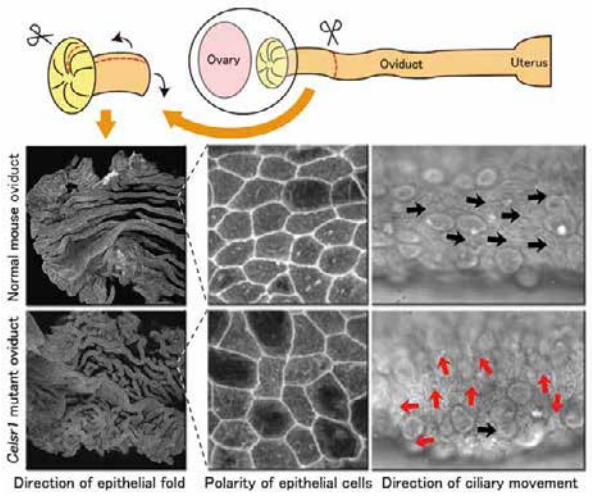


Figure 3. In the oviduct of mice that have lost the Celsr1 protein, various polarity impairments covering multiple levels are observed.

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, 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 (Figure 3). Our mosaic analysis analyses with *Celsr1*-deficient cells in the wild type oviduct epithelium suggest that the geometry of epithelial cells, but not the morphology of the epithelial fold, is primarily regulated by Celsr1.

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.

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 are 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.

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 information 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.

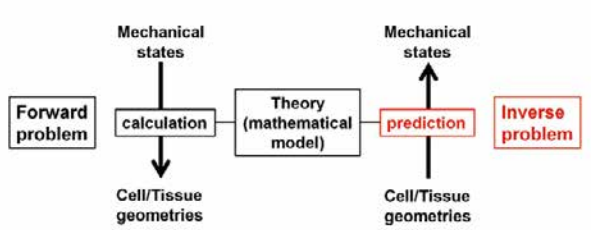


Figure 4. Theoretical estimation of cellular/tissue mechanical states. Schematic illustration of estimation.

Publication List

〔Original papers〕

- Imuta, Y., Koyama, H., Shi, D., Eiraku, M., Fujimori, T., and Sasaki, H. (2014). Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos. Mech. Dev. *132*, 44-58.
- Ishino, Y., Hayashi, Y., Naruse, M., Tomita, K., Sanbo, M., Fuchigami, T., Fujiki, R., Hirose, K., Toyooka, Y., Fujimori, T., Ikenaka, K., and Hitoshi, S. (2014). Bre1a, a histone H2B ubiquitin ligase, regulates the cell cycle and differentiation of neural precursor cells. J. Neurosci. *34*, 3067-3078.
- Nakagawa, S., Shimada, M., Yanaka, K., Mito, M., Arai, T., Takahashi, E., Fujita, Y., Fujimori, T., Standaert, L., Marine, J.C., and Hirose, T. (2014). The lncRNA Neat1 is required for corpus luteum formation and the establishment of pregnancy in a subpopulation of mice. Development *141*, 4618-4627.
- Shi, D., Komatsu, K., Hirao, M., Toyooka, Y., Koyama, H., Tissir, F., Goffinet, A.M., Uemura, T., and Fujimori, T. (2014). Celsr1 is required for the generation of polarity at multiple levels of the mouse oviduct. Development *141*, 4558-4568.

DIVISION OF GERM CELL BIOLOGY

Professor
YOSHIDA, Shosei

Assistant Professor:	*HARA, Kenshiro*
	KITADATE, Yu
NIBB Research Fellow:	*NAKAMURA, Yoshiaki**
Technical Staff:	*MIZUGUCHI, Hiroko*
Postdoctoral Fellow:	*NAKAMURA, Yoshiaki*
	IKAMI, Kanako
	TOKUE, Moe
SOKENDAI Graduate Student:	*IKAMI, Kanako**
	*TOKUE, Moe**
	NONAMI, Yuta
	ISHIZAKA, Miho
Technical Assistant:	*INADA, Kana*
	KON, Yayoi
	MARUYAMA, Ayumi
Secretary:	*KUBOKI, Yuko*

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 stem cell system.

The goal of the Division of Germ Cell Biology is to fully understand the mammalian sperm stem cell system under the context of *in vivo* tissue architecture. We have successfully revealed some characteristics of this interesting stem cell system. First, differentiating germ cells that had been believed to be irreversibly committed for differentiation still retain their self-renewing potential and can contribute to stem cell pool maintenance ("potential stem cells"). Secondly, "reversion" from potential stem cells occurs at a higher frequency when testicular tissue is damaged and regeneration is induced. Thirdly, the undifferentiated spermatogonia populations including both "actual" and "potential" stem cells are preferentially localized near vasculature (vasculature-associated niche). Lastly, stem cells turn over at an unexpectedly frequent and stochastic manner under a steady-state situation that continuously produces sperm (Nakagawa *et al*., Dev. Cell 2007; Yoshida *et al*., Science 2007; Nakagawa *et al*., Science 2010; Klein *et al*., Cell Stem Cell 2010). These observations have thrown doubt on the prevailing stem cell model, the "A_s model", postulating that stem cell function is restricted to the singly isolated spermatogonia (A_s cells).

I. Background: Testicular architecture and sperm stem cell theories

In mammalian testis, a huge number of sperm are continuously produced over the reproduction period (1-2 years in mice), which relies on stem cell activity. For decades, the cellular identity and behavior of spermatogenic stem cells has been argued as a fundamental question in the field of spermatogenesis.

The process of spermatogenesis takes place in seminiferous tubules of the testis (Figure 1). In mice, mitotic stages of spermatogenic cells, called "spermatogonia", are divided into "undifferentiated" and "differentiating" populations. "Undifferentiated spermatogonia" are found as singly isolated cells (A_s), or syncytia consisting mainly of 2 (A_{pr}) or more (A_{al}) 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.

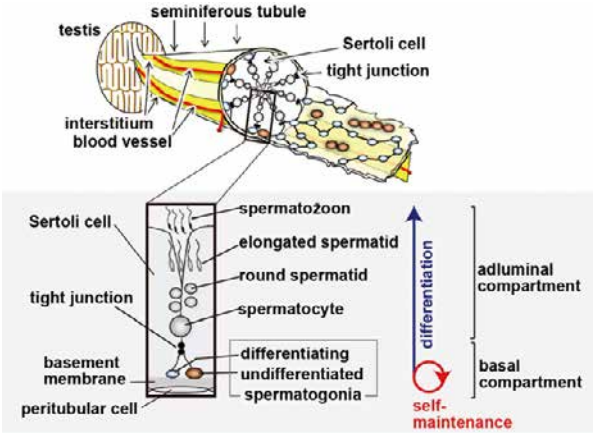


Figure 1. Spermatogenesis in seminiferous tubules. Spermatogenesis progresses from the basal- to adluminal compartment. Stem cells are thought to reside primarily within undifferentiated spermatogonia (brown) which are distributed sparsely on the basement membrane. Modified from Hara *et al*., Cell Stem Cell (2014).

The prevailing stem cell model, called the "A_s model", was proposed in 1971, suggesting that stem cell activity is restricted to the population of A_s spermatogonia, 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).

On the other hand, over the last decade, studies from some groups, including ours, have shown that the population of undifferentiated spermatogonia can be divided on the basis of their heterogeneous gene expression. In undisturbed steady-state spermatogenesis, the GFRα1+ subpopulation (mainly A_s, A_{pr}, and fewer A_{al}) is thought to reside on the top of the hierarchy: As well as maintaining their own population, GFRα1+ cells also give rise to the second, Ngn3+, subpopulation of undifferentiated spermatogonia (comprised of more A_{al} and fewer A_s and A_{pr}). Ngn3+ cells, although retaining an ability to revert into GFRα1+ cells that becomes apparent during regeneration after tissue insult, mostly differentiate into differentiating spermatogonia and then meiotic spermatocytes and haploid spermatids in undisturbed situations.

Seminiferous tubules represent a typical "open" stem cell niche. This makes a stark contrast to the well-investigated "closed" niche-supported tissues, such as *Drosophila* gonads or mammalian small intestine. The "closed" niche is a definitive, specified region of a tissue which typically tethers the stem cells, while cells that exit this niche region will differentiate. In contrast, seminiferous tubules do not harbor such a defined structure, but the stem cells (GFRα1+ spermatogonia) are scattered in the basal compartment with some, but significant, preference to the vasculature between the tubules. It remains an important unsolved question how

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

stem cell fate is regulated (whether differentiated or remaining undifferentiated) in an open niche system.

In 2014, we questioned the cellular identity and the dynamics of the stem cells, as well as the mechanisms regulating stem cell fate in the recently proposed open niche model, as described below.

II. Cellular identity and the dynamics of spermatogenic stem cells

The aforementioned studies illustrate that the dynamics of GFRα1+ spermatogonia are an essential problem for a full understanding of the identity and behavior of mouse sperm stem cells. To address this, we performed single-cell-level analyses of *in vivo* dynamics of GFRα1+ spermatogonia, taking advantage of pulse-labeling and live-imaging studies, combined with biophysical modeling, in collaboration with Dr. Ben Simons of the University of Cambridge, UK..

GFRα1+ spermatogonia lie scattered unevenly on the basement membrane of seminiferous tubules (Figure 3), but their local density over a prolonged tubule length is remarkably constant in adult mice. By pulse-labeling the GFRα1+ spermatogonia with GFP, we obtained clonal fate data of GFRα1+ spermatogonia from several days to over a year, and showed that, although the entirety of GFRα1+ spermatogonia maintained their own population while giving rise to differentiating progeny, the individual GFRα1+ spermatogonia followed highly variable and intricate fate behaviors. These findings suggest that the maintenance of GFRα1+ spermatogonia is achieved by "population asymmetry", in which balanced stem cell maintenance and production of differentiating progeny are achieved at a population-level.

We next investigated the behavior of GFRα1+ spermatogonia, by means of *in vivo* live-imaging. From the numerous 3-day-long observations that altogether effectively cover one year of spermatogonial behavior, it is indicated that GFRα1+ cells continually change their morphological states between A_s, A_{pr} and A_{al} spermatogonia through a combination of "incomplete cell division (leading to syncytial extension)" and "syncytial fragmentation (through breakage of intercellular bridges)", while giving rise to differentiating progeny from all of these morphological states.

The live-imaging measurements indicate that the rates of "incomplete division" and "syncytial fragmentation" may be constant regardless of whether they are syncytia or A_s. We were then motivated to try to capture these cells using a biophysical modeling scheme, which essentially depends only on the rates of "incomplete division" and "syncytial fragmentation". Intriguingly, the model accurately predicted the wide range of intricate clonal fate behaviors in steady-state over wide time scales from several days to over a year, and the recovery from tissue insult observed in laboratory experiments.

The results together indicate that the entirety of GFRα1+ spermatogonia (including both A_s and syncytia) comprise a single equipotent stem cell pool and cells continually interconvert between A_s and syncytial states, while giving rise to Ngn3+ progeny (Figure 2). This novel paradigm would challenge the "A_s model" that was proposed over 40 years ago.

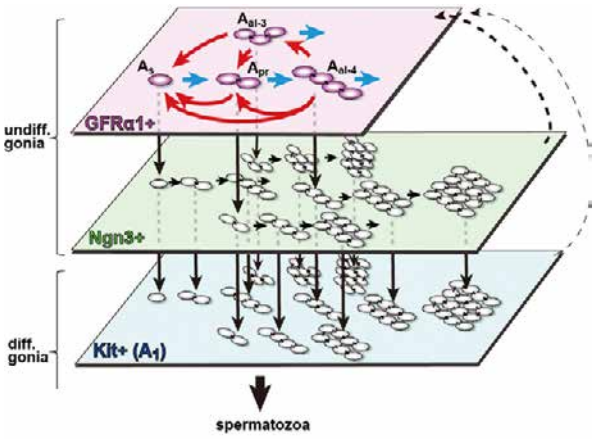


Figure 2. A proposed stem cell dynamics. GFRα1+ spermatogonia comprise a single stem cell pool, in which cells continually and reversibly interconvert between states of A_s, A_{pr} and A_{al} spermatogonia through incomplete cell division (blue arrows) and syncytial fragmentation (red arrows), while giving rise to Ngn3+ cells. Thus, 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, such as after tissue insult or during regeneration from damage etc. (broken arrows). Reproduced from Hara *et al*., Cell Stem Cell (2014).

II. Undifferentiated spermatogonia show heterogeneous differentiation competence in response to retinoic acid.

As described above, seminiferous tubules represent a typical open niche of stem cells. In particular, GFRα1+, Ngn3+, and Kit+ spermatogonial subpopulations are intermingled in the basal compartment (Figure 3). In addition, these are likely to be ubiquitously bathed with retinoic acid (RA), a strong differentiation-inducer, that occurs periodically along with the cycle of seminiferous epithelium (8.6 days per a cycle). So, a fundamental question is raised: Out of the entire population of undifferentiated spermatogonia with stem cell potential on the basis of the activity of post-transplantation colony formation, why do Ngn3+ cells preferentially differentiate and why do GFRα1+ cells maintain their own population (self-renewal)?

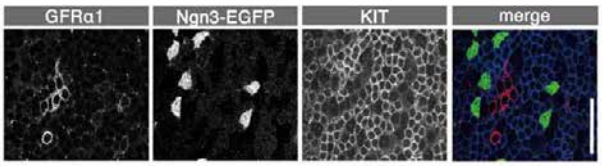


Figure 3. Localization of GFRα1+, Ngn3+, and KIT+ spermatogonia. Representative images of triple immuno-stained whole-mount seminiferous tubules of an *Ngn3-EGFP* mouse. Scale bars = 50 μm.

By pulse-labeling the Ngn3+ and GFRα1+ populations by means of the tamoxifen-inducible cre-loxP system, we tested the response of these cells to RA. The results first 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 the Ngn3+ cells in an RA-independent manner. Thus, GFRα1+ and Ngn3+ cells show distinctive differentiation competence in response to RA.

We then questioned the molecular mechanism that caused such differential responses to RA. To this end, the transcriptomes of GFRα1+ and Ngn3+ fractions were compared by gene expression microarray analysis. Among a number of genes involved in RA signal reception, particularly, retinoic acid receptor gamma (*Rarγ*), expression was preferentially observed in Ngn3+ cells over GFRα1+ cells. This was also true for the RARγ protein (Figure 4). On the other hand, most of the other RA-related genes showed similar levels of expression.

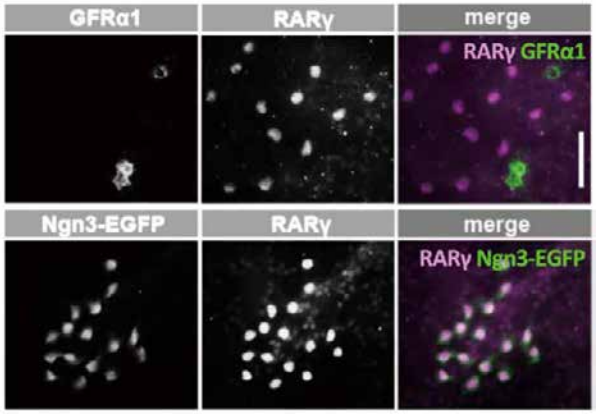


Figure 4. Expression of RARγ in Ngn3+, but not in GFRα1+, spermatogonia. Representative images of double immuno-stained whole-mount seminiferous tubules of an *Ngn3-EGFP* mouse. Scale bars = 50 μm. Modified from Ikami *et al*., Development *142*, 1-11 (2015).

We then addressed the roles of RARγ by ectopic expression in GFRα1+ cells that normally do not express this gene. Intriguingly, enforced expression of RARγ provided the GFRα1+ cells with differentiation competence in response to RA. This indicates the key role of RARγ in the heterogeneous differentiation competence and the subsequent fate selection of undifferentiated spermatogonia.

From these results, heterogeneous differentiation competence, combined with the periodically but ubiquitously distributed RA, appears to allocate the undifferentiated cells' fates for the continuity of spermatogenesis (Figure 5). We propose that heterogeneous differentiation competence combined with spatially ubiquitous distribution of differentiation-inducing signals would be paradigmatic for stem cell regulation in an opened niche based model.

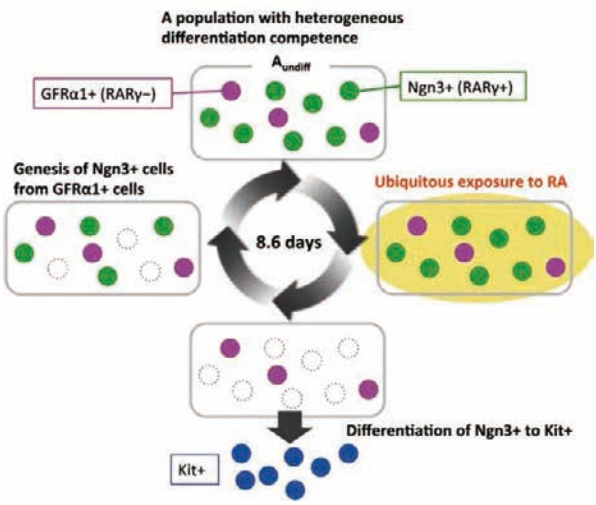


Figure 5. A scheme for the fate allocation of undifferentiated spermatogonia along the cycle of seminiferous tubules. See text for details.

Publication List

〔Original paper〕

- Hara, K., Nakagawa, T., Enomoto, H., Suzuki, M., Yamamoto, M., Simons, B.D., and Yoshida, S. (2014). Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states. Cell Stem Cell *14*, 658-672.

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

- 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. Low temperature-induced circulating triiodothyronine accelerates seasonal testicular regression. Endocrinology 2014 Nov 18.

Laboratory of Molecular Genetics for Reproduction

Associate Professor
TANAKA, Minoru

NIBB Research Fellow:	*YAMAMOTO, Yasuhiro**
Postdoctoral Fellow:	*YAMAMOTO, Yasuhiro*
	FUJIMORI, Chika
SOKENDAI Graduate Student:	*NISHIMURA, Toshiya*
	SAKAE, Yuta
	KIKUCHI, Mariko
	OTAKE, Norihito
Technical Assistant:	*KINOSHITA, Chie*
	WATAKABE, Ikuko
Secretary:	*TAKEDA, Manami*
	SHIRAISHI, Naomi

Laboratory scope

Reproduction is a universal and fundamental system for organisms to produce generations. To accomplish this purpose efficiently, organisms develop sexual 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 conserved among vertebrates.

Vertebrates, however, exhibit a variety of reproductive systems. 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 reproduction and a variety of reproductive systems. Currently, our lab focuses on the mechanisms of sex differentiation. The many modes of sex differentiation are a main component that contributes to variety, and we are addressing the role of each cell lineage in the context of sex differentiation.

We use medaka fish (*Oryzias latipes*) and 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 importance of germ cells not only in sex differentiation but also in reproduction. Germ cells have been generally considered to be a permissive cell type that are completely regulated by the surrounding somatic cells. But we have found that the germ cells are not specialized for gametogenesis but are more self-determinant cells in reproduction.

I. Developmental origin of primordial germ cells

Development of germ cells essentially rely on the presence of a cytoplasmic structure, called germplasm or alternatively nuage. The lack of germplasm results in defective or loss of the germ cells. Two separate systems controlling the development of germ cells are known for vertebrates. Some vertebrate species possess a maternal origin of germplasm and the germplasm localized in a specific cell type is essential for germ cell development. In these species, therefore, cells that develop into germ cells can be identified by the presence of germplasm. On the other hand in the second method, like in mammals, germ cells are induced at the gastrulation stage. The germplasem-equivalent structures in these cells are also formed at a later stage.

Our previous studies indicated that, in medaka, germ cells are established as primordial germ cells (PGCs) during gastrulation. However, it is not known how a small population of blastodermal cells are specified as cells to produce PGCs, or if germ cells are induced like those seen in mammals. We have cloned a cDNA encoding Bucky ball, a component of germplasm. Since we have found *bucky ball* expressed in fertilized eggs, CFP-fused Bucky balls allow us to keep track of cells that could generate PGCs during early embryogenesis by confocal microscopy.

The RNA encoding *cfp*-fused *bucky ball* was injected into fertilized eggs. We found that the protein is readily translated and was observed as early as the onset of the first division. The CFP-fused Bucky ball protein is localized as several particles uniformly in the blastodisc. Very interestingly, the particles seem to be anchored at yolk layers until the late morula stage and seem independent of cell division in the blastodisc. The inhibition of the cleavage plane by nocodazol did not change any morphology or distribution of the particles, supporting the independence of the cleavage plane.

Nanos3 protein is known to be expressed in the established PGCs. Co-injection of *dsred-nanos*3'UTR RNA and *cfp*-fused *buckey ball* RNA indicates that DsRed-derived fluorescence begins to be detected in the cells that retain large CFP-fused Bucky ball particles. This clearly indicates that the cells retaining the Bucky ball particles are the presumptive germ cells that have the potential to be established as primordial germ cells.

Interestingly, at one or two stages, numerous Bucky ball granules are present with a range of different sizes, but, as

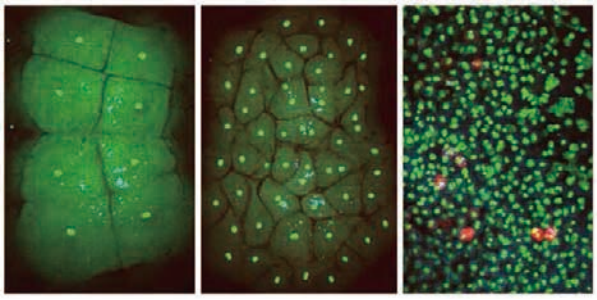


Figure 1. The localization of CFP-fused Bucky ball protein (blue) reveals presumptive primordial germ cells after fertilization. Green dots indicate histone3 signals in the nuclei. The cells expressing *nanos3* are established as primordial germ cells at the late morula stage, which are clearly recognized as the cells possessing large blue Bucky ball particles. Red cells are *nanos3*-expressing cells. The pictures are time-lapse shots of a live imaging movie. Left: 8 cell stage, Middle: 64 cell stage and Right: late morula stage.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

development proceeds, small particles are getting eliminated. As a result, at the later morula stage, approximately 20-30 cells possess granules larger than 25 μm^3 and become PGCs. As the PGCs are established, the translation of nanos3 and vasa genes is activated. These translated proteins begin to be detected on the granules of Bucky balls.

II. Sexually different characteristics of primordial germ cells

Since 2013, we have been characterizing female and male primordial germ cells and have identified genes showing sexually different expression in the PGCs. Last year we reported that the sexually different expression of one of the identified genes, *sdgc*, is due to the presence of Y chromosomes but is not dependent on the medaka sex determination gene, *DMY*/*Dmrt1bY*.

This year, we continued to analyze the function of this gene and found that the gene regulates proliferation of primordial germ cells. Primordial germ cells isolated from male embryos proliferate more than those from female embryos in the culture dish. Loss of gene function by injection of morpholino results in a reduced proliferation rate in males while injection of *sdgc* RNA shows increasing activity of proliferation *in vitro*. These results suggest that the expression of *sdgc* confers primordial germ cells with the potential to regulate proliferation.

Furthermore, we have mapped *sdgc* on the medaka genome and found that *sdgc* is located very close to the *DMY*/*Dmrt1bY* locus. The *DMY*/*Dmrt1bY* locus is known to be the only region that is specific to Y chromosomes and where recombination is repressed. Consistent with this, we could find the sex-specific SNPs in the promoter and the intron regions of *sdgc*. *Sdgc* might represent the evolutional way of the Y chromosome differentiating into a more specialized chromosome.

Collectively, a series of analyses of *sdgc* indicate that cells have the ability to express sexually different characteristics autonomously, which is independent of the expression of sex determination gene.

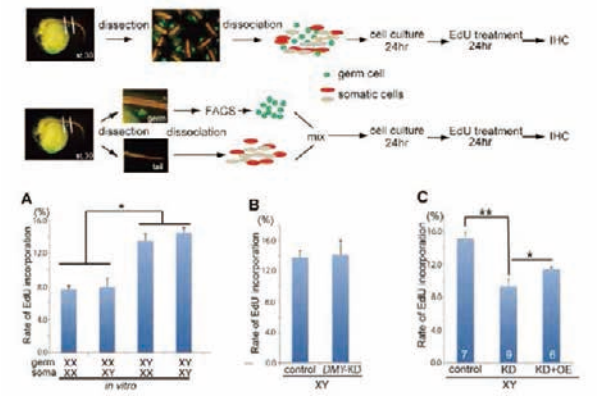


Figure 2. Sexually different behavior of primordial germ cells. Upper schema shows the way of primordial germ cell isolation and culture with somatic cells. A: Germ cell behavior (incorporation of EdU) is not affected by the sex of somatic cells but is determined cell-autonomously. B: The germ cell behavior is not dependent on the expression of sex determination gene, *DMY*/*Dmrt1bY*. C: The germ cell behavior (activity of proliferation) is reduced by knockdown of *sdgc*, which is recovered by overexpression of *sdgc*.

III. Sexually different development of steroidenic cells

Estrogen expression is important for feminization of the gonad, especially forming the ovarian cavity. Aromatase is a critical enzyme for producing estrogen from its precursor steroid, testosterone, and is encoded by the *cyp19a1* gene. Unlike mammals, during development the first emerging cells that express *cyp19a1* are the precursors of theca cells (in mammals, granulosa cells first express *cyp19a1*).

We have examined the development of steroidenic cells that produce testosterone and found that *ftz-f1* cells may be the precursor of testosterone-producing cells. Currently, two types of *ftz-f1* cells are observed in the developing gonads and precise lineage analysis is under investigation using time-lapse movies with the primary culture system.

Publication List

〔Original papers〕

- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, *Sdgc*, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development *141*, 3363-3369.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.

〔Review articles〕

- Tanaka, M. (2013). Vertebrate female germline—the acquisition of femaleness. WIREs Dev. Biol. doi: 10.1002/wdev.131
- Nishimura, T., and Tanaka, M. (2014). Gonadal development in fish. Sex. Dev. *8*, 252-261.
- Tanaka, M. (2014). Molecular and cellular bases of sexual flexibility in vertebrates. In 'New Principles in Developmental Processes (eds: Kondoh, H., and Kuroiwa, A.)' Springer Japan, pp. 265-278.

DIVISION OF MOLECULAR NEUROBIOLOGY

Professor
NODA, Masaharu

Associate Professor
SHINTANI, Takafumi

Assistant Professor:	*SAKUTA, Hiraki*
	HIYAMA, Takeshi
Technical Staff:	*TAKEUCHI, Yasushi*
NIBB Research Fellow:	*KUBOYAMA, Kazuya**
	NOMURA, Kengo
Postdoctoral Fellow:	*FUJIKAWA, Akihiro*
	SUZUKI, Ryoko
	MATSUMOTO, Masahito
	KUBOYAMA, Kazuya
	LIN, Chia-Hao
SOKENDAI Graduate Student:	*MATSUDA, Takashi*
	YU, Yang
	TANGA, Naomi
	HIGASHI, Satoru
Technical Assistant:	*MIURA, Seiko*
	NAKANISHI, Norie
	DOKYO, Yumi
	WADA, Kotoe
	KONISHI, Mie
	ISOSHIMA, Yoshiko
	HASHIMOTO, Terumi
Secretary:	*KODAMA, Akiko*

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

Topographic maps are fundamental features of neural networks in the nervous system. We have long studied the molecular mechanisms for regional specification in the developing retina as the basis of the topographic retinotectal projection. We are now focusing our attention on the molecular mechanisms underlying axonal navigation, branching, and arborization for synapse formation, along with elimination of mistargeted axons and branches. Among the region-specific molecules in the developing retina, we have already found several molecules that induce abnormal branching or arborization when their expression was experimentally manipulated *in vivo*.

One is adenomatous polyposis coli 2 (APC2), which 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 behaviors of growth cones 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. We are now investigating a mutation in human *APC2* gene identified in patients with intellectual disabilities.

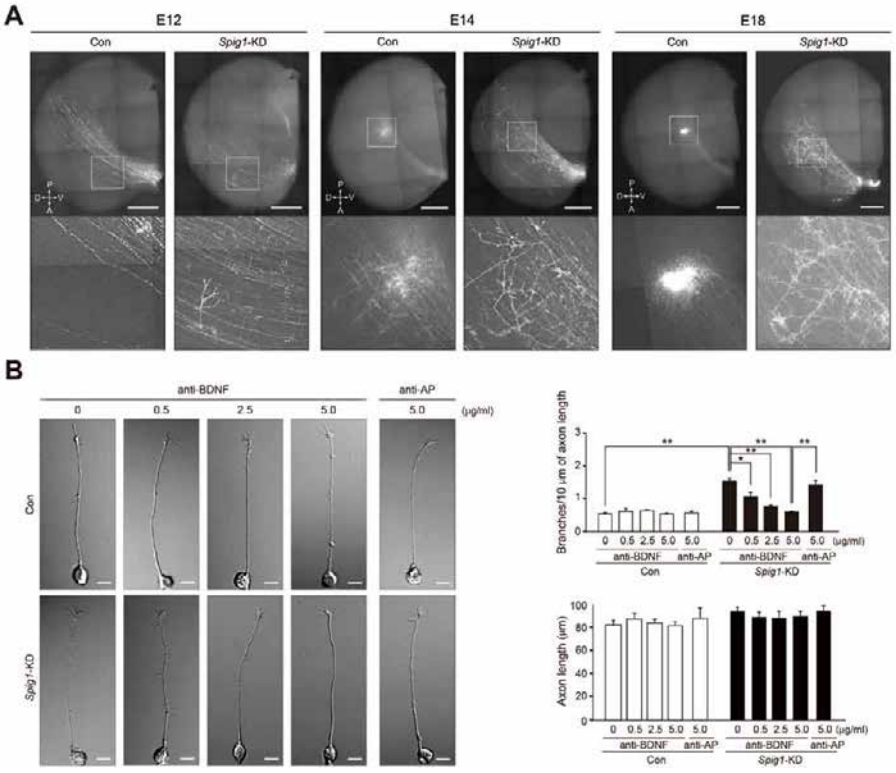


Figure 1. Effect of *Spig1*-knockdown (KD) in the developing chick retina. **A**, Projection pattern of dorsal RGC axons by DiI labeling in control (Con) and *Spig1*-KD embryos. Higher magnification of the boxed area is shown in the lower part of each panel. A, anterior; P, posterior; D, dorsal; V, ventral. Scale bars, 1 mm. **B**, Effect of a neutralizing antibody for BDNF on the elongation and branching of RGC axons. After the electroporation of *Spig1*-shRNA or control retroviral construct at HH stage 9-10, retinal cells were dissociated from the dorsal one-third of the retina at E8. The branch number and axon length of RGC axons were quantified. Data are shown as the mean ± SE of four independent experiments. $*p < 0.05$, $**p < 0.001$ (ANOVA with Scheffé's *post hoc* tests). Scale bars, 10 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

Another molecule is SPARC-related protein containing immunoglobulin domains 1 (SPIG1, also known as Follistatin-like protein 4), which is a secretory protein expressed in a dorsal-specific manner in the developing chick retina. The knockdown of *Spig1* in the retinal ganglion cells (RGCs) of developing chick embryos induced robust ectopic branching of dorsal RGC axons and failed to form a tight terminal zone at the proper position on the tectum (Figure 1A). The knockdown of *Spig1* in RGCs also led to enhanced axon branching *in vitro*. However, this was canceled by the addition of a neutralizing antibody against brain-derived neurotrophic factor (BDNF) to the culture medium (Figure 1B). SPIG1 and BDNF were colocalized in vesicle-like structures in cells. SPIG1 bound with the proform of BDNF (proBDNF) but very weakly with mature BDNF *in vitro*. The expression and secretion of mature BDNF were significantly decreased when SPIG1 was exogenously expressed with BDNF in HEK293T or PC12 cells. The amount of mature BDNF proteins as well as the tyrosine phosphorylation level of the BDNF receptor, tropomyosin-related kinase B (TrkB), in the hippocampus were significantly higher in *Spig1*-knockout mice than in wild-type mice (Figures 2A and B). Furthermore, the spine density of CA1 pyramidal neurons was consistently increased (Figure 2C). Together, these results suggest that SPIG1 negatively regulated BDNF maturation by binding to proBDNF, thereby suppressing axonal branching and spine formation.

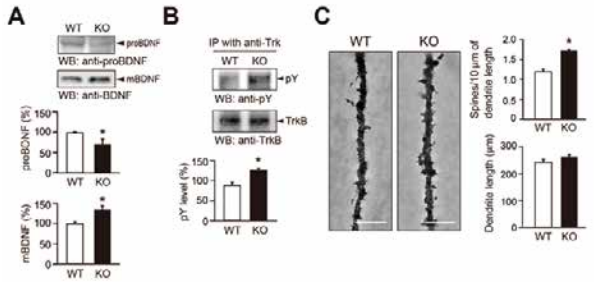


Figure 2. Functional interactions between SPIG1 and BDNF in hippocampal cells. **A**, Comparison of *BDNF* expression in the hippocampus of wild type (WT) and *Spig1*-knockout (KO) mice at postnatal day 14 (P14). The amounts of BDNF are presented by densitometric units normalized to the value for the WT. Data are shown as the mean ± SE (n = 14 for each). *$p < 0.05$ (Student's t test). **B**, Increased tyrosine phosphorylation of TrkB in the hippocampus of *Spig1*-KO mice. The tyrosine phosphorylation of TrkB proteins immunoprecipitated with an anti-Trk antibody from the hippocampus at P14 was analyzed using 4G10 and anti-TrkB. Densitometric data are presented as a percentage of the WT control (bottom). Data are mean ± SE (WT, n = 6; *Spig1*-KO, n = 8). *$p < 0.05$ (Student's t test). **C**, Golgi staining of the hippocampal tissues from WT and *Spig1*-KO mice at P10. We analyzed the dendritic segments of CA1 pyramidal neurons. Spine density and dendritic length are shown as the mean ± SE (n = 6 for each). *$p < 0.01$ (Student's t test). Scale bars, 10 mm.

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 the R3 and R5 subfamilies of RPTPs.

2-1 R3 RPTP subfamily

The human genome contains 58 and 20 genes for RPTKs and RPTPs, respectively. In some studies including ours, RPTPs have been shown to be involved in the regulation of RPTKs through dephosphorylation as substrates. However, our understanding about the roles of individual RPTPs in the regulation of RPTKs is still limited.

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 RPTK subfamilies. We revealed that multiple RPTKs are recognized as substrates by the R3 RPTPs. We also demonstrated that the R3 members showed differences in substrate specificity toward individual RPTKs. On the basis of the enzyme-substrate relationships identified, we are now investigating the physiological roles of the R3 RPTP subfamily by using their knockout mice.

2-2 R5 RPTP subfamily

Protein-tyrosine phosphatase receptor type Z (Ptprz) is predominantly expressed in glial and neuronal cells in the central nervous system (CNS). We are now focusing our efforts on determining the roles of Ptprz signaling in the regulation of hippocampal synaptic plasticity, dopamine transporter internalization, oligodendrocyte differentiation, etc.

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 concentration-sensitive Na channel.

In the brain, Na_x channels are preferentially expressed in astrocytes and ependymal cells in the sensory circumventricular organs, such as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), where Na_x-positive glial cells are involved in sensing an increase in $[Na^+]$ in body fluids. *Na_x*-deficient mice do not stop ingesting salt even when dehydrated, while wild-type mice avoid salt. This behavioral defect of *Na_x*-deficient mice is recovered by a site-directed transfer of the *Na_x* gene with an adenoviral vector into the SFO. The threshold value of Na_x for $[Na^+]_o$ was ~150 mM *in vitro*. In the SFO, however, endothelin-3 (ET-3) shifts the $[Na^+]_o$

dependency of Na_x activation to the lower-concentration side in a dose-dependent manner. This shift enables Na_x to gate even when $[Na^+]_o$ is in the physiological range (135–145 mM). Na_x thus functions as the brain's Na^+-level sensor for the homeostatic control of $[Na^+]$ in body fluids.

We can summarize the cellular mechanisms for $[Na^+]$-sensing and $[Na^+]$-dependent regulation of neural activities in the SFO as presented in Figure 3. The sensory CVOs, including the SFO, are characterized by the extensive networks of fenestrated capillaries which allow ingredients of plasma to release to the intercellular space. Their ventricular side is partitioned by an ependymal cell layer facing the third ventricle. Na_x channels populate perineural processes of astrocytes and ependymal cells in the SFO. Even under hydrated (normal) conditions, ET-3 level expressed in the SFO can modulate the $[Na^+]_o$ dependency of Na_x and make Na_x sensitive to an increase in $[Na^+]_o$ in the physiological range. When animals are dehydrated, $[Na^+]$ in plasma and CSF significantly increases above the usual level. Under such conditions, the $[Na^+]_o$ exceeds the threshold of Na_x, Na_x channels open, and the $[Na^+]_i$ in these Na_x-bearing cells is increased. This leads to activation of Na^+/K^+-ATPase in these cells. Activated Na^+/K^+-ATPase consumes ATP higher than the usual level to pump out Na^+. To fuel Na^+/K^+-ATPase with ATP, the glial cells enhance glucose uptake to stimulate anaerobic glycolysis. Lactate, the end product of the anaerobic glycolysis, is released from the glial cells and supplied to neurons, including GABAergic neurons, through the processes enveloping them. Lactate stimulates the activity of the GABAergic neurons through production of ATP, which presumably leads to the regulation of hypothetic neurons involved in the control of salt-intake behavior. In dehydrated *Na_x*-deficient mice, the $[Na^+]$-dependent stimulation of glycolysis is impaired and the activity of the GABAergic neurons is not promoted.

Na_x is also expressed in non-myelinating Schwann cells of the adult peripheral nervous system, but the pathophysiological role remained unclear. Recently, we found that functional recovery of the hind paw responses from the sciatic nerve transection was delayed in *Na_x*-deficient mice. Our studies revealed that Na_x is involved in the regeneration process of injured peripheral nerves by enhancing lactate release from non-myelinating Schwann cells, where Na_x was activated by ET-1 through ET_BR signaling. This finding may bring new strategies to promote peripheral nerve regeneration.

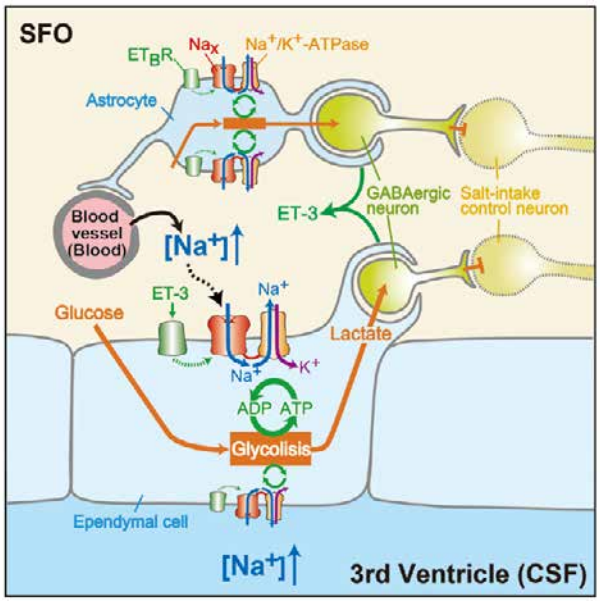


Figure 3. Overview of the $[Na^+]$-sensing mechanism and Na_x-dependent regulation of neuronal activity in the SFO.

Publication List

〔Original papers〕

- Suzuki, R., Matsumoto, M., Fujikawa, A., Kato, A., Kuboyama, K., Shintani, T., Sakuta, H., and Noda, M. (2014). SPIG1 negatively regulates BDNF maturation. J. Neurosci. *34*, 3443-3453.
- Unezaki, S., Katano, T., Hiyama, T.Y., Tu, N.H., Yoshii, S., Noda, M., and Ito, S. (2014). Involvement of Na_x sodium channel in peripheral nerve regeneration via lactate signaling. Eur. J. Neurosci. *39*, 720-729.

〔Review article〕

- Noda, M., and Hiyama, T.Y. (2014). The Na_x channel: What it is and what it does. The Neuroscientist DOI: 10.1177/1073858414541009.

DIVISION OF BRAIN BIOLOGY †

Professor
YAMAMORI, Tetsuo

Associate Professor
WATAKABE, Akiya

Assistant Professor:	*KOMINE, Yuriko*
	SADAKANE, Osamu
NIBB Research Fellow:	*OHTSUKA, Masanari**
Technical Staff:	*OHSAWA, Sonoko*
Postdoctoral Fellow:	*TAKAJI, Masafumi*
	OHTSUKA, Masanari
	HATA, Katsusuke
	NAKAGAMI, Yuki
SOKENDAI Graduate Student:	*SHUKLA, Rammohan*
Technical Assistant:	*NAKAMURA, Tohru*
	MORITA, Junko
	IMAI, Akiko
	KON, Yayoi

This year we have reported the following results. (1) Distribution of serotonin receptor subtypes and serotonergic terminations in the marmoset brain, (2) Simultaneous visualization of extrinsic and intrinsic axon collaterals in the mouse brain, (3) Characterization of claustral neurons, (4) Distinct motor impairments of dopamine D1 and D2 receptor knockout mice.

I. Distribution of serotonin receptor subtypes and serotonergic terminations in the marmoset brain

We examined the mRNA expression patterns of all the 13 members of the serotonin receptor (5HTR) family, by *in situ* hybridization (ISH) and the distribution of serotonergic terminations by serotonin transporter (SERT) protein immunohistochemical analysis. Ten of the 13 5HTRs showed significant mRNA expression in the marmoset brain. Our study showed several new features of the organization of serotonergic systems in the marmoset brain. (1) The thalamus expressed only a limited number of receptor subtypes compared with the cortex, hippocampus, and other subcortical regions. (2) In the cortex, there is layer-selective and area-selective mRNA expression of 5HTRs (Figure 1). (3) Highly localized mRNA expressions of 5HT1F and 5HT3A were observed. (4) There was a conspicuous overlap of the mRNA expression of receptor subtypes known to have somatodendritic localization of receptor proteins with dense serotonergic terminations in the visual cortex, the central lateral (CL) nucleus of the thalamus, the presubiculum, and the medial mammillary nucleus of the hypothalamus. This suggests a high correlation between serotonin availability and receptor expression at these locations. (5) The 5HTRs show differences in mRNA expression patterns between the marmoset and mouse cortices whereas the patterns of both the species were very similar in the hippocampus. We discussed the possible roles of 5HTRs in the marmoset brain revealed by the analysis of their overall mRNA expression patterns (Published in Front. Neural Circuits, 19 May 2014 | doi: 10.3389/fncir.2014.00052).

II. Simultaneous visualization of extrinsic and intrinsic axon collaterals in Golgi-like detail for mouse corticothalamic and corticocortical cells: a double viral infection method

We reported a novel tracing technique to stain projection neurons in Golgi-like detail by double viral infection. We used retrograde lentiviral vectors and adeno-associated viral vectors (AAV) to drive the "TET-ON/TET-OFF system" in neurons connecting two regions (Figure 2).

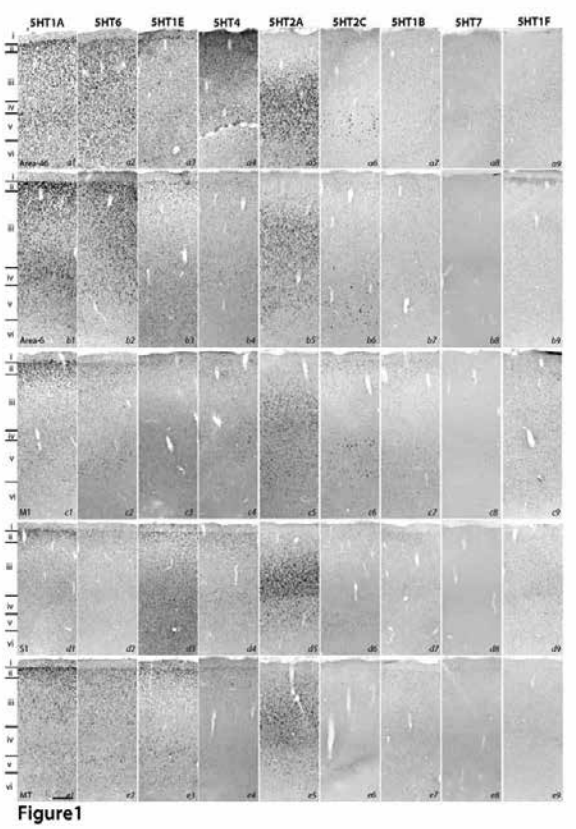


Figure1

Figure 1. ISH expression profiles of 5HTRs in the cortex. Area 46, area 6, primary motor cortex (M1), primary somatosensory cortex (S1), and V5 (MT). Layers identified by Nissl staining (not shown) are indicated on the left. Note that all images of a given gene are grouped together and presented at the same contrast level. Scale bar: 100 μm. (Cited from Shukla et al., Front. Neural Circuits, 8: 52, 2014).

Using this method, we successfully labeled the corticothalamic (CT) cells of the mouse somatosensory barrel field (S1BF) and motor cortex (M1) in their entirety (Figure 3). We also labeled contra- and ipsilaterally-projecting corticocortical (CC) cells of M1 by targeting contralateral M1 or ipsilateral S1 for retrograde infection. The strength of this method is that we can observe the morphology of specific projection neuron subtypes en masse. We found that the group of CT cells extended their dendrites and intrinsic axons extensively below but not within the thalamorecipient layer in both S1BF and M1, suggesting that the primary target of this cell type is not layer 4. We also found that both ipsi- and contra-lateral targeting CC cells in

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).
†: This laboratory was closed on 31 March, 2015.

M1 commonly exhibit widespread collateral extensions to contralateral M1 (layers 1–6), bilateral S1 and S2 (layers 1, 5 and 6), perirhinal cortex (layers 1, 2/3, 5, and 6), striatum, and claustrum. These findings not only strengthened the previous findings of single cell tracings but also extended them by enabling cross-area comparison of CT cells or comparison of CC cells of two different labeling systems (Published in Watakabe et al., Front. Syst. Neurosci. 8: 110, 2014).

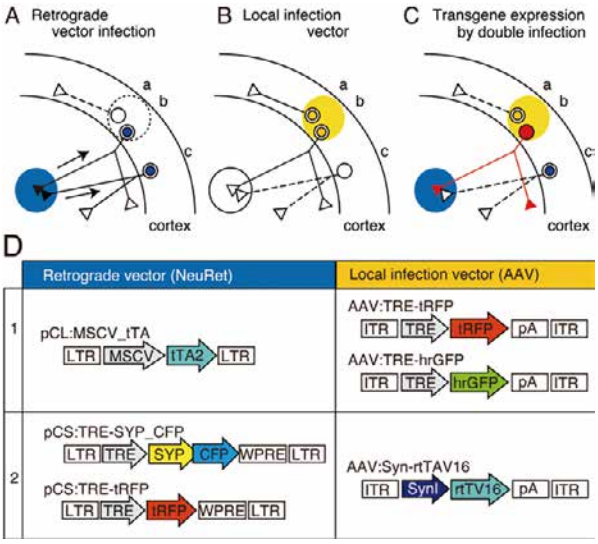


Figure 2. Schematic view of the TET double infection strategy and the viral vector constructs used for the experiments. (A–C) Schematic view of our double infection method, which utilizes the TET system. In this scheme, AAV is injected into the cortex (represented by yellow shading) as the local infection vector, while the NeuRet vector is injected into the subcortical region (represented by blue shading) as the retrograde vector. These vectors contain either tTA or TRE-transgene as depicted in **(D)**. In these panels, three neurons indicated as "a," "b," and "c" are shown. In panel **(A)**, the neurons infected with the retrograde vector ("b" and "c") are indicated by blue nuclei. The arrows represent the retrograde transport of the viral particles. In **(B)**, the neurons infected with the local infection vector ("a" and "b") are indicated by yellow nuclei. Panel **(C)** shows the consequence of double infection. Since the tTA and TRE-transgene are both present in neuron "b," high-level expression of the transgene takes place and fills the entire neuron with the transgene product (represented by red coloring). No transgene expression occurs when the neurons are infected by only one of the two viral vectors (e.g., neurons "a" and "c"). **(D)** Schematic representations of the viral constructs we used in this study. CFP, celulean; SynI, human synapsin I promoter; SYP, synaptophysin; tTA2, "TET-Off" tetracyclin transactivator; rtTV16, "TET-ON" reverse tetracyclin transactivator; tRFP, turboFP635; LTR, long terminal repeat; ITR, inverted terminal repeat; TRE, tetracycline responsive element; pA, poly(A) site; WPRE, woodchuck hepatitis virus post-transcriptional regulatory element (Cited from Watakabe et al., Front Neural Circuits. 8:110, 2014).

III. Characterization of claustral neurons by comparative gene expression profiling and dye-injection analyses

We investigated the identity of the claustrum as a part of the cerebral cortex, and in particular of the adjacent insular cortex, by connectivity features and patterns of gene expression. We mapped the cortical and claustral expression of several cortical genes in rodent and macaque monkey brains (*nurr1, latexin, cux2, and netrinG2*) to further assess shared features between the cortex and the claustrum. In

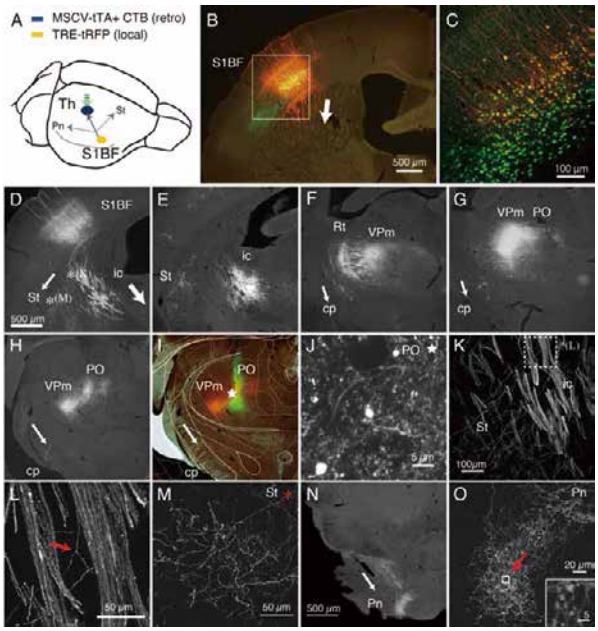


Figure 3. The identity of the claustrum as a part of the cerebral cortex, CT cells were efficiently labeled by TET

(A) Schematic view of double injection. The NeuRet vector encoding tTA (MSCV_tTA) was injected into the thalamus (represented by the green arrow) as a retrograde vector, together with CTB-Alexa488. The AAV vector carrying TRE-tRFP was injected into S1BF. We observed collateral projections to the thalamus (Th), striatum (St) and pons (Pn). (B) The coronal section at the level of S1BF showing CT cells that were labeled red by expression of tRFP. The green signals are also CT cells retrogradely labeled by CTB-Alexa488. The arrow indicates the direction of corticothalamic projections.

(C) Magnified view of the white square in (B). The contrast of this confocal image is adjusted so that we can identify each labeled cell body. (D–I) Coronal images of the tRFP signals that follow those in (B) were aligned in order from anterior to posterior. The arrows indicate the directions of the collateral projections labeled by this strategy. (D) The striatal collateral splits at the level of (D) [*(K) and *(M); higher magnification views in (K,M)]. In (E–H), the thalamic projection that proceeded in the internal capsule (ic) innervate reticular thalamic nucleus (Rt), VPm and PO, while the split collaterals proceed within the cerebral peduncle (cp). (I) Multicolor merged view for (H), which is just around the thalamic injection site. The green signals indicate the local deposit of CTB-Alexa 488, which mark the injection center for the retrograde vector. The asterisk shows the position of (J). The Paxinos atlas (Paxinos and Franklin, 2003) was superimposed to the image in (H) to make (I), to show the identification of the spots of concentrated tRFP signals. (J) A magnified view of the injection site indicated by asterisks in (I). Note that we can examine the fine branches with boutons even around the injection site (K) A magnified view of the striatal collaterals that branched out of the CT bundles of internal capsule (see D for low magnification view). The dotted square [denoted as *(L)] is magnified in (L) to indicate the example of collateral branching (indicated by the red arrow). (M) A magnified view of the striatal collaterals that arborized at the final destination (see D for low magnification view). (N) A low power view of the pontine collaterals. (O) A magnified view of the terminal arborization of pontine collaterals. The boxed region indicated by a red arrow is magnified in the inset, which exhibits a cluster of large boutons. The images used in (C, J–O) are maximal projection stacks of confocal sections.

Definitions: S1BF, somatosensory barrel field; Th, thalamus; Pn, pons; St, striatum; ic, internal capsule; cp, cerebral peduncle; Rt, reticular thalamic nucleus; VPm, ventral posteromedial nucleus; PO, posterior thalamic nuclear group (Cited from Watakabe et al., Front. Neural Circuits *8*:110, 2014).

mice, these genes were densely expressed in the claustrum, but very sparsely in the cortex and not present in the striatum. To test whether the cortical vs. claustral cell types

can be distinguished by co-expression of these genes, we performed a panel of double ISH in mouse and macaque brains. NetrinG2 and nurr1 genes were co-expressed across the entire cortex and claustrum, but cux2 and nurr1 were co-expressed only in the insular cortex and claustrum. Latexin was expressed, in the macaque, only in the claustrum. The nurr1+ claustral neurons expressed VGluT1, a marker for cortical glutamatergic cells and send cortical projections. Taken together, our data suggest a partial commonality between claustral neurons and a subtype of cortical neurons in the monkey brain. Moreover, in the embryonic (E110) macaque brain, many nurr1+ neurons were scattered in the white matter between the claustrum and the insular cortex, possibly representing their migratory history. In a second set of experiments, we injected Lucifer Yellow intracellularly in mouse and rat slices to investigate whether dendrites of insular and claustral neurons can cross the border of the two brain regions. Dendrites of claustral neurons did not invade the overlying insular territory. In summary, the gene expression profile of the claustrum is similar to that of the neocortex, in both rodent and macaque brains, but with modifications in density of expression and cellular co-localization of specific genes (Published in Watakabe, et al., Front Syst Neurosci. 8:98, 2014).

IV. Distinct motor impairments of dopamine D1 and D2 receptor knockout mice revealed by three types of motor behavior

We examined the behavioral difference among N10 congenic D1R and D2R KO, and wild type (WT) mice. Both D1R and D2R knock out (KO) mice of the major dopamine receptors show significant motor impairments. However, there are some discrepant reports, which may be due to differences in genetic background and experimental procedures. In addition, only a few studies directly compared the motor performance of D1R and D2R KO mice. First, we examined spontaneous motor activity in the home cage environment for 5 consecutive days. Second, we examined motor performance using the rota-rod task, a standard motor task in rodents. Third, we examined motor ability with the Step-Wheel task in which mice were trained to run in a motor-driven turning wheel adjusting their steps on foothold pegs to drink water. The results showed clear differences among the mice of three genotypes in three different types of behavior. In monitoring spontaneous motor activities, D1R and D2R KO mice showed higher and lower 24 h activities, respectively, than WT mice. In the rota-rod tasks, at a low speed, D1R KO mice showed poor performance but later improved, whereas D2R KO mice showed good performance early, without further improvement. When first subjected to a high speed task, the D2R KO mice showed poorer rota-rod performance at low speeds than the D1R KO mice. In the Step-Wheel task, across daily sessions, D2R KO mice increased their running speed sufficiently to reach the waterspout, and decreased time between touching the floor due to missing the peg, and decreased the number of times the wheel was stopped, which was much better performance than that of D1R KO mice. These incongruent results between the two tasks for D1R and D2R KO mice may be due to differences in motivation for the rota-rod and Step-Wheel tasks, i.e. aversion- and reward-driven, respectively. The Step-Wheel system may become a useful tool for assessing the motor ability of WT and mutant mice (Published in Nakamura et al., Front Integr. Neuroscience. 8: 56, 2014).

Publication List

〔Original papers〕

- Nakamura, T., Sato, A., Kitsukawa, T., Momiyama, T., Yamamori, T., and Sasaoka, T. (2014). Distinct motor impairments of dopamine D1 and D2 receptor knockout mice revealed by three types of motor behavior. Front. Integr. Neurosci. *8*, 56.
- Shukla, R., Watakabe, A., and Yamamori, T. (2014). mRNA expression profile of serotonin receptor subtypes and distribution of serotonergic terminations in marmoset brain. Front. Neural Circuits *8*, 52.
- Watakabe, A., Ohsawa, S. Ichinohe, N., Rockland, K.S., and Yamamori, T. (2014). Characterization of claustral neurons by comparative gene expression profiling and dye-injection analyses. Front. Syst. Neurosci. *8*, 98.
- Watakabe, A., Takaji, M., Kato, S., Kobayashi, K., Mizukami, H., Ozawa, K., Ohsawa, S., Matsui, R., Watanabe, D., and Yamamori T. (2014). Simultaneous visualization of extrinsic and intrinsic axon collaterals in Golgi-like detail for mouse corticothalamic and corticocortical cells: a double viral infection method. Front. Neural Circuits *8*, 110.

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

- Watakabe, A., Ohtsuka, M, Kinoshita, M. Takaji, M. Isa, K Mizukami, H., Ozawa, K. Isa, T., and Yamamori, T. Comparative analyses of adeno-associated viral vector serotypes 1, 2, 5, 8 and 9 in marmoset, mouse and macaque cerebral cortex. Neurosci. Res. 2014 Sep 18.

DIVISION OF BRAIN CIRCUITS

Professor
MATSUZAKI, Masanori

Assistant Professor:	*WAKE, Hiroaki*
	HIRA, Riichiro
	MASAMIZU, Yoshito
NIBB Research Fellow:	*TANAKA, Yasuhiro**
	EBINA, Teppei
Technical Staff:	*OHSAWA, Sonoko*
Postdoctoral Fellow:	*MASAMIZU, Yoshito**
	TANAKA, Yasuyo
	TANAKA, Yasuhiro
	TAKEDA, Yuta
SOKENDAI Graduate Student:	*OHKUBO, Fuki*
	HASEGAWA, Ryota
	SUZUKI, Ayami
Visiting Scientist:	*KATO, Daisuke*
	KONDO, Masashi
	HIRAKAWA, Reiko
Visiting Graduate Student:	*TERADA, Shin-Ichiro*
	SUGAWARA, Yuki
Technical Assistant:	*HIMENO, Miki*
	SAITO, Junko
	KOBAYASHI, Tomoko
	KOTANI, Keiko
	TAKAHASHI, Yoichi
	IWASE, Etsuko
	IMOTO, Eiko
	SUGIURA, Haruka
Secretary:	*SUGIYAMA, Tomomi*

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. One of the most important problems in neuroscience is how a variety of spatio-temporally heterogeneous neural activity in the cortex emerges moment-by-moment at multiple stages of a movement.

I. Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task.

The primary motor cortex (M1) possesses two intermediate layers upstream of the motor-output layer: Layer 2/3 (L2/3) and layer 5a (L5a). Although repetitive training often improves motor performance and movement coding by M1 neuronal ensembles, it is unclear how neuronal activities in L2/3 and L5a are reorganized during motor task learning. We conducted two-photon calcium imaging in the mouse M1 during 14 training sessions of a self-initiated lever-pull task (Figure 1).

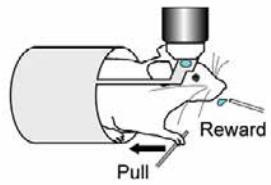


Figure 1. Schematic showing the self-initiated lever-pull task for a head-restrained mouse.

Mice were trained over 14 sessions (1 h/d) to use their right forelimb to pull a lever over a distance of 5 mm for 700 ms to acquire a water reward. Training was initiated 2–3 weeks after injection of an adeno-associated virus (AAV) 2/1 encoding GCaMP3 into the left forelimb M1. Two-photon calcium imaging was performed on the left forelimb M1 of 9 mice without apparent deterioration of the neuronal responsiveness over repeated sessions in L2/3 or L5a.

We assessed the effect of repetitive training on the neuronal coding of lever movement because the lever movement provided a good representation of forelimb movement. We determined whether the accuracy of the lever trajectory predicted from neuronal ensemble activity improved with learning. To evaluate the predictive information carried by the neurons ($I_{ensemble}$), we calculated the mutual information between predicted and recorded lever trajectories. In L2/3, there was no significant change in $I_{ensemble}$ from early sessions (sessions 1-4) to late sessions (sessions 11-14; Figure 2). By contrast, in L5a, $I_{ensemble}$ was significantly higher in the late sessions than in the early sessions (Figure 2). No consistent changes in L2/3 $I_{ensemble}$ were observed as learning progressed, whereas increases in L5a $I_{ensemble}$ were associated with improved task performance.

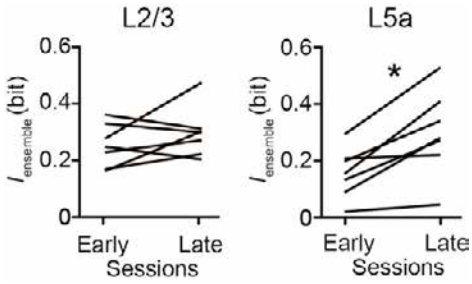


Figure 2. $I_{ensemble}$ averaged over early and late sessions for each field in L2/3 (left; n=7) and L5a (right; n=7). *$P < 0.05$.

Next, to determine how each neuron changed its predictive information (I_{single}) during learning, we analyzed I_{single} of neurons. Neurons that increased or decreased I_{single} across sessions were defined as increase- or decrease-neurons, respectively. In L2/3, there was a similar proportion of increase- and decrease-neurons (Figure 3), suggesting that the overall change in L2/3 I_{single} was balanced across all imaged fields. By contrast, in L5a, there was a higher proportion of increase-neurons than decrease-neurons (Figure 3). Many L5a increase-neurons displayed an increase in activity associated with lever-pull movements during learning. Increase-neurons were critical for the correlation between L5a $I_{ensemble}$ and task performance.

Each neuron within a field in a given session was ranked according to I_{single} and the rank was then normalized between 1 (top) and 100 (bottom). The $I_{ensemble}$ of the top 20% of ranked neurons was larger than the $I_{ensemble}$ of the bottom 50%

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2014. The former title is indicated by an asterisk (*).

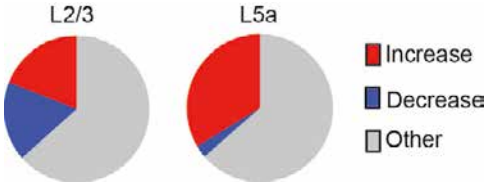


Figure 3. Proportion of increase-, decrease-, and other-neurons across all fields in L2/3 (left) and L5a (right).

of ranked neurons in > 85% of sessions in L2/3 and L5a. Thus, the top 20% of ranked neurons were considered to be highly ranked neurons that strongly contributed to the ensemble representation. L2/3 increase-neurons rose through the ranks from the early sessions to occupy 31% of the top 20% of ranked neurons in the late sessions. Forty-six percent of the top 20% of ranked neurons in the early sessions were also in the top 20% of ranked neurons in the late sessions. We call these neurons stable, highly ranked neurons. The mean activity of the stable, highly ranked neurons in L2/3 during successful lever-pull movements did not significantly change from the early to late sessions. These results suggest that L2/3 $I_{ensemble}$ was maintained by balanced activity of increase- and decrease-neurons, and by activity of stable, highly ranked neurons. In contrast to L2/3, the rank of L5a increase-neurons gradually increased during the middle to the late sessions, and increase-neurons accounted for 73% of the top 20% of ranked neurons in the late sessions. In L5a, only 23% of the top 20% of ranked neurons in the early sessions were in the top 20% of ranked neurons in the late sessions. These results indicate that the increase in L5a $I_{ensemble}$ was supported by the conversion of increase-neurons into highly ranked neurons during the middle to late sessions.

We also found that 31% of the examined L5a crossed corticostriatal (CCS) neurons were increase-neurons and 3% were decrease-neurons. Of the top 20% of the ranked CCS neurons observed in the early sessions, only 17% were in the top 20% of the CCS neurons observed in the late sessions. Twenty-six percent of examined L5a corticospinal (CSp) neurons were increase-neurons and 5% were decrease-neurons. Of the top 20% of the ranked CSp neurons observed in the early sessions, 100% were in the top 20% of the CSp neurons observed in the late sessions. These results suggest that increase-neurons were more prevalent than decrease-neurons for both L5a CCS and CSp neurons and that a subset of L5a CCS neurons was likely recruited into the newly formed ensemble that represented the lever-pull movement.

The balanced changes in L2/3 neurons could reflect the fact that L2/3 neurons with highly plastic activity process a variety of information from other areas of the brain to maintain a constant overall level of network activity for homeostasis. L2/3 neurons may play an important role in rapid acquisition of novel movements or rapid adaptation to some disturbance by combining sensory feedback and motor primitives. As such, L2/3 may work as a driver for motor output throughout learning. L5a $I_{ensemble}$ increased during learning, and this was maintained by a gradual increment in I_{single} ranks of a subset of L5a neurons. These neurons were directly associated with lever-pull movement and presumably worked as a potent driver for motor output in the late sessions. Changes in L5a neurons in M1 may represent the formation of motor memory that accompanies memory transfer from the prefrontal area to M1 and the basal ganglia. Stable, highly ranked CSp neurons may be directly associated with muscle movements or motor primitives. The distinct dynamic networks in L2/3 and L5a, the two intermediate layers of M1, are clearly core elements that drive the L5b motor output for well-learned movements.

II. Reward-timing-dependent bidirectional modulation of cortical microcircuits during optical single neuron operant conditioning

Animals rapidly adapt to environmental change. To reveal how cortical microcircuits are rapidly reorganized when an animal recognizes novel reward contingency, we conducted two-photon calcium imaging of layer 2/3 motor cortex neurons in mice and simultaneously reinforced the activity of a single cortical neuron with water delivery.

Head-restrained mice were trained to perform a self-initiated lever-pull task using the right forelimb before single-neuron operant conditioning by two-photon calcium imaging (2pSNOC). After 5–14 lever-pull task training sessions, two-photon calcium imaging of L2/3 motor cortical neurons was performed during SNOC. Each 2pSNOC session consisted of three periods: a pre-conditioning period (10 min), a conditioning period (15 min), and a post-conditioning period (10 min). 2pSNOC was performed during the conditioning period. Active neurons during the pre-conditioning period were reconstructed and classified into two groups: neurons with high activity during lever-related periods, and other neurons ("lever-unrelated neurons"). One neuron was targeted in the conditioning period. During the conditioning period, the mouse performed a 2pSNOC task. During the 2pSNOC task, water drops were successfully delivered to the mouse immediately after the $\Delta F/F$ of a single target neuron increased above a threshold (Figure 4).

During the conditioning period, for lever-unrelated target neurons, activity increased by approximately 50%, whereas, for lever-related target neurons, activity did not change (Figure 5). The reward frequency increased by approximately 50% when lever-unrelated neurons were targeted, and did not change when lever-related neurons were targeted. The rapid operant conditioning of single lever-unrelated neurons was successful even though the activity of non-target neurons slightly changed. When lever-related neurons were targeted, the mouse performed goal-directed lever-pull movements from the onset of the conditioning period without recognition of SNOC. This

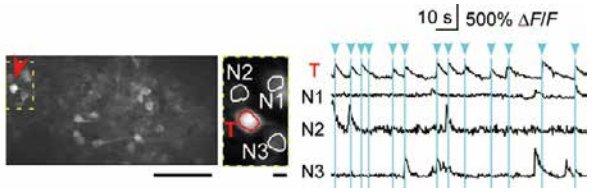


Figure 4. Left: A time-averaged image of a L2/3 field during 2pSNOC. Red arrowhead, a target neuron. Scale bar, 100 μm. Middle: Expanded image of the rectangle in left. Scale bar, 10 μm. Right: $\Delta F/F$ of the target (T) and the three neighboring (N1, N2, and N3) neurons. Cyan bars, reward delivery.

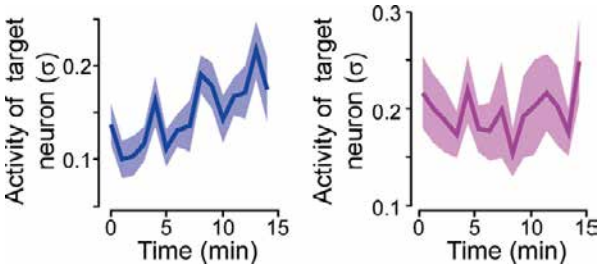


Figure 5. Time course of the activity of lever-unrelated (left; n=11) and lever-related (right; n=13) target neurons during the conditioning period. The activity is Z scored for each neuron. Thick lines and shading indicate the mean ± s.e.m.

may explain why the activity gain (the ratio of the mean activity in the last 5 min of the conditioning period to the mean activity in the first 5 min of the conditioning period minus 1) for lever-related target neurons was not significantly positive. In the following analyses, only data from sessions with a lever-unrelated target neuron were used.

Non-target neurons whose activity was temporally, but not spatially, associated with the target neuron increased their activity. Non-target neurons should have been affected by the water delivery because it was the reinforcer. For each non-target neuron, the ratio of the sum of the activity in a 0.33-s time bin t s after reward delivery to the sum of the remaining activity in the same bin was defined as the reward synchronization index (t) (RSI(t)). For each time bin, activity gain was averaged across the non-target neurons with RSI values in the top 5%. The activity gain of non-target neurons with RSI in the top 5% was significantly larger than zero when t ranged from –0.33 s to 0.33 s, and significantly smaller than zero when t was 2.3, 3, and 4 s. We refer to this phenomenon as reward-timing-dependent bidirectional modulation (RTBM), and refer to neurons that had RSI (–0.33 s to 0.33 s) in the top 5% as SR neurons and neurons that had RSI (2 s to 4 s) in the top 5% as AR neurons. The activity gain of SR and AR neurons was substantially positive and negative, respectively. The percentage of lever-related and lever-unrelated neurons was similar between SR and AR neurons. The distance of SR and AR neurons from the target neuron was similar to the distance of other neurons from the target neuron. The pairwise correlation with the target neuron was higher for SR neurons than for AR neurons and other neurons during both the conditioning period and pre-conditioning period without water delivery. This indicates that SR neurons were frequently active together with the target neuron. By contrast, AR neurons did not appear different from other non-target neurons in terms of their associations with the target neuron. Neither SR nor AR neurons were specifically related to licking. Likewise, neither SR nor AR neurons were specifically related to lever-pull in response to the reward delivery.

To validate whether the activity timing relative to the reward delivery is sufficient to induce the neuronal activity changes, we performed repetitive pairing of the activity of a random set of neurons and reward delivery with different time intervals. In each photostimulation session, 60 photostimuli were delivered during a 15 min period to induce firing in channelrhodopsin-2-expressing neurons in the field of view and a reward was given 0.25 s after or 2.5 s before each photostimulation (potentiation protocol and depression protocol, respectively). The photostimuli-induced activity of photostimuli-responsive neurons significantly increased and decreased in the potentiation and depression protocols, respectively (Figure 6). The activity of the other neurons did not change in either protocol. Thus, repetitive pairing of neuronal activity with reward delivery at different time intervals was sufficient to recreate RTBM.

Our results suggest that the microcircuit has the capability to strengthen the activity of neurons that were previously included in the same ensemble without strong spatial constrains. RTBM may be one of possibly many processes that underlie rapid reorganization of the L2/3 cortical microcircuit during fast adaptation to environmental changes, which occurs during BMI/BCI learning, motor adaptation, and skill learning.

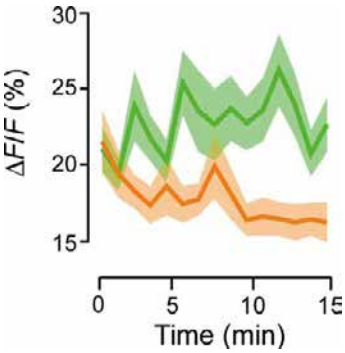


Figure 6. Time course of the mean $\Delta F/F$ of photostimuli-responsive neurons in the potentiation (green, n=78 neurons) and depression (orange, n=40 neurons) protocols. Thick lines and shading indicate the mean ± s.e.m.

Publication List

〔Original papers〕

- Hira, R., Ohkubo, F., Masamizu, Y., Ohkura, M., Nakai, J., Okada, T., and Matsuzaki, M. (2014). Reward-timing-dependent bidirectional modulation of cortical microcircuits during optical single neuron operant conditioning. Nature Commun. *5*, 5551.
- Masamizu, Y., Tanaka, Y.R., Tanaka, Y.H., Hira, R., Ohkubo, F., Kitamura, K., Isomura, Y., Okada, T., and Matsuzaki, M. (2014). Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task. Nature Neurosci. *17*, 987-994.

LABORATORY OF NEUROPHYSIOLOGY

Associate Professor
WATANABE, Eiji

NIBB Research Fellow: *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 the 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).

Last and this year, 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 biological motion could prominently induce shoaling behavior. We have shown what aspects of motion are critical in the induction of shoaling behavior. Motion or 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 the information which is vital for their survival (Nakayasu & Watanabe, 2014).

This year, we have developed novel method for behavior analysis by using 3D computer graphics. The fine control of various features of living fish have been difficult to achieve in studies of fish behaviors. However, computer graphics allow us to manipulate morphological and motion cues systematically. Therefore, we have constructed 3D computer graphics 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.

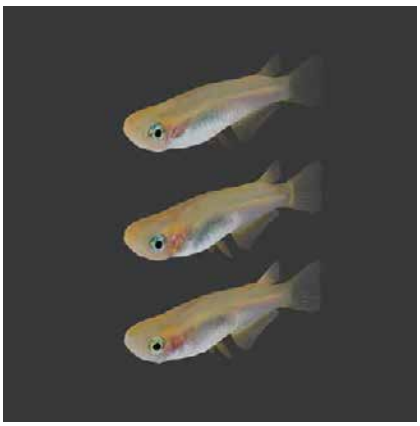


Figure 1. Virtual Medaka fish constructed of 3D polygonal models and photo textures.

II. Psychophysical study of Human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual illusions of 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). This year, we have made a more developed novel visual illusion, the shelf-shadow illusion (Figure 2).

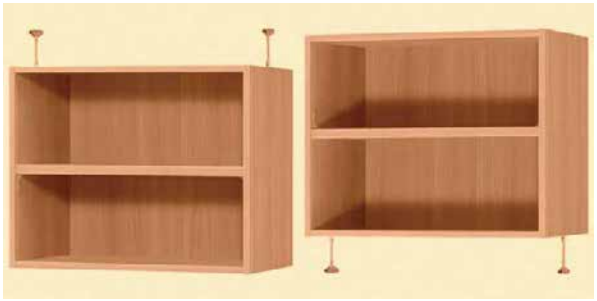


Figure 2. Shelf-Shadow Illusion (Photograph version). Upward shadows look darker than downward shadows even when the shadows are actually the same brightness. Third place award of The 5th Illusion Contest in Japan.

Publication List

〔Original paper〕

● Nakayasu, T., and Watanabe, E. (2014). Biological motion stimuli are attractive to medaka fish. Animal Cognition *17*, 559-575.

Division of Evolutionary Biology

Professor
HASEBE, Mitsuyasu

Associate Professor
MURATA, Takashi

Assistant Professor: *TAMADA, Yosuke*
ISHIKAWA, Masaki
Technical Staff: *KABEYA, Yukiko*
NIBB Research Fellow: *IMAI, Akihiro*
Postdoctoral Fellow: *MANO, Hiroaki*
SHIBATA, Tomoko
TAMAOKI, Daisuke
TORIBA, Taiyo
SOKENDAI Graduate Student:
FUKUSHIMA, Kenji
KAMIDA, Chiharu
KOSHIMIZU, Shizuka
LI, Chen
MORISHITA, Mio
SUGAYA, Tomomi
ZHANG, Liechi
HORIUCHI, Yuta
Visiting Graduate Student: *GU, Nan*
Visiting Scientist: *Čuperová, Zuzana*
KAWASHIMA, Takeshi
Technical Assistant: *AOKI, Etsuko*
GOTO, Misako
KAJIKAWA, Ikumi
MORI, Asuka
MATSUZAKI, Yoko
NISHI, Tayo
OOI, Shoko
MASUOKA, Tomoko
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 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 leads to generation of a new individual, which is an effective strategy for propagation. The ability to reprogram is different from species to species but the reason is unknown. The moss *Physcomitrella patens* has a rapid reprogramming ability (see http://www.nibb.ac.jp/evodevo/0.TopE.html) and is feasible for use in experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours. We reported that two paralogous *P. patens WUSCHEL-related homeobox 13-like* (*PpWOX13L*) genes, homologs of stem cell regulators in flowering plants, are transiently upregulated and required for the initiation of cell growth during stem cell formation. Concordantly, Δ*ppwox13l* deletion mutants fail to upregulate genes encoding homologs of cell wall loosening factors during this process. During the moss life cycle, most of the Δ*ppwox13l* mutant zygotes fail to expand and initiate an apical stem cell to form the embryo. Our data show that *PpWOX13L* genes are required for the initiation of cell growth specifically during stem cell formation, in analogy to WOX stem cell functions in seed plants, but using a different cellular mechanism (Sakakibara *et al*. 2014).

To perform fine live imaging during the reprogramming process, we now try to apply Adaptive optics (AO) to the observation of Physcomitrella cells. AO is the technique to cancel the aberration caused by atmospheric turbulence and to perform diffraction- limited observation of celestial bodies from the ground. Applying AO to microscopy, we can cancel the aberration caused by cellular structures and perform high-resolution live imaging. To construct the AO system, we first analyzed the optical properties of Physcomitrella cells. Live-cell imaging with bright field and phase contrast microscopies as well as image degradation analysis using fluorescent beads demonstrated that chloroplasts are the main source of the disturbance in the cell (Tamada *et al*. 2014). According to this information, we constructed a prototype of an AO microscope that can correct the aberration caused by chloroplasts. Images of chloroplasts that are located at the opposite side from the objective lens in the leaf cell are degraded due to aberration from cellular structures including chloroplasts themselves. With AO microscopy, we successfully obtained fine images of the chloroplast where the grana structure was observed more clearly. This study was mainly conducted by Yosuke Tamada.

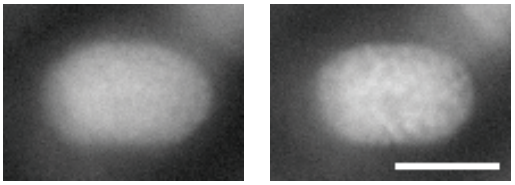


Figure 1. AO correction of a chloroplast image. Left, no correction; right, with AO. Bar, 5 μm.

III. Evolution of Regeneration: Master Regulator for Reprogramming STEMIN

Animal somatic cells can be reprogrammed to induce pluripotent stem (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. On the other hand, differently from *STEMIN1*, induction of *STEMIN2* or *STEMIN3* in gametophores did not change leaf cells into chloronema apical stem cells. These indicate that *STEMIN1* has an enough ability to change intact leaf cells to stem cells, but its paralogous genes do not. Masaki Ishikawa was this study's main researcher.

IV. Evolution of Elaborated Cell Division Machinery: Spindle body

At mitosis, all eukaryotic cells divide chromosomes to two daughter cells using a mitotic spindle, which is composed of microtubules. For accurate distribution of the chromosomes, the spindle has two poles. The centrosomes, which act as microtubule organizing centers, ensure formation of the two poles in metazoan cells. In contrast, the cells of land plants and their sister group, charophycean green algae, form a 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. It is challenging, however, to visualize microtubule reorganization during spindle formation in living plant cells, because large numbers of microtubules rapidly redistribute in 3-dimensional space. 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. Our data shows that microtubules elongate from template microtubules on the nuclear envelope upon nuclear envelope breakdown. The template microtubules are distinct from the spindle microtubules because they disappear during spindle development. The data suggests that microtubules which had been organized before spindle formation are the organizers of the bipolar spindle. Takashi Murata was this study's main researcher.

V. Evolution of water conducting systems

The development of a water conducting system was one of the most important requirements for land plants. Recent studies implicate a group of NAC domain transcription factors including VND6 and VND7 of *Arabidopsis thaliana* as key regulators of formation of xylem vessels, water-conducting cells in vascular plants. However, molecular mechanisms for development of other types of water-conducting cells are still unclear. We collaborated with Prof. Taku Demura in NAIST and showed that their *Physcomitrella patens* homologues, named PpVNS1 to PpVNS8, play a crucial role during the development of hydroids, specialized water-conducting cells in bryophytes. *PpVNS* genes are expressed in the midrib of developing leaves, in which hydroids are developed. Overexpression of *PpVNS* genes induced cell death in *P. patens* and ectopic formation of vessel-like cells in the vascular plant *Arabidopsis*. From these observations, we proposed that the last common ancestor of bryophytes and vascular plants had developed a system using VNS homologues to induce cell death during water-conducting cell formation. Our findings also suggest that the water-conducting cells in bryophytes and vascular plants are homologous, and that current land plants share a conserved genetic basis with VNS family proteins for development of water conducting systems (Xu *et al*. 2014).

VI. 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 histological, physiological, biochemical, and behavioral approaches, the lack of reverse 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). This new technique is currently applied for live imaging of actin cytoskeleton and calcium dynamics, both of which participate in the seismonastic movement through as-yet-unidentified mechanisms. Our transgenic technique also enabled us to develop a CRISPR/Cas-mediated gene knock-out system in this species. To investigate adaptive meanings of the seismonastic movement, we now try to produce immotile mutants using the CRISPR/Cas system. This study was conducted mainly by Hiroaki Mano.

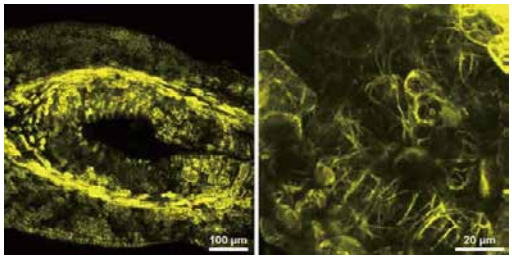


Figure 2. Visualization of actin cytoskeleton in leaflets of *Mimosa pudica* using a Lifeact-Venus protein

VII. 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. On the other hand, the oxidized form of xanthommatin was found in mantises with brown body color. To further elucidate the mechanism underlying

the different body coloration, we are now analyzing the ultrastructure of ommochrome granules in pigment cells of these mantises by transmission electron microscopy. This work was mainly done by Hiroaki Mano.

VIII. Evolution of pitcher leaves in carnivorous plants

Carnivorous plants form specialized leaves that are capable of attracting, trapping, and digesting prey and absorbing nutrients. The unusual plants evolved from non-carnivorous plants but their evolutionary process is mostly unknown. To understand the genomic changes associated with the evolution of carnivory, we sequenced 2-Gbp genome of the Australian pitcher plant *Cephalotus follicularis* in collaboration with Beijing Genomics Institute. This study was conducted mainly by Kenji Fukushima and Tomoko Shibata.

Publication List

〔Original papers〕

- Furuta, Y., Namba-Fukuyo, H., Shibata, T.F., Nishiyama, T., Shigenobu, S., Suzuki, Y., Sugano, S., Hasebe, M., and Kobayashi, I. (2014). Methylome diversification through changes in DNA methyltransferase sequence specificity. PLoS Genet. *10*, e1004272.
- Gusev, O., Suetsugu, Y., Cornette, R., Kawashima, T., Logacheva, M.D., Kondrashov, A.S., Penin, A.A., Hatanaka, R., Kikuta, S., Shimura, S., Kanamori, H., Katayose, Y., Matsumoto, T., Shagimardanova, E., Alexeev, D., Govorun, V., Wisecaver, J., Mikheyev, A., Koyanagi, R., Fujie, M., Nishiyama, T., Shigenobu, S., Shibata, T.F., Golygina, V., Hasebe, M., Okuda, T., Satoh, N., and Kikawada, T. (2014). Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. Nature Commun. *5*, 4784.
- Hieno, A., Naznin, H.A., Hyakumachi, M., Sakurai, T., Tokizawa, M., Koyama, H., Sato, N., Nishiyama, T., Hasebe, M., Zimmer, A.D., Lang, D., Reski, R., Rensing, S.A., Obokata, J., and Yamamoto, Y.Y. (2014). ppdb: plant promoter database version 3.0. Nucleic Acids Res. *42*, D1188-1192.
- Ishida, T., Tabata, R., Yamada, M., Aida, M., Mitsumasu, K., Fujiwara, M., Yamaguchi, K., Shigenobu, S., Higuchi, M., Tsuji, H., Shimamoto, K., Hasebe, M., Fukuda, H., and Sawa, S. (2014). Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. EMBO Rep. *15*, 1209-1215.
- Kishi-Kaboshi, M., Muto, H., Takeda, A., Murata, T., Hasebe, M., and Watanabe, Y. (2014). Localization of tobacco germin-like protein 1 in leaf intercellular space. Plant Physiol. Biochem. *85*, 1-8.
- Mano, H., Fujii, T., Sumikawa, N., Hiwatashi, Y., and Hasebe, M. (2014). Development of an agrobacterium-mediated stable transformation method for the sensitive plant *Mimosa pudica*. PLoS One *9*, e88611.
- Sakakibara, K., Reisewitz, P., Aoyama, T., Friedrich, T., Ando, S., Sato, Y., Tamada, Y., Nishiyama, T., Hiwatashi, Y., Kurata, T., Ishikawa, M., Deguchi, H., Rensing, S.A., Werr, W., Murata, T., Hasebe, M., and Laux, T. (2014). WOX13-like genes are required for reprogramming of leaf and protoplast cells into stem cells in the moss *Physcomitrella patens*. Development *141*, 1660-1670.
- Takeshita, K., Shibata, T.F., Nikoh, N., Nishiyama, T., Hasebe, M., Fukatsu, T., Shigenobu, S., and Kikuchi, Y. (2014). Whole-genome sequence of *Burkholderia* sp. strain RPE67, a bacterial gut symbiont of the bean bug *Riptortus pedestris*. Genome Announc. *2*, e00556-14.
- Tamada, Y., Murata, T., Hattori, M., Oya, S., Hayano, Y., Kamei, Y., and Hasebe, M. (2014). Optical property analyses of plant cells for adaptive optics microscopy. International J. Optomechatronics *8*, 1-11.
- Xu, B., Ohtani, M., Yamaguchi, M., Toyooka, K., Wakazaki, M., Sato, M., Kubo, M., Nakano, Y., Sano, R., Hiwatashi, Y., Murata, T., Kurata, T., Yoneda, A., Kato, K., Hasebe, M., and Demura, T. (2014). Contribution of NAC transcription factors to plant adaptation to land. Science *343*, 1505-1508.
- Yoshida, K., Makino, T., Yamaguchi, K., Shigenobu, S., Hasebe, M., Kawata, M., Kume, M., Mori, S., Peichel, C.L., Toyoda, A., Fujiyama, A., and Kitano, J. (2014). Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. PLoS Genet. *10*, e1004223.

〔Review articles〕

- Fukushima, K., and Hasebe, M. (2014). Adaxial-abaxial polarity: the developmental basis of leaf shape diversity. Genesis *52*, 1-18.
- Horio, T., and Murata, T. (2014) The role of dynamic instability in microtubule organization. Front. Plant Sci. doi: 10.3389/fpls.2014.00511
- Kofuji, R., and Hasebe, M. (2014). Eight types of stem cells in the life cycle of the moss Physcomitrella patens. Curr. Opin. Plant Biol. *17*, 13-21.
- Sessa, E., Banks, J., Barker, M., Der, J., Duffy, A., Graham, S., Hasebe, M., Langdale, J., Li, F., Marchant, D., Pryer, K., Rothfels, C., Roux, S., Salmi, M., Sigel, E., Soltis, D., Soltis, P., Stevenson, D., and Wolf, P. (2014). Between two fern genomes. Gigascience *3*, 15.
- Sparks, E.E., Imai, A., Hasebe, M., and Benfey, P.N. (2014). Developmental regulation and *de novo* formation of stem cells in plants. In Stem Cells: From Basic Research to Therapy, Volume 1: Basic Stem Cell Biology, Tissue Formation during Development, and Model Organisms. Pp. 405-434, CRC Press.
- Tomescu, A.M., Wyatt, S.E., Hasebe, M., and Rothwell, G.W. (2014). Early evolution of the vascular plant body plan - the missing mechanisms. Curr. Opin. Plant Biol. *17*, 126-136.

Division of Symbiotic Systems

Professor
KAWAGUCHI, Masayoshi

Assistant Professor:	*TAKEDA, Naoya*
	SUZAKI, Takuya
Technical Staff:	*FUKADA-TANAKA, Sachiko*
NIBB Research Fellow:	*SOYANO, Takashi*
Postdoctoral Fellow:	*FUJITA, Hironori*
	TATEMATSU, Kiyoshi
	HANDA, Yoshihiro
	KOBAYASHI, Yuki
	NAGAE, Miwa
	MATSUOKA, Hikaru
SOKENDAI Graduate Student:	*SASAKI, Takema*
	YORO, Emiko
	TSUZUKI, Shusaku
	NISHIDA, Hanna
	FUKUHARA, Mai
	LIU, Meng
Visiting Undergraduate:	*MIZUTANI, Yoshiyuki*
Technical Assistant:	*SUZAKI, Momoyo*
	ICHIKAWA, Michiko
	OGAWA, Yuko
	YOSHINORI, Yumi
Secretary:	*SANJO, Kazuko*
	OKUBO, Masayo

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 mechanisms of both symbiotic systems.

I. Nodulation

1-1 Genetic mechanism involved in early and late nodule development

In response to appropriate inductive conditions, plants have the capacity of forming new organs from differentiated cells. Root nodulation is one such unique developmental process that predominantly occurs in leguminous plants. In this process, signaling initiated by symbiotic bacterial infection alters the fate of differentiated cortical cells and causes formation of new organs.

The *L. japonicus vagrant infection thread 1* (*vag1*) is a novel mutant involved in nodule development. *VAG1* encodes a protein orthologous to *Arabidopsis* ROOT HAIRLESS 1, which functions as a subunit of DNA topoisomerase VI. Analyses focusing on nuclear size found the emergence of a few cortical cells with enlarged nuclei during initiation of cortical cell division in wild type. In the *vag1* mutants, these potentially endoreduplicated cells are not observed and subsequent cortical cell division is severely compromised. Thus, it is possible that DNA topoisomerase VI is involved in the endoreduplication of cortical cells, which can trigger the onset of cortical cell division. During late nodule development, endoreduplication results in the formation of enlarged rhizobia-colonized cells in mature nodules. In wild type *L. japonicus*, there are enlarged rhizobia-colonized cells in the inner region of the nodule, and smaller rhizobia-infected (as yet uncolonized) cells in the surrounding region (Figure 1). In *vag1* nodules, the number of these small rhizobia-infected cells is higher whereas the number of rhizobia-colonized cells is lower (Figure 1). This suggests that the *vag1* mutant has a defect in differentiation from small rhizobia-infected cells to enlarged rhizobia-colonized cells. Overall, our data indicate that endoreduplication mediated by DNA topoisomerase VI may be a prerequisite regulator for the control of two key nodule developmental processes: the first is related to the onset of nodule organogenesis during early nodule development, and the second is associated with the differentiation of rhizobia-colonized infected cells in late nodule development.

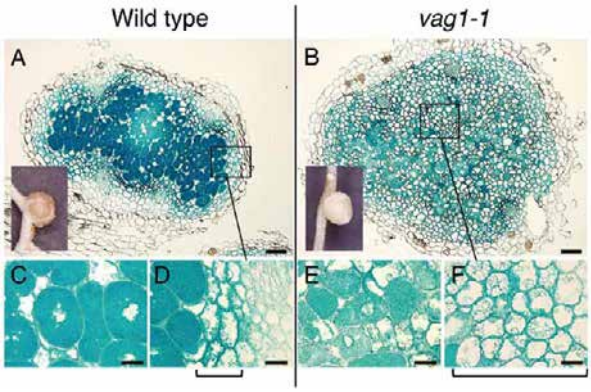


Figure 1. The effect of the *vag1* mutation on nodule structure. Sections through nodules of wild type (A, C, D) and *vag1-1* (B, E, F) at 21 days after inoculation with rhizobia that constitutively express the *LacZ* reporter gene. (C, E) Rhizobia-colonized infected cells located at the inner region of nodules. (D, F) In wild type, small rhizobia-infected (as yet uncolonized) cells (bracket) are located surrounding the region of rhizobia-colonized cells. In contrast, the inner region of *vag1-1* nodule comprises a large number of these small rhizobia-infected cells. Scale bars: 100 μm in A, B; 20 μm in C-F.

1-2 A presumptive post-translational modification enzyme, PLENTY controls nodulation and root growth in *L. japonicus*

Legumes can survive even in nitrogen-deficient environments depending on root nodules symbiosis with rhizobia; however, forming nodules consumes energy, requiring nodule number to be strictly controlled. The previous studies of hypernodulation mutants, *har1*, *klv*, and *tml*, have proposed long-distance control of nodulation (Figure 2), via systemic mobile signals, CLE-RS1/2 peptides. Recently, we found that, at least, the CLE-RS2 peptide is arabinosylated and this modification is essential for nodule inhibition (Okamoto et al., 2013). In *Arabidopsis*, the post-translational modifications of hormone-like small peptides and their critical roles in biological activities have been

gradually found. Further, one of the modification enzymes, hydroxyproline O-arabinosyltransferase (HPAT), is identified and its homolog in *L. japonicus* is thought to be a strong candidate for the modification enzyme of CLE-RS2.

We identified the HPAT homolog in *L. japonicus*, PLENTY, as a responsible factor for the already isolated *plenty* mutant. PLENTY is localized to the Golgi, suggesting a similarity of protein functions between *Lj*PLENTY and *At*HPAT. However, overexpression of *CLE-RS1/2* is still effective on nodule inhibition in *plenty*, suggesting that PLENTY itself does not mainly mediate the arabinosylation of CLE-RS1/2. In addition, *plenty har1* double mutant showed an additive nodulation. These suggest a novel HAR1-independent nodulation controlling pathway mediated by an unknown possible substrate of PLENTY which is a putative post-translational modification enzyme.

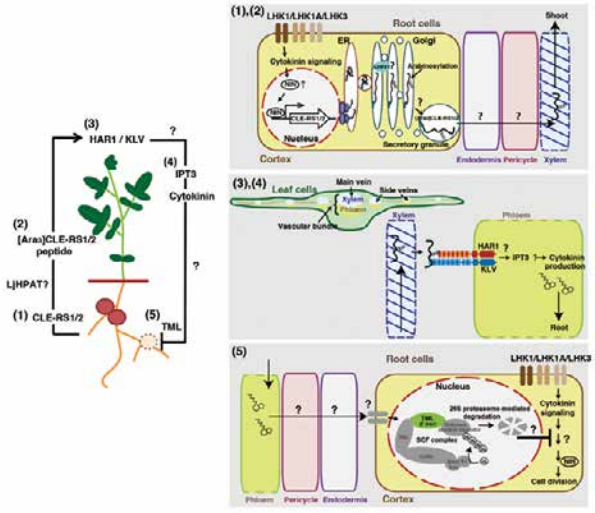


Figure 2. A model of the long-distance control of nodulation. (1) Nodulation signaling pathway downstream of cytokinin receptors activates *NIN* expression. The NIN transcription factor activates *CLE-RS1/2* expression through direct binding to their promoter. (2) It is likely that the CLE-RS1/2 peptides are posttranslationally modified with triarabinoside, a reaction mediated by an enzyme similar to HPAT in the Golgi apparatus. These modified CLE-RS peptides are transported to the xylem. (3) These peptides are transmitted from roots to shoots and directly bind to HAR1 in the phloem of leaf cells. (4) Downstream of the CLE-RS/HAR1 signaling pathway, activated IPT3 produces cytokinin, which is transported to roots through phloem tissue. (5) Shoot-derived cytokinin is directly or indirectly involved in proteasome-mediated degradation of an unidentified positive regulator of nodule organogenesis.

II. Arbuscular mycorrhiza symbiosis

Arbuscular mycorrhiza is a plant-fungus interaction that confers great advantage to growth and survival on the land. AM fungi enter into the host root and elongate the hyphae between the root cells. The intraradical hyphae form symbiotic structures called 'arbuscule' and 'vesicle' (Figure 3A). AM fungi supply phosphate to the host plant through the symbiotic structures and in return, they obtain photosynthetic products form the host. To obtain insights about molecular mechanisms of AM development, we are studying symbiotic signaling factors that regulate symbiotic gene expression and AM fungal infection.

We performed transcriptome analysis in wild-type and symbiotic mutants of *L. japonicus*. This analysis showed that plant hormone gibberellin (GA) biosynthesis and metabolism genes were induced during AM development. The GA biosynthesis gene expression was disturbed in the symbiotic mutants that showed abnormal AM fungal colonization in the host roots, indicating that *de novo* biosynthesis of GAs has some function in AM development. Functional analysis of GA in AM development revealed that GA has a negative effect on some AM-induced gene expressions, but also has a positive effect on other expressions (Figure 3B). *RAM1* and *RAM2* that function in AM entry processes are suppressed by GA signaling, on the other hand, GA signaling promotes or maintains *SbtM1* expression that is required for AM fungal colonization in the host root. This indicated that GA signaling interferes with the symbiotic signaling pathway, which decreases or enhances expression levels of AM-induced genes. In addition, treatment with GA or GA biosynthesis inhibitor disturbed the GA signaling and caused an inhibitory effect on AM hyphal entry into the host root or the hyphal branching in the root cortical cell layer. These studies revealed the host plant controls GA signaling level by induction of GA biosynthesis and metabolism genes during AM development and the GA signaling regulates AM fungal colonization in the host root.

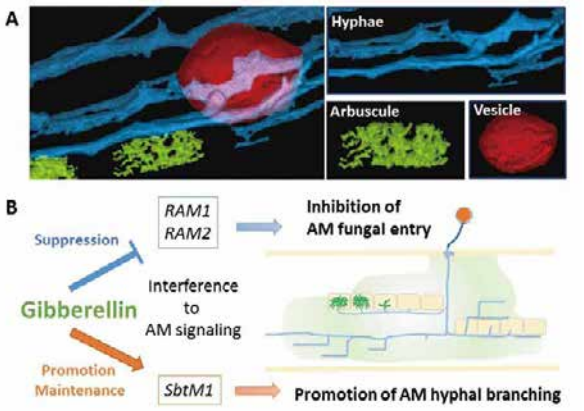


Figure 3. AM fungal colonization and interference of GA signaling with the symbiotic gene expression. (A) AM fungal structures in the host root. (B) GA signaling is enhanced by GA biosynthesis during AM development and differentially interferes with symbiotic signaling pathways. The interference affects and regulates the AM colonization in the host root.

III. Computational modeling for pattern formation of stomatal differentiation in *Arabidopsis* leaves

Stomata are epidermal structures that mediate gas exchange in plants, and are usually formed separately from each other (Figure 4A). In the initial step of stomatal development, stomatal lineage cells are self-organized from a homogeneous field, and thus this is a good example for two-dimensional pattern formation in living systems. Stomatal differentiation is promoted by transcription factors SPCH and SCRM, but in contrast is suppressed by diffusible peptide EPF2, which interacts with its membrane receptors to stimulate the degradation of SPCH/SCRM. In addition, this regulatory network involves feedback loops; SPCH•SCRM

heterodimer activates SCRM expression (positive feedback), and also stimulates EPF2 expression leading to suppression of SPCH/SCRM (negative feedback). This regulatory framework is similar to that of the activator–inhibitor system, a well-known model for pattern formation, and is predicted to be essential for stomatal patterning.

Thus, we constructed and examined a mathematical model, which is based on experimental results. Our model can explain many experimental observations, such as that stomatal lineage cells are formed separately in the wild type but are clustered in *tmm* mutant and *er erl1 erl2* mutants (Figure 4B). Furthermore, our model also explains the opposite response that exogenous application of EPF2 peptide completely eliminates stomatal differentiation in the wild type but does not affect the *tmm* mutant (Figure 5C). These results suggest that stomatal pattern formation is basically understood by the activator-inhibitor mechanism, that is, local activation and lateral inhibition.

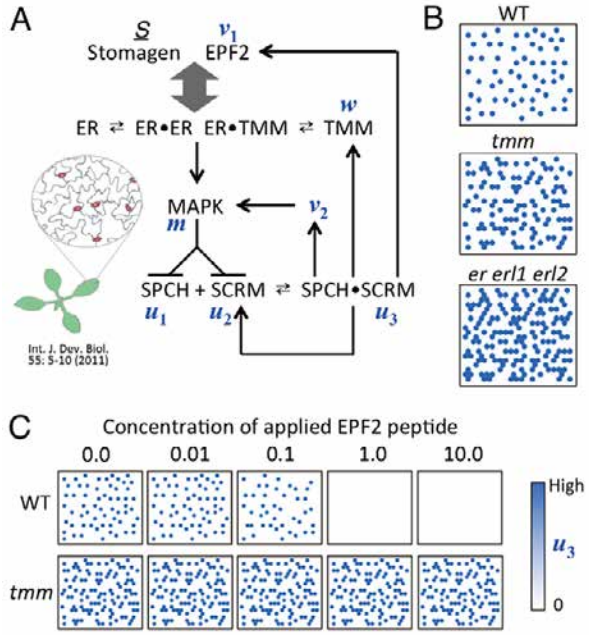


Figure 4. (A) Regulatory network for the differentiation of stomatal lineage cells. Our model can generate various stomatal patterns including the wild type, *tmm*, and *er erl1 erl2* (B), and also explains the opposite response to EPF2 peptide between the wild type and *tmm* (C).

Publication List

〔Original papers〕

- Chungopast, S., Hirakawa, H., Sato, S., Handa, Y., Saito, K., Kawaguchi, M., Tajima, S., and Nomura, M. (2014). Transcriptomic profiles of nodule senescence in *Lotus japonicus* and *Mesorhizobium loti* symbiosis. Plant Biotech. *31*, 345-349.
- Daum, G., Medzihradszky, A., Suzaki, T., and Lohmann J.U. (2014). A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. Proc. Natl. Acad. Sci. USA *111*, 14619-14624.
- Fujita, H., Aoki, S., and Kawaguchi, M. (2014). Evolutionary dynamics of nitrogen fixation in the legume-rhizobia symbiosis. PLoS One *9*, e93670.
- Kikuchi, Y., Hijikata, N., Yokoyama, K., Ohtomo, R., Handa, Y., Kawaguchi, M., Saito, K., and Ezawa, T. (2014). Polyphosphate accumulation is driven by transcriptome alterations that lead to near-synchronous and near-equivalent uptake of inorganic cations in an arbuscular mycorrhizal fungus. New Phytol. *204*, 638-649.
- Kojima,T., Saito, K., Oba, H., Yoshida, Y., Terasawa, J., Umehara, Y., Suganuma, N., Kawaguchi, M., and Ohtomo, R. (2014). Isolation and phenotypic characterization of *Lotus japonicus* mutants specifically defective in arbuscular mycorrhizal formation. Plant Cell Physiol. *55*, 928-941
- Nagae, M., Takeda, N., and Kawaguchi, M. (2014). Common symbiosis genes CERBERUS and NSP1 provide additional insight into the establishment of arbuscular mycorrhizal and root nodule symbioses. Plant Signal. Behav. *9*, e28544.
- Sasaki, T., Suzaki, T., Soyano, T., Kojima, M., Sakakibara, H., and Kawaguchi, M. (2014). Shoot-derived cytokinins systemically regulate root nodulation. Nature Commun. *5*, 4983.
- Soyano, T., Hirakawa, H., Sato, S., Hayashi, M., and Kawaguchi, M. (2014). NODULE INCEPTION creates a long-distance negative feedback loop involved in homeostatic regulation of nodule organ production. Proc. Natl. Acad. Sci. USA *111*, 14607-14612.
- Suzaki, T., Ito, M., Yoro, E., Sato, S., Hirakawa, H. Takeda, N., and Kawaguchi, M. (2014). Endoreduplication- mediated initiation of symbiotic organ development in *Lotus japonicus*. Development *141*, 2441-2445.
- Wakabayashi, T., Oh, H., Kawaguchi, M., Harada, K., Sato, S., Ikeda, H., and Setoguchi, H. (2014). Polymorphisms of E1 and GIGANTEA in wild populations of *Lotus japonicus*. J. Plant Res. *127*, 651-660.
- Yoro, E., Suzaki, T., Toyokura, K., Miyazawa, H., Fukaki, H., and Kawaguchi, M. (2014). A positive regulator of nodule organogenesis, NODULE INCEPTION, acts as a negative regulator of rhizobial infection in *Lotus japonicus*. Plant Physiol. *165*, 747-758.

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

- Takeda, N., Handa, Y., Tsuzuki, S., Kojima, M., Sakakibara, H., and Kawaguchi, M. Gibberellins interfere with symbiosis signaling and gene expression, and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol. 2014 Dec 19.

〔Review articles〕

- Kawaguchi, M. (2014). Genes for autoregulaton of nodulation. In The *Lotus japonicus* Genome, S. Tabata and J. Stougaard, eds. (Springer), pp. 73-78.
- Kawaguchi, M., and Sandal, N. (2014). Wild accessions and mutant resources. In The *Lotus japonicus* Genome, S. Tabata and J. Stougaard, eds. (Springer), pp. 211-220.
- Soyano, T., and Kawaguchi, M. (2014). Systemic regulation of root nodule formation. Review. In Advances in Biology and Ecology of Nitrogen Fixation, T. Ohyama, ed. (InTech), pp. 89-109.
- Suzaki, T., and Kawaguchi, M. (2014). Root nodulation: a developmental program involving cell fate conversion triggered by symbiotic bacterial infection. Curr. Opin. Plant Biol. *21*, 16-22.

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 during 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 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 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.

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

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.

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

Associate Professor
NARUSE, Kiyoshi

Assistant Professor:	*TAKEHANA, Yusuke*
Postdoctoral Fellow:	*SASADO, Takao*
	NAKAMOTO, Masatoshi
	OKUYAMA, Teruhiro
	NOZU, Ryo
	CHISADA, Shinichi
Research Fellow:	*KANEKO, Hiroyo*
Technical Assistant:	*KOIKE, Yukari*
	TORII, Naoko
	AJIOKA, Rie
	KOIKE, Chieko
	TESHIMA, Yuuko
	HARA, Ikuyo
	ISHIKAWA, Hiroe
	TAKAKI, Chikako
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 primordial germ cell (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.

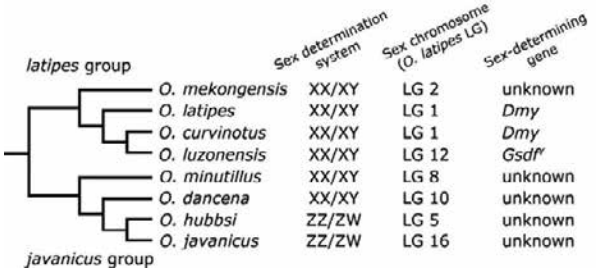


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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 PGCs, 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 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. 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.

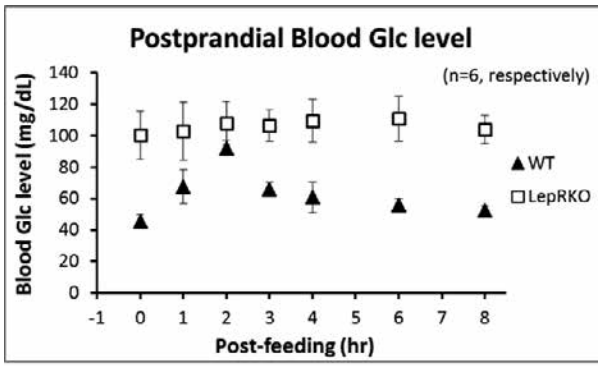


Figure 1. Postbrandial blood glucose levels of WT and LepRKO medaka.

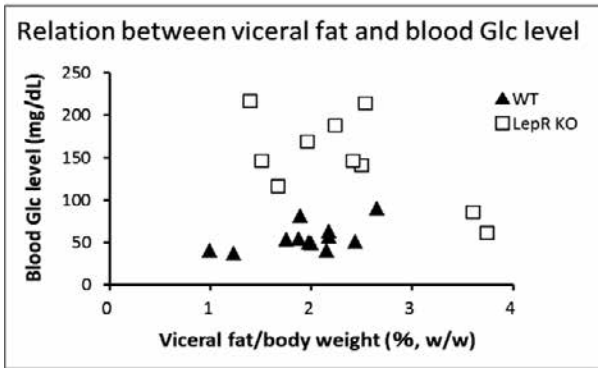


Figure 2. Relation between visceral fat and blood glucose levels of WT and LepRKO medaka.

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

Figure 3. NBRP Medaka website.

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 3). 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. 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〕

- Chisada, S., Kurokawa, T., Murashita, K., Ronnestad, I., Yaniguchi, T., Toyoda, A., Sakaki, Y., Takeda, S., and Yoshiura, Y. (2014). Leptin receptor-deficient (knockout) medaka, *Oryzias latipes*, show chronical up-regulated levels of orexigenic neuropeptides, elevated food intake and stage specific effects on growth and fat allocation. Gen. Comp. Endocrinol. *195*, 9-20.
- Guan, G., Zhang, X., Naruse, K., Nagahama, Y., and Hong, Y. (2014). Gene Replacement by Zinc Finger Nucleases in Medaka Embryos. Marine Biotechnol. *16*, 739-747.
- Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y., Yamamoto, S., Yabe, T., Takada, S., Kinoshita, M., Kuroiwa, A., and Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. Proc. Natl. Acad. Sci. USA *111*, 7343-7348.
- Maruyama, A., Oshima, Y., Kajiura-Kobayashi, H., Nonaka, S., Imamura, T., and Naruse, K. (2014). Wide field intravital imaging by two-photon-excitation digital-scanned light-sheet microscopy (2p-DSLM) with a high-pulse energy laser. Biomed. Opt. Express *5*, 3311-3325.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., and Hara, I. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genet. *10*, e1004246.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., and Morishita, S. (2014). Analysis of a novel gene, *Sdgc*, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development *141*, 3363-3369.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.
- Spivakov, M., Auer, T.O., Peravali, R., Dunham, I., Dolle, D., Fujiyama, A., Toyoda, A., Aizu, T., Minakuchi, Y., and Loosli, F. (2014). Genomic and Phenotypic Characterization of a Wild Medaka Population: Towards the Establishment of an Isogenic Population Genetic Resource in Fish. G3-Genes Genom. Genet. *4*, 433-445.
- Takehana, Y., Matsuda, M., Myosho, T., Suster, M.L., Kawakami, K., Shin, T., Kohara, Y., Kuroki, Y., Toyoda, A., and Fujiyama, A. (2014). Co-option of *Sox3* as the male-determining factor on the Y chromosome in the fish *Oryzias dancena*. Nature Commun. *5*, 4157.
- Tsuboko, S., Kimura, T., Shinya, M., Suehiro, Y., Okuyama, T., Shimada, A., Takeda, H., Naruse, K., Kubo, T., and Takeuchi, H. (2014). Genetic Control of Startle Behavior in Medaka Fish. PLoS One *9*, e112527.
- Zhang, X., Guan, G., Chen, J., Naruse, K., and Hong, Y. (2014). Parameters and efficiency of direct gene disruption by Zinc Finger Nucleases in Medaka Embryos. Marine Biotechnol. *16*, 125-134.

LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Thus, perception of the nutrient environment is also essential for cells. To recognize cellular nutrient conditions, eukaryotic cells employ Tor (target of rapamycin) protein kinase. Tor forms two distinct protein complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as protein synthesis, cell cycle and autophagy. TORC1 is thought to act as a nutrient sensor, because rapamycin, a TORC1 inhibitor, mimics a starved condition. On the other hand, TORC2, whose function is insensitive to rapamycin, 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 Tor receives nutrient 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 pathway (Figure 1).

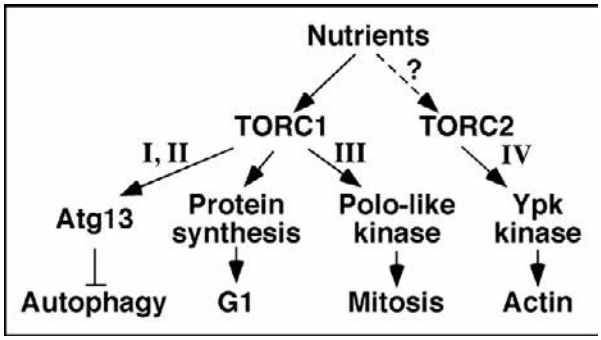


Figure 1. Tor signaling pathway of the budding yeast. Our group has found three branches of the Tor pathway.

I. TORC1 phosphorylates Atg13, the molecular switch of autophagy

Autophagy is mainly a response to nutrient starvation, and TORC1 negatively regulates autophagy. The Atg1 kinase and its regulators, i.e. Atg13, Atg17, Atg29, and Atg31 collaboratively function in the initial step of autophagy induction, downstream of TORC1. Atg1 is a Ser/Thr protein kinase, the activity of which is essential for autophagy and is largely enhanced following nutrient starvation or the addition of rapamycin. This regulation involves phosphorylation of Atg13.

We found that Atg13 is directly phosphorylated by TORC1. Phosphorylated Atg13 (during nutrient-rich conditions) loses its affinity to Atg1, resulting in repression of autophagy. On the other hand, under starvation conditions Atg13 is immediately dephosphorylated and binds to Atg1 to form Atg1 complex. Atg1 complex formation confers Atg1 activation and consequently induces autophagy. We determined phosphorylation sites of Atg13 and generated an unphosphorylatable Atg13 mutant (Atg13-8SA). Expression of Atg13-8SA induces autophagy bypassing inactivation of TORC1, the same as through starvation treatment or rapamycin. These results demonstrate that Atg13 acts as a molecular switch for autophagy induction.

II. Monitoring in vivo activity of TORC1 by phosphorylation state of Atg13

Since Atg13 has turned out to be a substrate of TORC1, *in vivo* activity of TORC1 can be monitored by the phosphorylation state of Atg13. Various conditions and mutants have been examined to determine what kind of nutrients TORC1 recognizes and how nutrient signals are transmitted to TORC1.

III. How and why TORC1 regulates mitotic entry?

TORC1 regulates protein synthesis, which is important for promotion of the cell cycle at G1 (G0). Little is known, however, about whether or not TORC1 is involved in other stages of the cell cycle.

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. Recently 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. Ypk2 kinase acts downstream of TORC2 to control actin organization

Genetic studies have shown that TORC2 controls polarity of the actin cytoskeleton via the Rho1/Pkc1/MAPK cell integrity cascade. However, the target (substrate) of TORC1 was not yet identified. We found that Ypk2, an lethality of TORC2 dysfunction, is directly phosphorylated by TORC2.

Publication List

〔Original paper〕

● Sekiguchi, T., Kamada, Y., Furuno, N., Funakoshi, M., and Kobayashi, H. (2014). Amino acid residues required for Gtr1p-Gtr2p complex formation and its interactions with the Ego1p-Ego3p complex and TORC1 components in yeast. Genes Cells *19*, 449-463.

LABORATORY OF BIOLOGICAL DIVERSITY

MANO Group

Assistant Professor:	*MANO, Shoji*
Postdoctoral Fellow:	*KANAI, Masatake*
	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 biogenesis 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 biogenesis and functions, we have been analyzing a number of Arabidopsis mutants having aberrant peroxisome morphology (*apem* mutants) and peroxisome unusual poisoning (*peup* mutants). To date, *APEM1*, *2*, *3*, *4*, *9* and *10*, and *PEUP1*, *2* and *4* genes were identified, and based on the characterization using their gene-products a part of the mechanism of division, protein transport and degradation of peroxisomes, were revealed.

Recently, we found that peroxisome functions and biogenesis are involved in the reproductive process. Therefore, peroxisomes in gametes and gametophytes were visualized, and their dynamics are currently under investigation (Figure 1).

II. Accumulation mechanism of seed storage proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves as foods, feed, 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 Arabidopsis mutants showing different patterns of oil/protein ratios to elucidate the mechanisms controlling oil and protein contents in seeds.

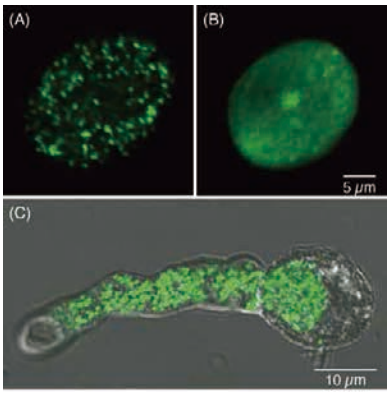


Figure 1. Visualization of peroxisomes in pollen and pollen tube.
The fusion gene of GFP with peroxisome targeting signal 1 is expressed in the wild-type (A) and *apem2*/*pex13* plants (B). (C) Peroxisomes move toward the tip region during pollen tube growth.

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 organelles' 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〕

- Goto-Yamada, S., Mano, S., Nakamori, C., Kondo, M., Yamawaki, R., Kato, A., and Nishimura, M. (2014). Chaperone and protease functions of LON protease 2 modulate the peroxisomal transition and degradation with autophagy. Plant Cell Physiol. *55*, 482-496.
- Mano, S., Nakamura, T., Kondo, M., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. (2014). The Plant Organelles Database 3 (PODB3) update 2014: integrating electron micrographs and new options for plant organelle research. Plant Cell Physiol. *55*, e1.
- Shibata, M., Oikawa, K., Mano, S., and Nishimura, M. (2014). Measurement of the number of peroxisomes. Bio-Protoc. *4*, e1284.

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

- Motomura, K., Le, Q.T.N., Hamada, T., Kutsuna, N., Mano, S., Nishimura, M., and Watanabe, Y. Diffuse DCP2 accumulates in DCP1 foci under heat stress in *Arabidopsis thaliana*. Plant Cell Physiol. 2014 Oct 22.

〔Review articles〕

- Goto-Yamada, S., Mano, S., and Nishimura, M. (2014). Interaction between chaperone and protease functions of LON2, and autophagy during the functional transition of peroxisomes. Plant Signal. Behav. *9*, e28838.
- Goto-Yamada, S., Mano, S., Oikawa, K., Shibata, M., and Nishimura, M. (2014). The role of peroxisomes in plant reproductive processes. In Sexual reproduction in animals and plants. – Edited by Sawada, H., Inoue, N., and Iwano, M. Springer Japan, pp. 419-429.
- Shibata, M., Oikawa, K., Yoshimoto, K., Goto-Yamada, S., Mano, S., Yamada, K., Kondo, M., Hayashi, M., Sakamoto, W., Ohsumi Y., and Nishimura, M. (2014). Plant autophagy is responsible for peroxisomal transition and plays an important role in the maintenance of peroxisomal quality. Autophagy *10*, 936-937.

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).

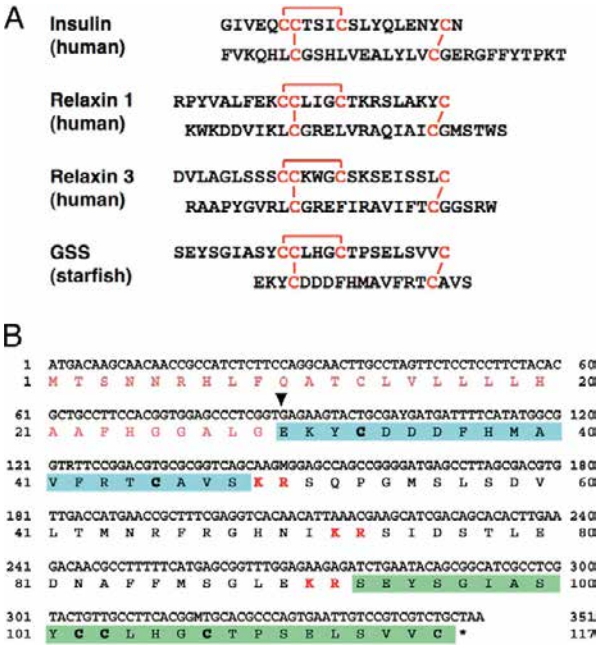


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of 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 the 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

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 such genome dynamisms 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 RNA in the whitish parts of the corolla. It is suggested that distinct regulation of small RNA causes 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 flower pigmentation. We are currently characterizing detailed molecular mechanisms of these mutations.

II. *de novo* sequencing of 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 the Tokyo-kokei standard line for genome sequencing, and employed shotgun sequencing using a single molecule real time sequencing system. We could recently obtain thousands of assembled sequences having reasonable size, and are going to characterize details of the genome sequence.

III. BioResource of morning glories

NIBB is the sub-center for 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 230 lines and 157,000 DNA clones.

IV. A novel flower color enhancing protein

Anthocyanin is a type of flavonoid pigment and is responsible for the colors of many flowers. The depth of flower color is determined by the amount of anthocyanin. We characterized *I. nil* mutants displaying pale colored flowers (Figure 1), and successfully isolated a novel enhancer of flavonoid production (EFP). The EFP deficient mutants fail in efficient flavonoid production resulting reduction of anthocyanin accumulation. The presence and action of EFP are conserved among diverse plants. The amino acid sequence of EFP resembles an enzyme catalyzing flavonoid biosynthesis, however, it has no enzymatic activities. We are currently studying a molecular mechanism of flavonoid production enhancement by EFP.

Figure 1. Wild type (right) and EFP deficient mutant of Japanese morning glory (left).

Publication List

〔Original papers〕

- Faraco, M., Spelt, C., Bliek, M., Verweij, W., Hoshino, A., Espen, L., Prinsi, B., Jaarsma, R., Tarhan, E., de Boer, A.H., Di Sansebastiano, G.-P., Koes, R., and Quattrocchio, F.M. (2014). Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. Cell Rep. *6*, 32-43.
- Morita, Y., Takagi, K., Fukuchi-Mizutani, M., Ishiguro, K., Tanaka, Y., Nitasaka, E., Nakayama, M., Saito, N., Kagami, T., Hoshino, A., and Iida, S. (2014). A chalcone isomerase-like protein enhances flavonoid production and flower pigmentation. Plant J. *78*, 294-304.
- Park, K.I., Hoshino, A., Saito, N., and Tatsuzawa, F. (2014). Anthocyanins in the flowers of Ipomoea tricolor Cav. (Convolvulaceae). Biochem. Syst. Ecol. *54*, 15-18.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*

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. nDart1-0-Inserted Mutants

It is essential to develop an easy detection method of the *nDart1-0*-inserted gene for forward and reverse genetics. For high-copy-number elements, transposon display (TD) based on an amplified fragment-length polymorphism (AFLP) technique is a powerful tool to visualize multiple transposons. We successfully visualized 13 copies of *nDart1* of Nipponbare through an *nDart1*-optimized TD method. In addition, an *nDart1-0*-specific iPCR procedure was also developed (Hayashi-Tsugane etl al. 2014). Among 8,984 lines, visible phenotypes were observed at the seedling, post-transplanting, reproductive, and mature stages. Fifty percent of the total tagged lines showed mutant phenotypes. Abnormal growth phenotypes, in particular, were easily detectable at the seedling stage, whereas both sterility and dwarf phenotypes were frequently observed at the mature stage. Some of these mutant phenotypes are shown in Figure 1. A variegated albino plant can survive under normal field conditions (a). Although chlorophyll mutants that revert to the wild phenotype are also observed at the seedling stage, these zebra mutants (b and c) retain a cross-banded phenotype even at the reproductive stage. A mutant (d) abnormally elongates the leaf and stem at the seedling stage, somewhat similar to the *Gibberella fujikuroi*-infected rice plant. Fuchsia leaf blades with revertant sectors (e) are observed under UV light. This is similar to the hcf (high chlorophyll fluorescence) mutant in maize. Under normal growth conditions, this mutant shows pale-green leaves and is lethal at the seedling stage. A mutant displaying an extremely short-statured phenotype (f) is named Thumbelina; this phenotype must be caused by the insertion of a DNA transposon because of its mutable phenotype (right in f), as a normally elongated shoot can often be observed in the thumbelina mutant (Tsugane et al., 2006). A semi-dominant mutant (g) shows a very short panicle with many tillers, and its heterozygous plant exhibits an intermediate phenotype between the wild type and the mutant. A mutant (right in h) has an abnormal ligule, resulting in an erect leaf. The short panicle phenotype is shown in (i) (right). The mutant shown in (j) expresses multiple glumes and high sterility, and a mutant (k) exhibiting the neck leaf-enveloped panicle phenotype, like the NECK LEAF1 mutant cloned by Wang et al. (2009), is observed. The mutant shown in (l) displays a phenotype similar to that of a PLASTCHRON mutant.

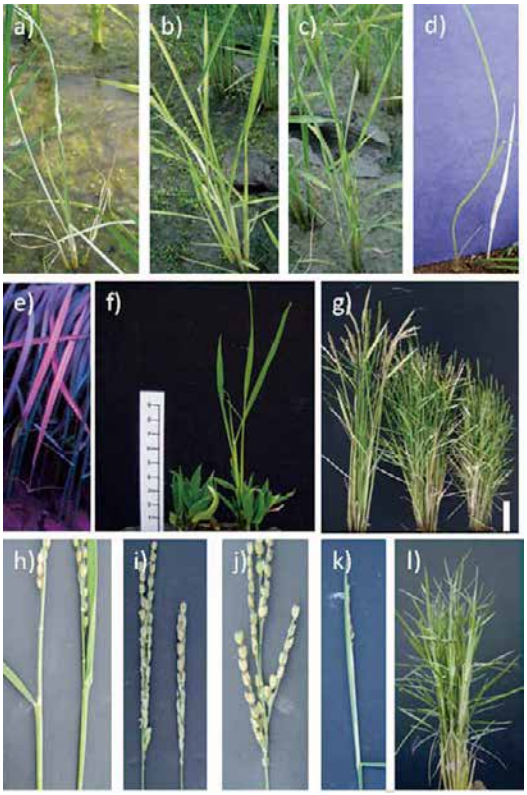


Figure 1. Phenotypes of *nDart*-tagged line mutants. Scale bar = 10 cm.

Publication List

〔Original paper〕

● Hayashi-Tsugane, M., Takahara, H., Ahmed, N., Himi, E., Takagi, K., Iida, S., Tsugane, K., and Maekawa, M. (2014). A mutable albino allele in rice reveals that formation of thylakoid membranes requires SNOW-WHITE LEAF1 gene. (2014). Plant Cell Physiol. 55, 3-15.

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.

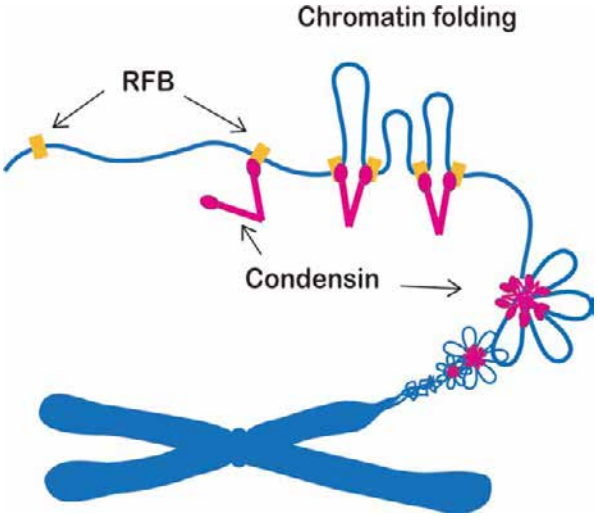


Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This leads to a folding of chromatin fibers between the sites, as a basic process of chromosome condensation.

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 developed software applications that are capable of describing cell dynamics out of 4D time-lapse imaging data sets by employing several approaches.

I. Automated cell 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 developed 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). Obtained cell dynamics are subjected to comparative analyses among wild type and several mutants to unveil genetic programs underlying organogenesis.

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).

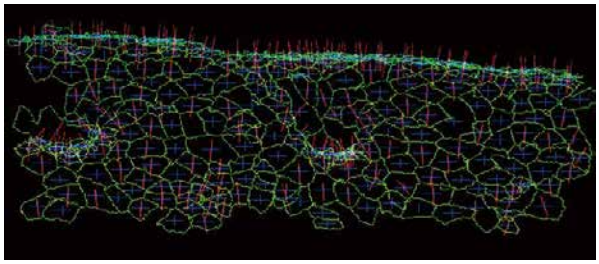


Figure 1. Visualized apical cell surface of *Drosophila* embryonic epidermal cells. A time-lapse confocal microscopic data set of fly embryo expressing E-cadherin-GFP was subjected to our automatic cell shape recognition system. Cell boundaries (green), center of gravity (blue) and normal vector (magenta)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

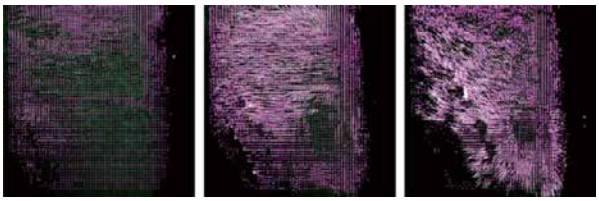


Figure 2. Collective cell migration of *Xenopus* neuroectodermal cells visualized as optical flow along a time-series. A modified PIV method successfully tracks uneven subcellular distribution of GFP signals over time. Dr. M. Suzuki (Prof. Ueno's laboratory at NIBB) performed the microscopy.

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/).

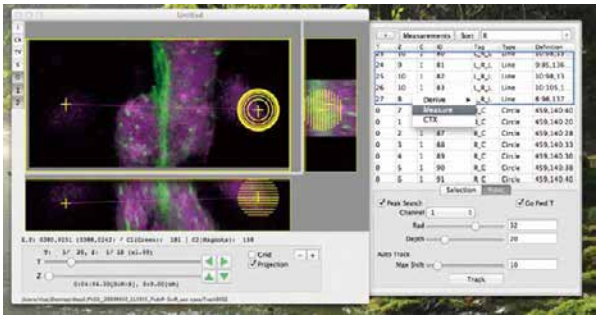


Figure 3. A lightweight/native 4D stack viewer equipped with functions for manual feature extraction.

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.

I. Novel image processing method based on mathematical morphology

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. Contrast enhancement plays an important role in image processing; it enhances structural features that are barely detectable to the human eye and allows automatic extraction of those features. To effectively recognize a region of interest, specific target structures must be enhanced while surrounding objects remain unmodified. A contrast enhancement technique which uses mathematical morphology enables selective enhancement of target structures. Based on set theory, mathematical morphology applies shape information to image processing.

Mathematical morphology operates by a series of morphological operations, which use small images called structuring elements (typically, a single structuring element is used). The structuring element acts as a moving probe that samples each pixel of the image. Since the structuring element moves in a fixed direction across the image, some intricate images (in particular, those whose structural details contain a variety of directional characters) may not be properly processed. Consequently, an artifact in the shape of structuring elements may be generated at the object periphery. Since objects in biomedical images consist of delicate structural features, this drawback is an especially serious problem.

To overcome this problem, we have proposed an extension of conventional mathematical morphology called rotational morphological processing (RMP). RMP based morphological filters have been applied to a wide variety of biomedical images, including electron micrographs, light micrographs and medical images such as mammographic images and chest X-ray images.

In this study, we have developed a novel RMP-based contrast enhancement method. The goal of the study is to enable enhancement of fine morphological features of a mass lesion with high suppression of surrounding tissues, such as mammary glands. The method uses a top-hat contrast operator, a well-known and commonly used morphological operation for extracting local features from a low-contrast image. The proposed method involves three steps: (1) selective extraction of target features by mathematical morphology, (2) enhancement of the extracted features by two contrast modification techniques and (3) segmentation of the target region (mass lesion) by using an automatic thresholding technique. The effectiveness of the method was quantitatively evaluated by the contrast improvement ratio (CIR) and its usefulness was demonstrated by applying it to various types of medical images. The results prove that the proposed method enables specific extraction and enhancement of mass lesions, which is essential for clinical diagnosis based on medical image analysis. Figure 1 shows a mammographic image enhanced by the proposed method.

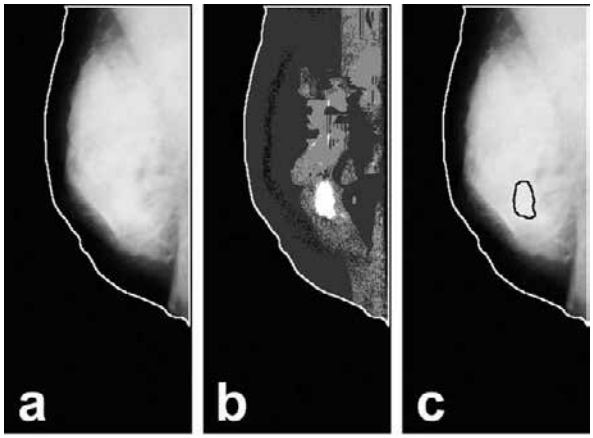


Figure 1. Image enhancement and target detection achieved by the proposed method for a mass lesion in a mammographic image. The images were obtained from the Mammographic Image Analysis Society (MIAS) database. (a) Original mammographic image (mdb179). (b) Enhancement result of candidates of mass lesion obtained by the proposed method. (c) Detection result of the lesion region.

Division of Molecular Environmental Endocrinology

Professor
IGUCHI, Taisen

Assistant Professor:	*MIYAGAWA, Shinichi*
	OGINO, Yukiko
NIBB Research Fellow:	*MIYAKAWA, Hitoshi*
Postdoctoral Fellow:	*HIRUTA, Chizue*
	MIYAKAWA (OKAMOTO), Misato
Technical Staff:	*MIZUTANI, Takeshi*
SOKENDAI Graduate Student:	*TOYOTA, Kenji*
	SUMIYA, Eri
	YATSU, Ryohei
Visiting Graduate Student:	*TOHYAMA, Saki*
Visiting Scientist:	*EVENSETH, Linn*
	DOHENY, Brenna
	OMORI, Akiko
Technical Assistant:	*HAYASHI, Tomoko*
	INABA, Kayo
Secretary:	*IMAIZUMI, Taeko*

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 of animals to sex hormones during critical windows of perinatal life caused the immune and nervous systems, bone, muscle, and the liver 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.

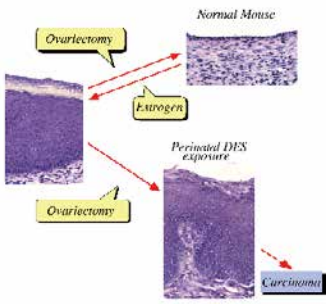


Figure 1. Scheme of estrogen- dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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.

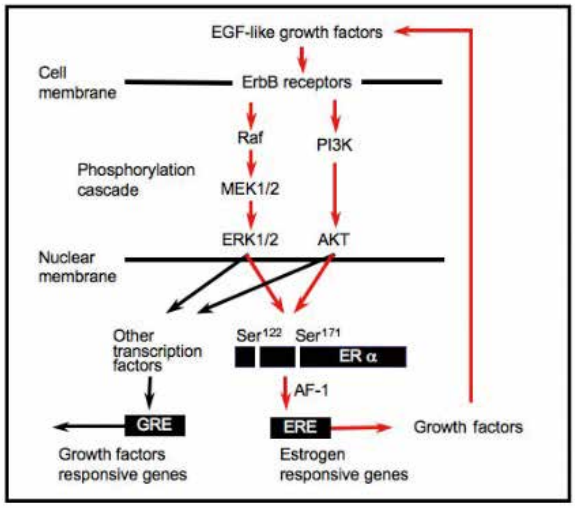


Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

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, lungfish, sturgeon, gar, roach, stickleback, mosquitofish, mangrove, *Rivulus*, catshark, whale shark, 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 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 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 androgenic 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 to *D. magna*. We established a *Daphnia* EST database and developed an oligonucleotide-based DNA

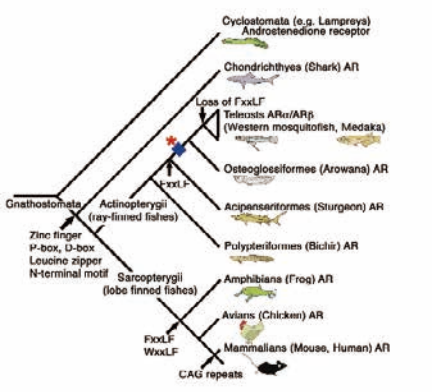


Figure 3. Evolutionary relationships of androgen receptor sequences.

microarray 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 sub-optimal, 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 JH receptor (heterodimer of methoprene-tolerant and steroid receptor co-activator) in daphnids and the function of ecdysone in molting and

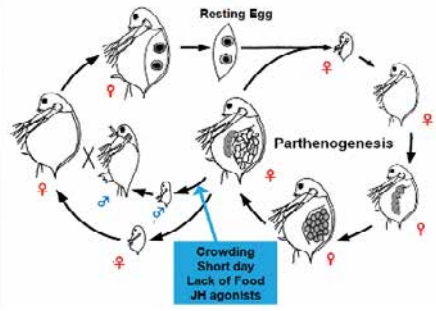


Figure 4. Life cycle of *Daphnia*.

ovulation in *D. magna*.

Sex determination mechanisms can broadly be categorized by either a genotypic or environmentally driven mechanism. Temperature-dependent sex determination (TSD), an environmental sex determination mechanism most commonly observed among vertebrates, has long been observed especially among reptiles. However, the temperature-dependent triggering mechanism of TSD and the subsequent differentiation cascade has long remained unknown. We are working on 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. We have successfully isolated and cloned the TRPV4 channel, and demonstrated its thermal activation at temperatures proximate to TSD-related temperature in alligators. 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 propose a novel mechanism for the sex determination pathway.

Publication List

〔Original papers〕

- Bhatia, H., Kumar, A., Ogino, Y., Gregg, A., Chapman, J., McLaughlin, M.J., and Iguchi, T. (2014). Di-n-butyl phthalate causes estrogenic effects in adult male Murray rainbowfish (*Melanotaenia fluviatilis*). Aquat. Toxicol. *149C*, 103-115.
- Bhatia, H., Kumar, A., Ogino, Y., Du, J., Gregg, A., Chapman, J., McLaughlin, M., and Iguchi, T. (2014). Effects of the commercial antiandrogen flutamide on the biomarkers of reproduction in male Murray rainbowfish (*Melanotaenia fluviatilis*). Environ. Toxicol. Chem. *33*, 1098-1107.
- Hamlin, H.J., Lowers, R.H., Kohno, S., Mitsui-Watanabe, N., Amano, H., Hara, A., Ohta, Y., Miyagawa, S., Iguchi, T., and Guillette, L.J.Jr. (2014). The reproductive hormone cycle of adult female American alligator from a Barrier island population. Reproduction *147*, 855-863.
- Hiruta, C., Ogino, Y., Sakuma, T., Toyota, K., Miyagawa, S., Yamamoto, T., and Iguchi, T. (2014). Targeted gene disruption by use of transcription activator-like effector nuclease (TALEN) in the water flea *Daphnia pulex*. BMC Biotechnol. *14*, 95.
- Ichikawa, M., Murai, E., Hashiguchi, Y., Iguchi, T., and Sato, T. (2014). Effects of diethylstilbestrol on luteinizing hormone-producing cells in the mouse anterior pituitary. Exp. Biol. Med. *239*, 311-319.
- Ihara, M., Ihara, M.O., Kumar, V., Narumiya, M., Hanamoto, S., Nakada, N., Yamashita, N., Miyagawa, S., Iguchi, T., and Tanaka, H. (2014). Co-occurrence of estrogenic and anti-estrogenic activities in wastewater: quantitative evaluation of balance by *in vitro* ERα reporter gene assay and chemical analysis. Environ. Sci. Technol. *48*, 6366-6373.
- Miyagawa, S., Harada, M., Matsumaru, D., Tanaka, K., Inoue, C., Nakahara, C., Haraguchi, R., Matsushita, S., Suzuki, K., Nakagata, N., Ng, R.C., Akita, K., Lui, V.C., and Yamada, G. (2014). Disruption of the temporally regulated cloaca endodermal b-catenin signaling causes anorectal malformation. Cell Death Differ. *21*, 990-997.
- Miyagawa, S., Lange, A., Hirakawa, I., Tohyama, S., Ogino, Y., Mizutani, T., Kagami, Y., Kusano, T., Ihara, M., Tanaka, H., Tatarazako, N., Ohta, Y., Katsu, Y., Tyler, C.R., and Iguchi, T. (2014). Differing species responsiveness of estrogenic contaminants in fish is conferred by the ligand binding domain of the estrogen receptor. Environ. Sci. Technol. *48*, 5254-5236.
- Nakamura, A., Takanobu, H., Tamura, I., Yamamuro, M., Iguchi, T., and Tatarazako, N. (2014). Verification of responses of Japanese medaka (*Oryzias latipes*) to antiandrogens, vinclozolin and flutamide, in short-term assays. J. Appl. Toxicol. *34*, 545-553.
- Ogino, Y., Hirakawa, I., Inohaya, K., Sumiya, E., Miyagawa, S., Tatarazako, N., Denslow, N., Yamada, G., and Iguchi, T. (2014). Bmp7 and Lef1 are the downstream effectors of androgen signaling in androgen-induced sex characteristics development in medaka. Endocrinology *155*, 449-462.
- Omori, A., Miyagawa, S., Ogino, Y., Harada, M., Ishii, K., Sugimura, Y., Ogino, H., Nakagata, N., and Yamada, G. (2014). Essential roles of epithelial bonee morphogentic protein signaling during prostatic development. Endocrinology *155*, 2534-2544.
- Sébillot, A., Damdimopoulou, P., Ogino, Y., Spirhanzlova, P., Miyagawa, S., Du Pasquier, D., Mouatassim, N., Iguchi, T., Lemkine, G., Demeneix, B.A., and Tindall, A.J. (2014). Rapid fluorescent detection of (anti-)androgens with *spiggin-gfp* medaka. Environ. Sci. Technol. *48*, 10919-10928.
- Sumiya, E., Ogino Y., Miyakawa, H., Hiruta, C., Toyota, K., Miyagawa, S., and Iguchi, T. (2014). Roles of ecdysteroids for progression of reproductive cycle in the fresh water crustacean *Daphnia magna.* Front. Zool. *11*, 60.
- Toyota, K., Kato, Y., Miyakawa, H., Yatsu, R., Mizutani, T., Ogino, Y., Miyagawa, S., Watanabe, H., Nishide, H., Uchiyama, I., Tatarazako, N., and Iguchi, T. (2014). Molecular impact of juvenile hormone agonists on neonatal *Daphnia magna*. J. Appl. Toxicol. *34*, 537-544.

〔Review articles〕

- Hiruta, C., Toyota, K., Miyakawa, H., Sumiya, E., and Iguchi, T. (2014). Sexual reproduction is a key element in the life history strategy of water fleas, *Daphnia magna* and *Daphnia pulex* - Casting a spotlight on male induction and its morphology -. In: El-Doma, M (ed.) Daphnia: Biology and Mathematics Perspectives, Nova Science Publishers, pp. 261-278.
- Kohno, S., Parrott, B.B., Yatsu, R., Miyagawa, S., Moore, B.C., Iguchi, T., and Guillette, L.J. Jr. (2014). Gonadal differentiation in reptiles exhibiting environmental sex determination. Sex. Devel. *8*, 208-226.
- Miyakawa, H., Iguchi, T., and Miura, T. (2014). Developmental process of defensive morph in *Daphnia pulex*. In: El-Doma, M. (ed). Daphnia: Biology and Mathematics Perspectives. Nova Science Publishers, pp. 245-260.
- Miyakawa, H., Toyota, K., Sumiya, E., and Iguchi, T. (2014). Comparison of JH signaling in insects and crustaceans. Curr. Opinion Insect Sci. *1*, 81-87.
- Zoeller, R.T., Bergman, Å., Becher, G., Bjerregaard, P., Bornman, R., Brandt, I., Iguchi, T., Jobling, S., Kidd, K.A., Kortenkamp, A., Skakkebaek, N.E., Toppari, J., and Vandenberg, L.N. (2014). A path forward in the debate over health impacts of endocrine disrupting chemicals. Environ. Health *13*, 118.

DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

Professor
MINAGAWA, Jun

Associate Professor
TAKAHASHI, Shunichi

Assistant Professor:	*TOKUTSU, Ryutaro*
Postdoctoral Fellow:	*KAMADA, Konomi*
	OHNISHI, Norikazu
	MARUYAMA, Shin-ichiro
	YAMASAKI, Hiroaki
	AIHARA, Yusuke
SOKENDAI Graduate Student:	*KAMRANI, Yousef Yari*
	KATO, Hiroki
	KOSUGE, Kotaro
Visiting Graduate Student:	*KISHIMOTO, Mariko*
Technical Assistant:	*YONEZAWA, Harumi*
	KADOWAKI, Tamaka
	HOSHI, Rie
Secretary:	*KOJIMA, Yoko*

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 the environmentally important photosynthetic organisms thrive in their ecological niche.

I. Macroorganization of photosynthetic machinery

Photosystem (PS) II is a multiprotein complex that splits water and initiates electron transfer in photosynthesis. The central part of PSII, the PSII core, is surrounded by light-harvesting complex II proteins (LHCIIs). In higher plants, two or three LHCII trimers are seen on each side of the PSII core whereas only one had been seen in the corresponding positions in a unicellular green alga *Chlamydomonas reinhardtii*. Recently, we re-examined the supramolecular organization of this PSII-LHCII supercomplex in *C. reinhardtii* by solubilizing the thylakoid membranes with n-dodecyl-α-D-maltoside and subjecting them to gel-filtration. This newly-prepared PSII-LHCII supercomplex bound twice as much LHCII than previously reported and retained higher oxygen-evolving activity. Single-particle image analysis of the electron micrographs revealed that the PSII-LHCII supercomplex had a novel supramolecular organization, with three LHCII trimers attached to each side of the core.

II. Acclimation of photosynthesis

Using *C. reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes such as state transitions and non-photochemical quenching by means of biochemistry, molecular genetics, optical spectroscopy, small-angle neutron scattering, and bio-imaging.

2-1 State transitions

Plants 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. It is acknowledged that state transitions induce substantial reorganizations of the PSs. However, their consequences on the chloroplast structure are more controversial. Here, we investigate how state transitions affect the chloroplast structure and function using complementary approaches for the living cells of *Chlamydomonas reinhardtii*. Using small-angle neutron scattering, we found a strong periodicity of the thylakoids in State 1, with characteristic repeat distances of approximately 200 Å, which was almost completely lost in State 2. As revealed by circular dichroism, changes in the thylakoid periodicity were paralleled by modifications in the long-range order arrangement of the photosynthetic complexes, which was reduced by approximately 20% in State 2 compared with State 1, but was not abolished. Furthermore, absorption spectroscopy reveals that the enhancement of PSI antenna size during State 1 to State 2 transition (approximately 20%) is not commensurate to the decrease in PSII antenna size (approximately 70%). This implies that a large part of the phosphorylated LHCIIs do not bind to PSI, but instead form energetically quenched complexes, which we have shown to be either associated with PSII supercomplexes or in a free form (Figure 1). Altogether these noninvasive in vivo approaches allowed us to present a more likely scenario for state transitions that explains their molecular mechanism and physiological consequences.

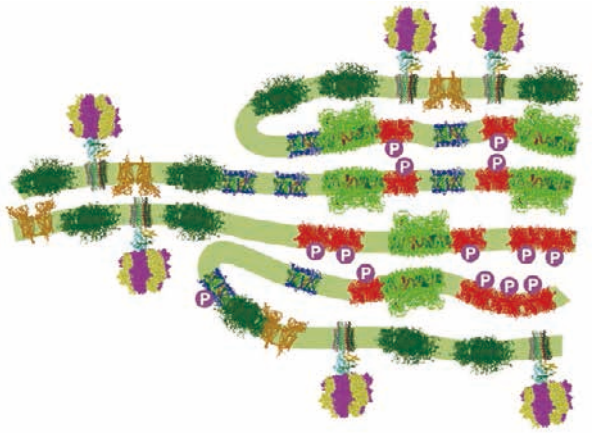


Figure 1. Hypothetical model for chloroplast remodeling during state transitions in *C. reinhardtii*. Side views of the membrane planes showing thylakoid ultrastructure and PS supercomplex composition in State 2. A number of LHCII proteins are phosphorylated, and the thylakoids are partially unstacked and undulated. The periodicity of the thylakoid membranes is weak. Most of the phosphorylated LHCIIs are in an energy-quenching state (red). They either remain associated with PSII so that a large part of the PSII–LHCII supercomplex array is preserved, or are unbound and aggregated.

2-2 Energy-dependent quenching of excess energy (qE 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 (dicyclohexylcarbodiimide), 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 (Figure 2).

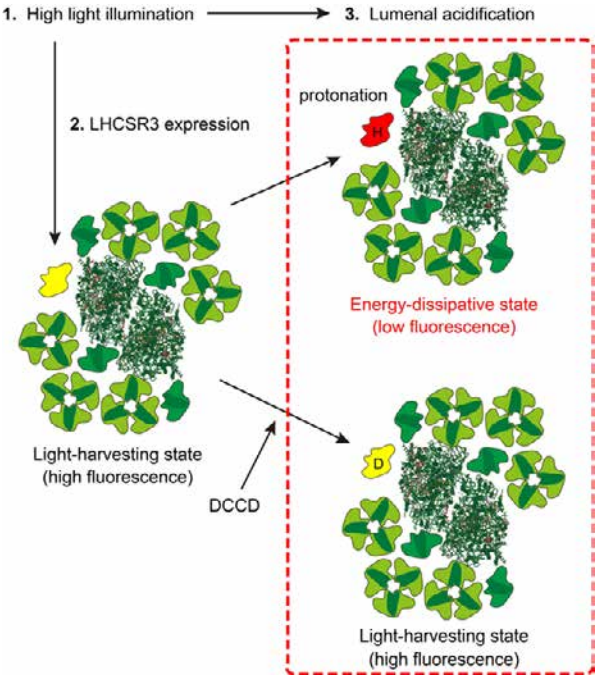


Figure 2. A model for the induction of qE in *C. reinhardtii*.

Although it has been known that two very closely related genes (*LHCSR3.1* and *LHCSR3.2*) encoding LHCSR3 protein and another paralogous gene *LHCSR1* are present in the *C. reinhardtii* genome, it was unclear how these isoforms are differentiated in terms of transcriptional regulation and functionalization. We showed that transcripts of both of the isoforms, *LHCSR3.1* and *LHCSR3.2*, are accumulated under high light stress. Reexamination of the genomic sequence and gene models along with a survey of sequence motifs suggested that these two isoforms shared an almost identical but still distinct promoter sequence and a completely identical polypeptide sequence, with more divergent 3'-untranscribed regions. Transcriptional induction under high light condition of both isoforms was suppressed by treatment with a PSII inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and a calmodulin inhibitor W7.

Despite a similar response to high light, the inhibitory effects of DCMU and W7 to *LHCSR1* transcript accumulation were limited compared to *LHCSR3* genes. These results suggest that the transcription of *LHCSR* paralogs in *C. reinhardtii* are regulated by light signals and differentially modulated via photosynthetic electron transfer and calmodulin-mediated calcium signaling pathway(s) (Figure 3).

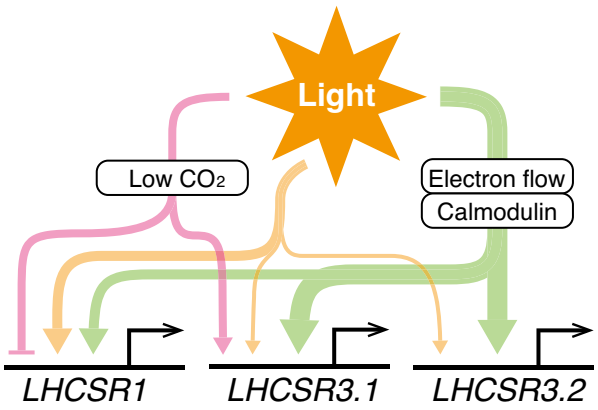


Figure 3. A model of transcriptional regulation of *LHCSR* genes in *C. reinhardtii*.

III. 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 4), 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.

Figure 4. 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*.

Publication List

〔Original papers〕

- Maruyama, S., Tokutsu, R., and Minagawa, J. (2014). Transcriptional regulation of the stress-responsive light harvesting complex genes in *Chlamydomonas reinhardtii*. Plant Cell Physiol. *55*, 1304-1310.
- Nagy, G., Ünnep, R., Zsiros, O., Tokutsu, R., Takizawa, K., Porcar, L., Moyet, L., Petroutsos, D., Garab, G., Finazzi, G., and Minagawa, J. (2014). Chloroplast remodeling during state transitions in *Chlamydomonas reinhardtii* as revealed by non-invasive techniques in vivo. Proc. Natl. Acad. Sci. USA *111*, 5042-5047.
- Shibata, Y., Katoh, W., Chiba, T., Namie, K., Ohnishi, N., Minagawa, J., Nakanishi, H., Noguchi, T., and Fukumura, H. (2014). Development of a novel cryogenic microscope with numerical aperture of 0.9 and its application to photosynthesis research. Biochim. Biophys. Acta *1837*, 880-887.
- Takahashi, H., Okamuro, A., Minagawa, J., and Takahashi, Y. (2014). Biochemical characterization of photosystem I associating light-harvesting complexes I and II isolated from State-2 cells of *Chlamydomonas reinhardtii*. Plant Cell Physiol. *55*, 1437-1449.

〔Review articles〕

- Finazzi, G., and Minagawa, J. (2014). High light acclimation in green microalgae. *In* "Non-Photochemical Quenching and Energy Dissipation in Plants, Algae, and Cyanobacteria" (B. Demmig-Adams, G. Garab G, W. Adams III and Govindjee, Eds.), pp.445-469, Advances in Photosynthesis and Respiration Vol.40, Springer, Dordrecht.
- Johnson, G. N., Cardol, P., Minagawa, J., and Finazzi, G. (2014). Regulation of electron transport in photosynthesis. *In* "Plastid Biology" (Theg, S., Wollman, F.-A., Eds.)" pp.437-464, Advances in Plant Biology Vol. 5, Springer, Dordrecht.

DIVISION OF SEASONAL BIOLOGY (ADJUNCT)

Adjunct Professor
YOSHIMURA, Takashi

Specially Appointed Assistant Professor: *SHINOMIYA, Ai*
SHIMMURA, Tsuyoshi
Visiting Graduate Student: *SENGA, Takumi*
ADACHI, Daisuke
SHIMO, Takayuki
Visiting Undergraduate: *NAKATSUKASA, Mana*
Technical Assistant: *BABA, Nayumi*
AKAMA, Akiko
Secretary: *OKUBO, Masayo*

Animals living outside the tropics adapt various physiology and behavior to seasonal changes in the environment. As animals use changes in day length and temperature as seasonal cues, these phenomena are referred to as photoperiodism and thermoperiodism, respectively. Medaka provides an excellent model to study these mechanisms because of their rapid and robust seasonal responses. In addition, genomic sequences and transgenic approaches are available in this species. In this division, we are trying to uncover the underlying mechanisms of seasonal adaptation.

I. Identification of deep brain photoreceptor

It has been known for more than a century that non-mammalian vertebrates receive light information directly within the deep brain to adapt to seasonal changes in day length. However, the identity of the deep brain photoreceptor remained unclear.

Cerebrospinal fluid (CSF)-contacting neurons extend knob-like dendrites into the ventricular cavity (Figure 1). This dendritic structure resembles those of photoreceptor cells in the developing retina and the pineal organ. Thus, the CSF-contacting neurons have been suggested to function as a deep brain photoreceptor.

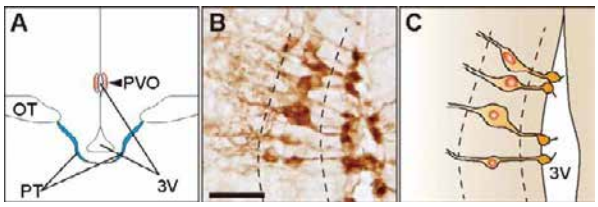


Figure 1. (A) Schematic drawing of the quail mediobasal hypothalamus. PVO: paraventricular organ; PT: pars tuberalis of the pituitary gland; 3V: third ventricle; OT: optic tract. (B) OPN5-positive CSF-contacting neurons in the PVO. (C) Schematic drawing of representative OPN5-positive CSF-contacting neurons in the panel (B).

1-1 Intrinsic photosensitivity of OPN5-positive CSF-contacting neuron

In a previous study, we demonstrated localization of a novel photopigment, OPN5 in the CSF-contacting neurons of quail. We used whole-cell patch-clamp analysis to demonstrate that OPN5-positive CSF-contacting neurons in the PVO are intrinsically photosensitive.

1-2 Involvement of OPN5-positive CSF-contacting neuron in the seasonal reproduction

We examined the effect of siRNA-mediated OPN5 knockdown on long day induction of springtime hormone, thyrotropin (thyroid stimulating hormone: TSH). Long day-induction of TSH was suppressed by the OPN5 knockdown, suggesting that OPN5 is involved in the seasonal regulation of reproduction. To our knowledge, this is the first demonstration of the intrinsic photosensitivity of deep brain CSF-contacting neurons.

II. Involvement of tissue-specific post-translational modification in seasonal time measurement

TSH is a glycoprotein secreted from the pituitary gland. Pars distalis-derived TSH stimulates the thyroid gland to produce thyroid hormones, whereas pars tuberalis-derived TSH acts on the hypothalamus to regulate seasonal physiology and behavior as a springtime hormone. However, it had not been clear how these two TSHs avoid functional crosstalk. We found that this regulation is mediated by tissue-specific glycosylation. Although pars tuberalis-derived TSH was released into circulation, it did not stimulate the thyroid gland. The pars distalis-derived TSH is known to have sulfated bi-antennary *N*-glycans, and sulfated TSH is rapidly metabolized in the liver. By contrast, pars tuberalis-derived TSH had sialylated multi-branched *N*-glycans; in circulation, it formed the macro-TSH complex with immunoglobulin or albumin, resulting in the loss of its bioactivity. Glycosylation is fundamental to a wide range of biological processes. However, this is the first demonstration of its involvement in preventing functional crosstalk of signaling molecules in the body.

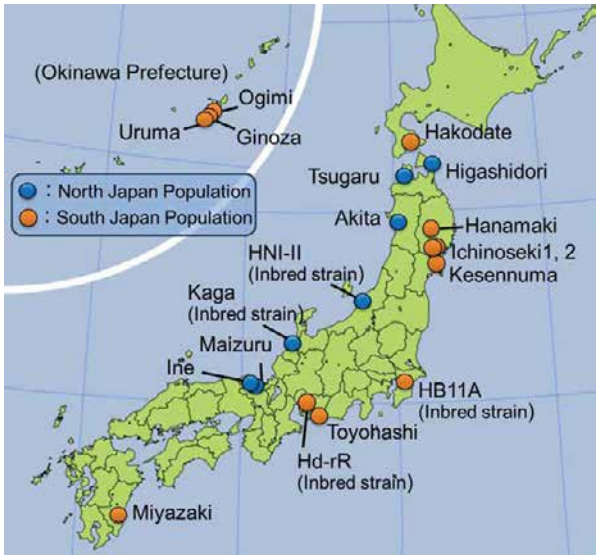


Figure 2. Medaka populations collected and used in our study.

III. Genome-wide association study 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 light period 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 planning to perform a genome-wide association study in medaka populations collected from various latitudes all over Japan.

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

To perform a genome-wide association study, we have collected thousands of medaka fish from all over Japan (Figure 2). We have examined the effects of changing day length and temperature to determine the critical day lengths and critical temperatures 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 3).

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. We identified hundreds of genes that respond to day length and temperature changes.

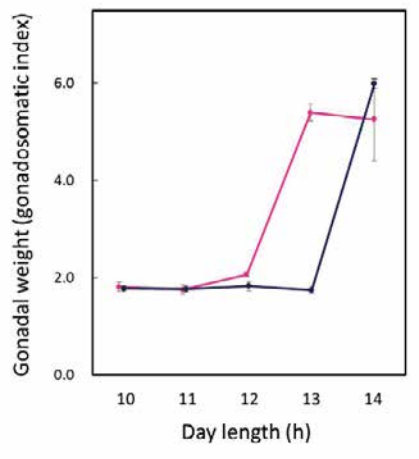


Figure 3. Different critical day length between medaka from higher latitudes and lower latitudes.

Publication List

〔Original papers〕

- Ikegami, K., Liao, X.H., Hoshino, Y., Ono, H., Ota, W., Ito, Y., Nishiwaki-Ohkawa, T., Sato, C., Kitajima, K., Iigo, M., Shigeyoshi, Y., Yamada, M., Murata, Y., Refetoff, S., and Yoshimura, T. (2014). Tissue-specific post-translational modification allows functional targeting of thyrotropin. Cell Reports 9, 801-809.
- Nakane, Y., Shimmura, T., Abe, H., and Yoshimura, T. (2014). Intrinsic photosensitivity of deep brain photoreceptor. Curr. Biol. 24, R596-R597.

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

- 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. Low temperature-induced circulating triiodothyronine accelerates seasonal testicular regression. Endocrinology 2014 Nov 18.

〔Review articles〕

- Nakane, Y., and Yoshimura, T. (2014). Universality and diversity in the signal transduction pathway that regulates seasonal reproduction in vertebrates. Front. Neurosc. 8, 115.
- Shinomiya, A., Shimmura, T., Nishiwaki-Ohkawa, T., and Yoshimura, T. (2014). Regulation of seasonal reproduction by hypothalamic activation of thyroid hormone. Front. Endocrinol. 5, 12.

LABORATORY OF GENOME INFORMATICS

Assistant Professor
UCHIYAMA, Ikuo

Postdoctoral Fellow: *CHIBA, Hirokazu*

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 algorithm (see Section II below). By means of this algorithm, 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.

Because of the rapid increase in microbial genome data owing to next generation sequencing technology, it becomes more challenging to maintain high quality orthology relationships while allowing users to utilize the latest genomic data available. Since recently accumulating sequences are mostly draft genome data, MBGD now stores them and allows users to incorporate them into a user specific ortholog database through the MyMBGD functionality. In MyMBGD, draft genome data as well as user genome data are incorporated into an existing ortholog table created from complete genome data in an incremental manner. In addition, to provide high quality orthology relationships, the standard ortholog table, which is first created by DomClust, is now refined using the DomRefine program (see Section II below).

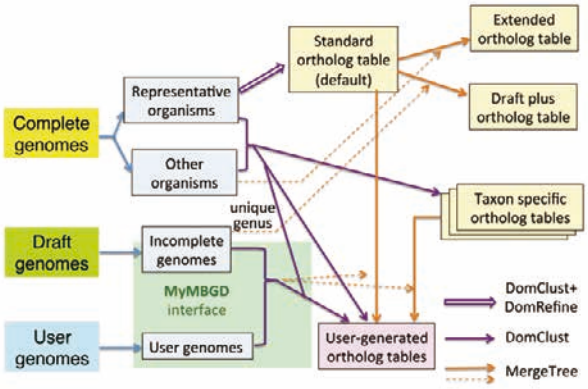


Figure 1. Overview of the data construction procedure in MBGD

II. Improvement of the methods for constructing orthologous groups 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 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 ortholog clustering results at the domain level as the sum total of domain-level alignment scores. We developed a refinement pipeline to improve domain-level clustering, DomRefine, by optimizing the DSP score. We applied DomRefine to domain-based ortholog groups created by DomClust using a dataset obtained from the MBGD database, and evaluated the results using COG and TIGRFAMs as the reference data. Thus, we observed that the agreement between the resulting classification and the classifications in the reference databases is improved in the refinement pipeline. Moreover, the refined classification showed better agreement than the classifications in the eggNOG databases when TIGRFAMs was used as the reference database.

III. Identification of the core structure conserved among moderately 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 bacterial taxa to define their core gene sets, and are now trying to utilize this information to characterize novel genomic and metagenomic data.

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.

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

Orthology is a key to integrate knowledge about various organisms through comparative analysis. Moreover, presence/absence of orthologs in each genome can be an important clue to understand the relationship between gene functions and species phenotype/habitat.

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). While the OrthO is a compact ontology for general use, it is designed to be extended to the description of database-specific concepts. 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, which accepts arbitrary queries specified by users. In this framework based on the OrthO, the biological data of different organisms can be integrated using the ortholog information as a hub.

This is part of the MicrobeDB project, a collaborative project under the National Bioscience Database Center.

VI. Identification of mobile genes and its application to characterizing *H. pylori* pan-genome repertoire

Gene contents of the same bacterial species can have great variation and thus the whole repertoire of genes in a bacterial species, termed the pan-genome, can be very large. 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 to define mobility of genes against the reference coordinate determined by the core alignment created by CoreAligner, and classified each accessory 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. 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.

Publication List

〔Original papers〕

- Chiba, H., and Uchiyama, I. (2014). Improvement of domain-level ortholog clustering by optimizing domain-specific sum-of-pairs score, BMC Bioinformatics *15*, 148.
- Kawai, M., Futagami, T., Toyoda, A., Takaki, Y., Nishi, S., Hori, S., Arai, W., Tsubouchi, T., Morono, Y., Uchiyama, I., Ito, T., Fujiyama. A., Inagaki, F. and Takami, H. (2014). High frequency of phylogenetically diverse reductive dehalogenase-homologous genes in deep subseafloor sedimentary metagenomes. Front. Microbiol. *5*, 80.
- Matsui, H., Takahashi, T., Murayama, S.Y., Uchiyama, I., Yamaguchi, K., Shigenobu, S., Matsumoto, T., Kawakubo, M., Horiuchi, K., Ota, H., Osaki, T., Kamiya, S., Smet, A., Flahou, B., Ducatelle, R., Haesebrouck, F., Takahashi, S., Nakamura, S., and Nakamura, M. (2014). Development of New PCR Primers by Comparative Genomics for the Detection of *Helicobacter suis* in Gastric Biopsy Specimens. Helicobacter *19*, 260-271.
- Toyota, K., Kato, Y., Miyakawa, H., Yatsu, R., Mizutani, T., Ogino, Y., Miyagawa, S., Watanabe, H., Nishide, H., Uchiyama, I., Tatarazako, N., and Iguchi, T. (2014). Molecular impact of juvenile hormone agonists on neonatal *Daphnia magna*. Appl. Toxicol. *34*, 537-544.

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

- Uchiyama, I., Mihara, M., Nishide, H., and Chiba, H. MBGD update 2015: microbial genome database for flexible ortholog analysis utilizing a diverse set of genomic data. Nucleic Acids Res. 2014 Nov 14.

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.

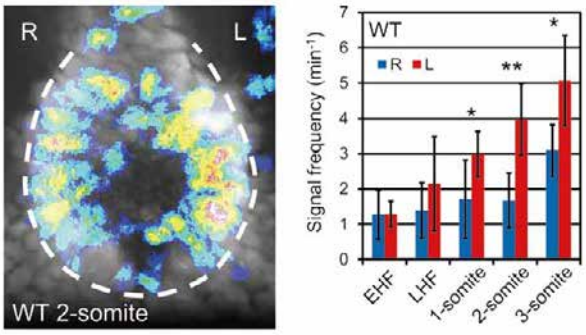


Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

During the analysis we generated transgenic lines carrying ultrasensitive Ca^{2+} sensor proteins. We demonstrated these lines are useful for visualizing Ca^{2+} dynamics of adult tissues, while they did not work in the embryonic tissues.

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 imaging acquisition. This method also has peculiar disadvantages, however. Specifically scattering of excitation light within the specimen and 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.

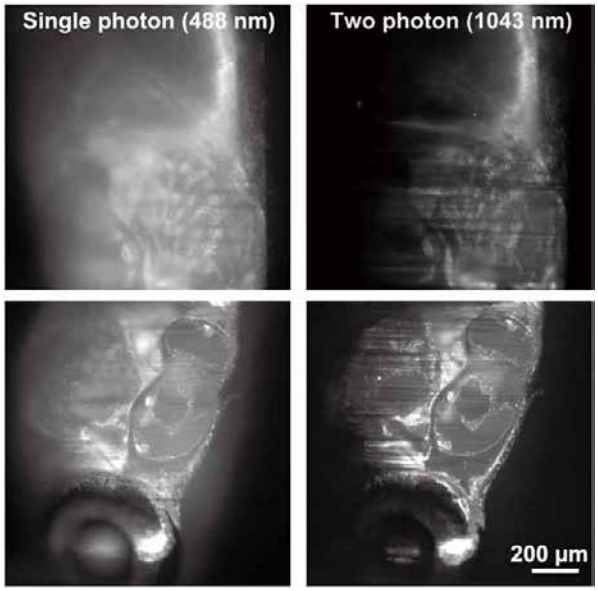


Figure 2. Two-photon light-sheet microscopy. Left: Fluorescent images of a medaka taken by a conventional (one-photon) light-sheet microscope. Right: Images of the same area taken by our two-photon light-sheet microscope.

Publication List

〔Original papers〕

- Ichikawa, T., Nakazato, K., Keller, P.J., Kajiura-Kobayashi, H., Stelzer, E.H., Mochizuki, A., and Nonaka, S. (2014). Live imaging and quantitative analysis of gastrulation in mouse embryos using light-sheet microscopy and 3D tracking tools. Nature Protoc. *9*, 575-585.
- Maruyama, A., Oshima, Y., Kajiura-Kobayashi, H., Nonaka, S., Imamura, T., and Naruse, K. (2014). Wide field intravital imaging by two-photon-excitation digital-scanned light-sheet microscopy (2p-DSLM) with a high-pulse energy laser, Biomed. Opt. Express *5*, 3311-3325.
- Oshima, Y., Imamura, T., Shintani, A., Kajiura-Kobayashi, H., Hibi, T., Nagai, T., Nonaka, S., and Nemoto, T. (2014). Ultrasensitive imaging of Ca^{2+} dynamics in pancreatic acinar cells of yellow cameleon-nano transgenic mice. Int. J. Mol. Sci. *15*, 19971-19986.

LABORATORY OF NUCLEAR DYNAMICS

Specially Appointed Associate Professor
MIYANARI, Yusuke

SOKENDAI Graduate Student: *SUGIYAMA, Kazuya*
Technical Assistant: *SANBO, Chiaki*
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.

I. 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.

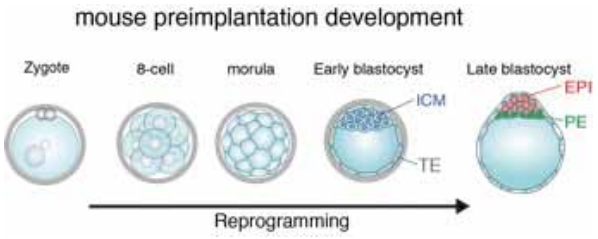


Figure 1. Lineage allocation in mouse preimplantation development

II. 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.

III. 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.

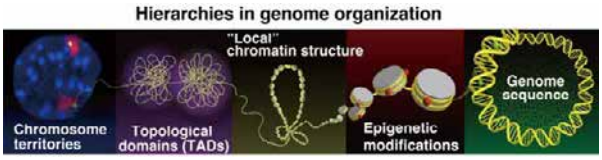


Figure 2. Hierarchical chromatin structure

IV. 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.

Publication List

〔Original paper〕

● Miyanari, Y. (2014). TAL effector-mediated genome visualization (TGV). Methods 69. 198-204.

NIBB Core Research Facilities

Head
YOSHIDA, Shosei

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

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

Functional Genomics Facility

Specially Appointed Associate Professor
SHIGENOBU, Shuji

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

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

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

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD5500xl (Life technologies), HiSeq2500, HiSeq1500, and MiSeq (Illumina), the Functional Genomics Facility is committed to joint research aiming to explore otherwise inaccessible new fields in basic biology.

During 2014 we carried out 37 NGS projects in collaboration with NIBB laboratories as well as the researchers of other academic institutions. These projects cover a wide range of species (bacteria, animals, plants, and humans) including both model and non-model organisms, and various applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

Figure 1. Next-generation sequencers

Proteomics

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

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

Other analytical instruments

- Cell sorter (SONY SH800)
- Bioimaging Analyzer (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser Capture Microdissection System (Arcturus XT)
- DNA Sequencer (ABI PRISM 310; ABI 3130xl)
- Real Time PCR (ABI 7500)
- Ultra Centrifuge (Beckman XL-80XP etc.)

Figure 2. A mass spectrometry system

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2014, we provided two three-day training courses on RNA-seq data analysis. These courses are designed to introduce the basic knowledge and skills of bioinformatics analysis to biologists who are not familiar with bioinformatics.

Figure 3. NIBB Genome Informatics Training Course

Publication List of Collaborative Research

〔Original papers〕

- Blankenburg, S., Balfanz, S., Hayashi, Y., Shigenobu, S., Miura, T., Baumann, O., Baumann, A., and Blenau, W. (2014). Cockroach GABAB receptor subtypes: Molecular characterization, pharmacological properties and tissue distribution. Neuropharmacology. 88, 134-144.
- Furuta, Y., Namba-Fukuyo, H., Shibata, T.F., Nishiyama, T., Shigenobu, S., Suzuki, Y., Sugano, S., Hasebe, M., and Kobayashi, I. (2014). Methylome diversification through changes in DNA methyltransferase sequence specificity. PLoS Genet. 10, e1004272.
- Ishida, T., Tabata, R., Yamada, M., Aida, M., Mitsumasu, K., Fujiwara, M., Yamaguchi, K., Shigenobu, S., Higuchi, M., Tsuji, H., Shimamoto, K., Hasebe, M., Fukuda, H., and Sawa, S. (2014). Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. EMBO Rep. 5, 1202-1209.
- Kaiwa, N., Hosokawa, T., Nikoh, N., Tanahashi, M., Moriyama, M., Meng, X.-Y., Maeda, T., Yamaguchi, K., Shigenobu, S., Ito, M., and Fukatsu, T. (2014). Symbiont-Supplemented Maternal Investment Underpinning Host's Ecological Adaptation. Curr. Biol. 24, 2465-2470.
- Kodama, Y., Suzuki, H., Dohra, H., Sugii, M., Kitazume, T., Yamaguchi, K., Shigenobu, S., and Fujishima, M. (2014). Comparison of gene expression of Paramecium bursaria with and without Chlorella variabilis symbionts. BMC Genomics 15, 183.
- Matsui, H., Takahashi, T., Murayama, S.Y., Uchiyama, I., Yamaguchi, K., Shigenobu, S., Matsumoto, T., Kawakubo, M., Horiuchi, K., Ota, H., Osaki, T., Kamiya, S., Smet, A., Flahou, B., Ducatelle, R., Haesebrouck, F., Takahashi, S., Nakamura, S., and Nakamura, M. (2014). Development of new PCR primers by comparative genomics for the detection of Helicobacter suis in gastric biopsy specimens. Helicobacter 19, 260–271.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, Sdgc, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development 141, 3363-3369.
- Uehara, M., Wang, S., Kamiya, T., Shigenobu, S., Yamaguchi, K., Fujiwara, T., Naito, S., and Takano, J. (2014). Identification and characterization of an arabidopsis mutant with altered localization of NIP5;1, a plasma membrane boric acid channel, reveals the requirement for D-galactose in endomembrane organization. Plant Cell Physiol. 55, 704-714.
- Yoshida, K., Makino, T., Yamaguchi, K., Shigenobu, S., Hasebe, M., Kawata, M., Kume, M., Mori, S., Peichel, C.L., Toyoda, A., Fujiyama, A., and Kitano, J. (2014). Sex chromosome turnover contributes to genomic divergence between incipient stickleback species. PLoS Genet. 10, e1004223.

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

Specially Appointed Associate Professor:	*SHIGENOBU, Shuji*
NIBB Research Fellow:	*MAEDA, Taro*
Postdoctoral ellow:	*HOJO, Masaru*
	OGAWA, Kota
Technical Assistant:	*SUZUKI, Miyuzu*

Symbiogenomics

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

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

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

Aphid species bear intracellular symbiotic bacteria in the cytoplasm of bacteriocytes, specialized cells for harboring the bacteria. The mutualism is so obligate that neither can reproduce independently. The 464 Mb draft genome sequence of the pea aphid, *Acyrthosiphon pisum*, in consort with that of bacterial symbiont *Buchnera aphidicola* illustrates the remarkable interdependency between the two organisms. Genetic capacities of the pea aphid and the symbiont for amino acid biosynthesis are complementary. The genome analysis revealed that the pea aphid has undergone characteristic gene losses and duplications. The IMB antibacterial immune pathway is missing several critical genes, which might account for the evolutionary success of aphids to obtain beneficial symbionts. Lineage-specific gene duplications have occurred in genes in a broad range of functional categories, which include signaling pathways, miRNA machinery, chromatin modification and mitosis. The importance of these duplications for symbiosis remains to be determined. We found several instances of lateral gene transfer from bacteria to the pea aphid genome. Some of

them are highly expressed in bacteriocytes.

We recently discovered a novel class of genes in the pea aphid genome that encode small cysteine-rich proteins with secretion signals that are expressed exclusively in bacteriocytes of the pea aphid, and named these bacteriocyte-specific cysteine-rich proteins (BCR). The BCR mRNAs are first expressed at a developmental time point coincident with the incorporation of symbionts strictly in the cells that contribute to the bacteriocyte and this bacteriocyte-specific expression is maintained throughout the aphid's life. Some BCRs showed an antibiotic activity. These results suggest that BCRs act within bacteriocytes to mediate the symbiosis with bacterial symbionts, which is reminiscent of the cysteine-rich secreted proteins of leguminous plants that also regulate endosymbionts. Employment of small cysteine-rich peptides may be a common tactic of host eukaryotes to manipulate bacterial symbionts.

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

Publication List

〔Original papers〕

- Gusev, O., Suetsugu, Y., Cornette, R., Kawashima, T., Logacheva, M.D., Kondrashov, A.S., Penin, A.A., Hatanaka, R., Kikuta, S., Shimura, S., Kanamori, H., Katayose, Y., Matsumoto, T., Shagimardanova, E., Alexeev, D., Govorun, V., Wisecaver, J., Mikheyev, A., Koyanagi, R., Fujie, M., Nishiyama, T., Shigenobu, S., Shibata, T.F., Golygina, V., Hasebe, M., Okuda, T., Satoh, N., and Kikawada, T. (2014). Comparative genome sequencing reveals genomic signature of extreme desiccation tolerance in the anhydrobiotic midge. Nature Commun. 5, 4784.
- Kaiwa, N., Hosokawa, T., Nikoh, N., Tanahashi, M., Moriyama, M., Meng, X.-Y., Maeda, T., Yamaguchi, K., Shigenobu, S., Ito, M., and Fukatsu, T. (2014). Symbiont-supplemented maternal investment underpinning host's ecological adaptation. Curr. Biol. 24, 2465-2470.
- Kodama, Y., Suzuki, H., Dohra, H., Sugii, M., Kitazume, T., Yamaguchi, K., Shigenobu, S., and Fujishima, M. (2014). Comparison of gene expression of Paramecium bursaria with and without Chlorella variabilis symbionts. BMC Genomics 15, 183.
- Takeshita, K., Shibata, T.F., Nikoh, N., Nishiyama, T., Hasebe, M., Fukatsu, T., Shigenobu, S., and Kikuchi, Y. (2014). Whole-genome sequence of Burkholderia sp. strain RPE67, a bacterial gut symbiont of the bean bug Riptortus pedestris. Genome Announc. 2, e00556-14.

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

- Bourguignon, T., Lo, N., Cameron, S.L., Sobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T., and Evans, T.A. The evolutionary history of termites as inferred from 66 mitochondrial genomes. Mol. Biol. Evol. 2014 Nov 10.

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

Technical Staff: *KONDO, Maki*
TANIGUCHI-SAIDA, Misako
UCHIKAWA, Tamaki
Technical Assistant: *ICHIKAWA, Chiaki*
ISHIKAWA, Azusa

The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use "Light". The facility also provides technical support through management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers by Dr. Y. Kamei (refer to the Collaborative Research Group Research Enhancement Strategy Office section). Among its tools are advanced microscopes for biology and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism, capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve; allowing exposure to strong monochromatic light. The facility's microscopes, which are cutting edge devices such as confocal and multi-photon excitation microscopes, are used by both internal and external researchers as vital equipment for core and collaborative research projects.

Representative Instruments:

Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum from 250 nm (ultraviolet) to 1,000 nm (infrared) onto its 10 m focal curve with an intensity of monochromatic light at each wavelength more than twice as much as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe *et al*., Photochem. Photobiol. *36*, 491-498, 1982).

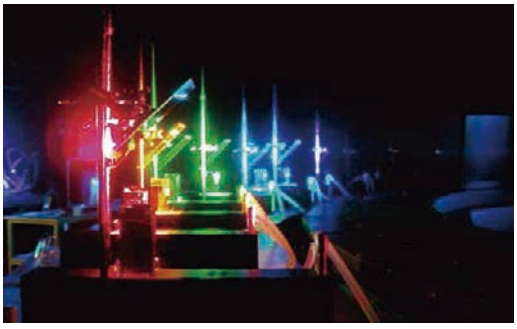


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

The spectrograph is dedicated to action spectroscopical studies of various light-controlled biological processes.

The NIBB Collaborative Research Program for the Use of the OLS supports about 10 projects every year conducted by both visiting scientists, including foreign researchers, as well as those in NIBB.

Action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted.

Microscopes

This facility also has Bioimaging machines such as widefield microscopes (Olympus IX-81, BX-63 and KEYENCE BZ-8000), confocal microscopes (Olympus FV1000, Nikon A1R, Nikon A1Rsi, Carl Zeiss Duo 5 and Yokogawa CSU-X1), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica 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. 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 2014, we held the 8th International training courses on frog and fish (refer to international practical course) and the 2nd 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 8th NIBB Bioimaging Forum focused on establishment of a network among Imaging Centers in Japan. In addition, we held a symposium focused on new microscope methods using adaptive optics, "Subaru microscope", with the National Astronomical Observatory of Japan (NAOJ).

Publication List of Collaborative Research

〔Original papers (Selected)〕

- Fang, X., Ide, N., Higashi, S., Kamei, Y., Toyooka, T., Ibuki, Y., Kawai, K., Kasai, H., Okamoto, K., Arimoto-Kobayashi, S., and Negishi, T. (2014). Somatic cell mutaions caused by 365 nm LED-UVA double-strand breaks through oxidative damage. Photochem. Photobiol. Sci. *13*, 1338-1346.
- Goto-Yamada, S., Mano, S., Nakamori, C., Kondo, M., Yamawaki, R., Kato, A., and Nishimura, M. (2014). Chaperone and protease functions of LON protease 2 modulate the peroxisomal transition and degradation with autophagy. Plant Cell Physiol. *55*, 482-496.
- Hayashi, S., Ochi, H., Ogino, H., Kawasumi, A., Kamei, Y., Tamura, K., and Yokoyama, H. (2014). Transcriptional regulators in the Hippo1 signaling pathway control organ growth in Xenopus tadpole tail regeneration. Dev. Biol. *396*, 31-41.
- Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y.I., Yamamoto, S., Yabe, T., Takada, S., Kinoshita, M., Kuroiwa, A., and Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. Proc. Natl. Accad. Sci. USA *111*, 7343-7348.
- Masamizu, Y., Tanaka, Y.R., Tanaka, Y.H., Hira, R., Ohkubo, F., Kitamura, K., Isomura, Y., Okada, T., and Matsuzaki, M. (2014). Two distinct layer-specific dynamics of cortical ensembles during learning of a motor task. Nature Neurosci. *17*, 987-994.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Wakamatsu, Y., Kelsh, R.N., Hibi, M., and Hashimoto, H. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genetics *10*, e1004246.
- Ogino, Y., Hirakawa, I., Inohaya, K., Sumiya, E., Miyagawa, S., Denslow, N., Yamada, G., Tatarazako, N., and Iguchi, T. (2014). Bmp7 and Lef1 are the downstream effectors of androgen signaling in androgen-induced sex characteristics development in medaka. Endocrinology *155*, 449-462.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.
- Tamada, Y., Murata, T., Hattori, M., Oya, S., Hayano, Y., Kamei, Y., and Hasebe, M. (2014). Optical property analyses of plant cells for adaptive optics microscopy. Int. Optomechatroni. *8*, 89-99.

● *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 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).

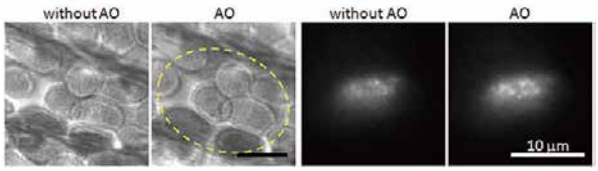


Figure 1. Effects of adaptive optics (AO) to the wide-field microscope images (bright field and fluorescence of plant cells).

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 *C. elegans*, *Drosophila*, medaka, zebrafish, *Xenopus* and *Arabidopsis*, to induce the heat shock response at a desired timing.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating. 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.

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). This year, our group and other collaborators were applying the same system to amphibians, *Xenopus* and salamanders, for regeneration studies.

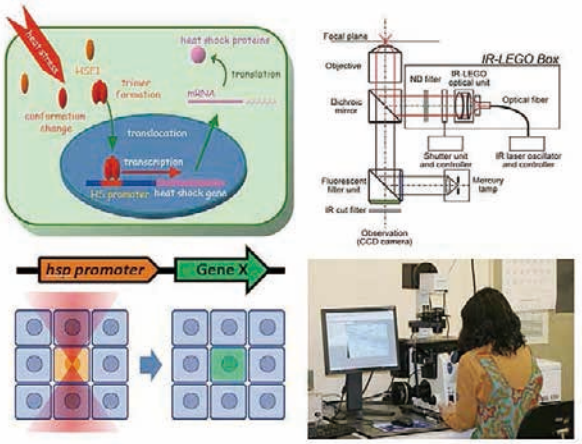


Figure 2. Schematic illustration of local gene induction system and an infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

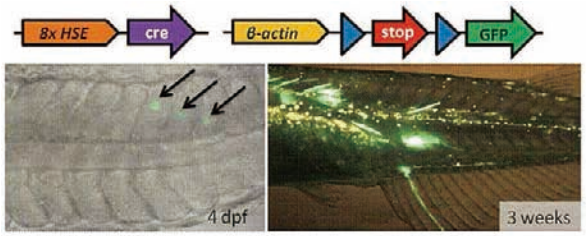


Figure 3. Examples of Cre-loxP mediated long-term GFP marking using IR-LEGO in living medaka individuals for cell linage tracing.

Publication List

〔Original papers〕

- Fang, X., Ide, N., Higashi, S., Kamei, Y., Toyooka, T., Ibuki, Y., Kawai, K., Kasai, H., Okamoto, K., Arimoto- Kobayashi, S., and Negishi, T. (2014). Somatic cell mutations caused by 365 nm LED-UVA double-strand breaks through oxidative damage. Photochem. Photobiol. Sci. *13*, 1338-1346.
- Hayashi, S., Ochi, H., Ogino, H., Kawasumi, A., Kamei, Y., Tamura, K., and Yokoyama, H. (2014). Transcriptional regulators in the Hippo1 signaling pathway control organ growth in Xenopus tadpole tail regeneration. Dev. Biol. *396*, 31-41.

- Murozumi, N., Nakashima, R., Hirai, T., Kamei, Y., Ishikawa-Fujiwara, T., Todo, T., and Kitano, T. (2014). Loss of follicle-stimulating hormone receptor function causes masculinization and suppression of ovarian development in genetically female medaka. Endocrinology *155*, 3136-3145.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Wakamatsu, Y., Kelsh, R.N., Hibi, M., and Hashimoto, H. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genetics *10*, e1004246.
- Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.
- Otozai, S., Ishikawa-Fujiwara, T., Oda, S., Kamei, Y., Ryo, H., Sato, A., Nomura, T., Mitani, H., Tsujimura, T., Inohara, H., and Todo T. (2014). p53-Dependent suppression of genome instability in germ cells. Mutat. Res. Fundam. Mol. Mech. Mutagen. *760*, 24-32.
- Tamada, Y., Murata, T., Hattori, M., Oyac, S., Hayano, Y., Kamei, Y., and Hasebe, M. (2014). Optical property analyses of plant cells for adaptive optics microscopy. Int. J. Optomechatroni. *8*, 89-99.

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,

Figure 1. Biological Information Analysis System

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/).

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. 70).

NIBB BioResource Center

Head
IGUCHI, Taisen

Vice 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*, makes it possible to produce genetically controlled organisms with markers placed by 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
	INADA, Yosuke
	FUJIMOTO, Daiji
	MATSUMURA, Kunihiro
	ATSUMI, Miho

Figure 1. Mouse (strain C57BL/6J)

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 staff 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 then. 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 2014 (from January 1 to December 31), 2,962 fertilized eggs (*in vitro* fertilization; 2,767 eggs of 10 lines in which 2,197 eggs of 10 lines were frozen for long-term storage,

Figure 2. Equipment for manipulating mouse eggs.

frozen eggs: 195 of 3 lines) and 4,948 mice were brought into the facility in the Yamate area, and 59,517 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 supports the activities of 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 2014 (from January 1 to December 31), 53 mice were brought into the facility in the Myodaiji area, and 3,759 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, birds, and insects)

The first floor of the center facility building in the Yamate area provides space and facilities to maintain small fish, chick embryos, and insects. 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 2014 (from January 1 to December 31), 11,002 medaka adults were brought to the facility and 94,135 medaka and zebrafish (88,592 fertilized eggs and 5,543 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 2014 we shipped 207 independent medaka strains, 233 cDNA/BAC/Fosmid clones, and 120 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. 44, 52, and 33).

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 by using xenon lamp) 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. 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.

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 green houses (44, 44, and 45 m^2) with heating are used for the sensitive carnivorous plants, and wild-type strains of medaka fish *Oryzias* sp. Seven green houses (4, 6, 6, 6, 6, 9, and 9 m^2) with air-conditioning are provided for the cultivation of a rice *Oryza* sp., *Lotus japonica* and related legume species, as well as mutant lines of the Japanese morning glory. Two green houses (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.

In 2014, several plant culture facilities came into use. Of these, the multiple precise environment control plant incubator system is composed of 10 incubators. Users can control all the incubators synchronously. A tissue culture rack with dimming LEDs and pulse-width modulation controllers is used for algae culture under precise light control.

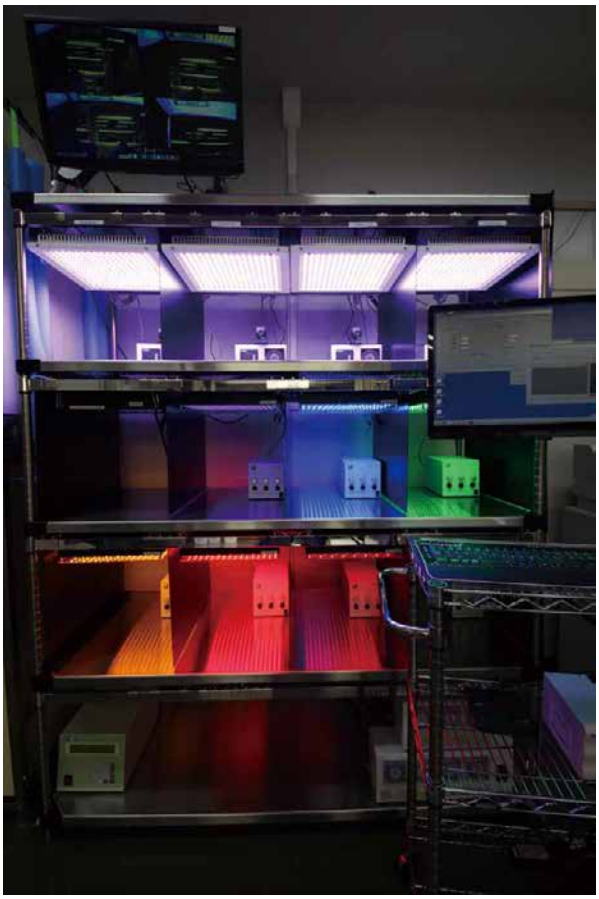


Figure 5. A tissue culture rack with dimming LEDs and pulse-width modulation controllers.

● *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 10,000 BAC clones, and provided 3 DNA clones and 28 *Ipomoea* lines to both local and foreign biologists this year.

Research activities of the assistant professor A. Hoshino are shown on the laboratory page (p. 57).

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.17).

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
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, The 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 working in the area in which each university satellite hub is responsible.

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 are cryopreservation 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 or other event 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.

Figure 1. IBBP Center

I. Current status of back up for biological resources

In 2014, IBBP Center stored 3,996 '384-well plates' consisting of 1,534,464 cDNA/BACs clones, 5,093 tubes for animal cells, plant and animal samples, proteins, genes, and microorganisms. In total 1,539,557 samples are stored.

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

Figure 3. Cryotube 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 cryobiological 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 cryopreservation 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 cryobiological 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 2014 we had ten applications and accepted all ten proposals. We are also working to establish a research center for cryobiological study thorough this Collaborative Research Project. In accordance with this aim we organized a Cryopreservation Conference in 2014 on October 23-24, 2014 at the Okazaki Conference Center, Okazaki, Japan. We had 107 participants from several fields from physics, chemistry, biology and technology.

Figure 4. Group photo of Cryopreservation Conference 2014

Research activity by D. Tanaka

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

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 methods, in combination with physiological factors, are the most influential determinants of survival.

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 5). 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).

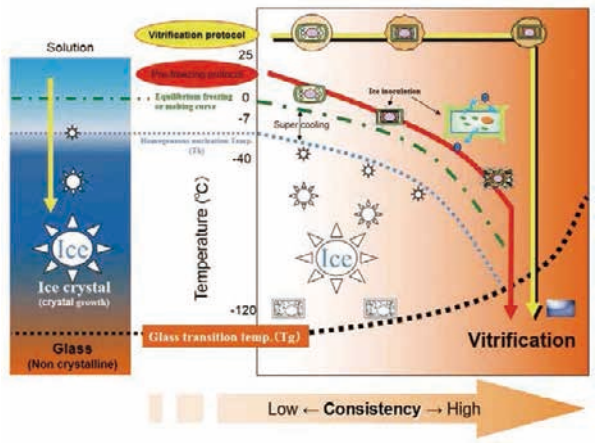


Figure 5. Phase diagram of vitrification of a solution.
Vitrification, a physical process, can be defined as the phase transition of an aqueous solution from a liquid into an amorphous glassy solid, or glass, at the glass transition temperature (Tg), while avoiding ice crystallization.

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 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 the behavior of water in cells and 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 gemmae of the liverwort, *Marchantia polymorpha* L., strain Takaragaike-1 that were cooled to the temperature of liquid nitrogen after exposure to various steps of the Cryoplate protocol (Figure 6).

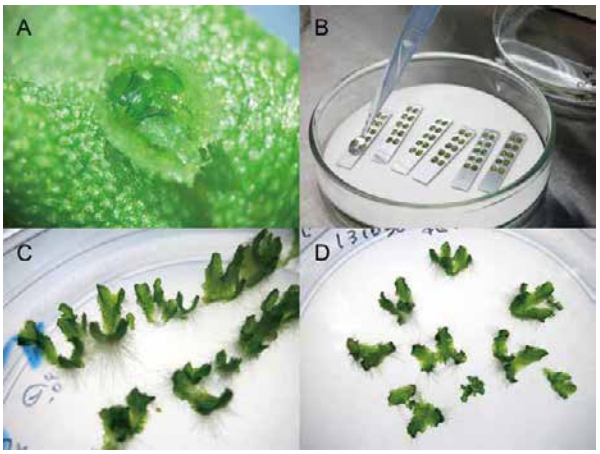


Figure 6. Cryopreservation protocol using aluminum cryoplate.
A: Preparation of *in vitro* grown gemmae of liverwort. B: Placing precultured gemmae in a cryoplate's wells. C: Regrowth of cryopreserved livewort line 'Takaragaike-1' 30 days after rewarming. D: Control (without cooling in liquid nitrogen).

Publication List

〔Original paper〕

- Kondo, T., Sakuma, T., Wada, H., Akimoto-Kato, A., Yamamoto, T., and Hayashi, S. (2014). TALEN-induced gene knock out in *Drosophila*. Dev. Growth Differ. *56*, 86-91.

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 (*Oryzias Latipes*) 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 is 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 7). Our findings provide clues for revealing diverse evolutionary mechanisms of pigment cell formation in animals.

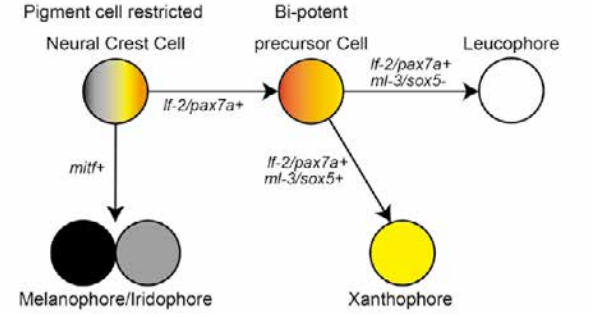


Figure 7. Model for leucophore and xanthophore development from neural crest cells.

Publication List

〔Original papers〕

- Kimura, T., Nagao, Y., Hashimoto, H., Yamamoto-Shiraishi, Y., Yamamoto, S., Yabe, T., Takada, S., Kinoshita, M., Kuroiwa, A., and Naruse, K. (2014). Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. Proc. Natl. Acad. Sci. USA *111*, 7343-7348.
- Nagao, Y., Suzuki, T., Shimizu, A., Kimura, T., Seki, R., Adachi, T., Inoue, C., Omae, Y., Kamei, Y., Hara, I., Taniguchi, Y., Naruse, K., Wakamatsu, Y., Kelsh, R.N., Hibi, M., and Hashimoto, H. (2014). Sox5 functions as a fate switch in medaka pigment cell development. PLoS Genet. *10*, e1004246.
- Nishimura, T., Herpin, A., Kimura, T., Hara, I., Kawasaki, T., Nakamura, S., Yamamoto, Y., Saito, T.L., Yoshimura, J., Morishita, S., Tsukahara, T., Kobayashi, S., Naruse, K., Shigenobu, S., Sakai, N., Schartl, M., and Tanaka, M. (2014). Analysis of a novel gene, Sdgc, reveals sex chromosome-dependent differences of medaka germ cells prior to gonad formation. Development *141*, 3363-3369.
- Tsuboko, S., Kimura, T., Shinya, M., Suehiro, Y., Okuyama, T., Shimada, A., Takeda, H., Naruse, K., Kubo, T., and Takeuchi, H. (2014). Genetic control of startle behavior in medaka fish. PLoS One *9*, e112527.

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:
ITO, Takayo
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, Ms. Ito, and Ms. Kamiya maintained the Myodaiji-Area. Ms. Sawada worked in the Yamate-Area. Dr. Kodama worked in both areas.

The following are CRF's notable activities in 2014.

1. The Common Facilities building I was repaired, therefore the radiation controlled area of the Myodaiji-Area was closed from April 2013 and re-opened on July 14th 2014. (Figure 1A, 1B, 1C)
2. Along with the re-opening of the Myodaiji-Area, the following areas were closed.

2-1 The radioisotope handling rooms at the NIPS building for animal experiments and gene-recombination experiments using radioisotopes.

2-2 The second radioactive waste storage room at the NIBB building.

The number of registrants and the number of users from January 2014 to December 2014 are shown in Table 1.

Users and visitors counted by the access control system of the controlled areas numbered 4,320 during this period. The radioisotope handling rooms' users and visitors counted by record sheet numbered 557. 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 2014. The Myodaiji-area was repaired.
A, A': The radioisotope laboratory (A: before, A': after)
B, B': The exhaust facility (B: before, B': after)
C, C': The drainage facility (C: before, C': after)

	Myodaiji-Area	Yamate-Area
registrants	69	87
users	21	41

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2014.

	controlled area			radioisotope
	Myodaiji-Area	Yamate-Area	total	handling room
users	754	748	1502	541
visitors	2651	167	2818	16
total	3405	915	4320	557

Table 2. Users and visitors who entered each controlled area and radioisotope handling room in 2014.

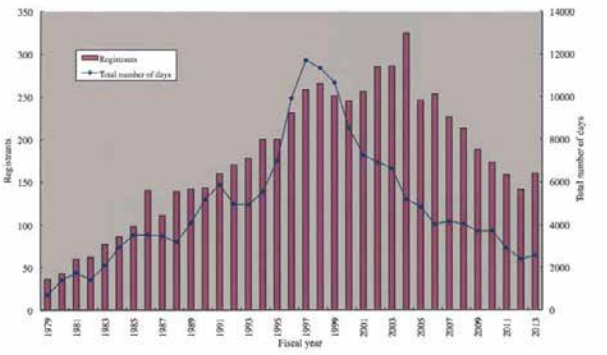


Figure 2. Annual changes in number of registrants and total number of days per fiscal year.

	Myodaiji-Area	Yamate-Area		total
		controlled area	radioisotope handling room	
^{125}I Received	0	0	—	0
^{125}I Used	0	55	—	55
^{35}S Received	74000	1850000	—	1924000
^{35}S Used	4218	1536915	—	1541133
^{33}P Received	38500	56250	—	94750
^{33}P Used	31775	49960	—	81735
^{14}C Received	174927	0	—	174927
^{14}C Used	13277	0	1480	14757
^{3}H Received	544049	1859250	—	2403299
^{3}H Used	42353	2778811	8800	2829964

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area and radioisotope handling room in 2014.

training courses	place	numbers of participant
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 courses

Table 4. Training courses for radiation workers in 2014.

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 2014

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
Group Adviser: *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), the Max Planck Institute for Plant Breeding Research (MPIPZ, Germany), 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.

This group supports and coordinates NIBB's activities related to international research collaborations, through organizing the above-mentioned various types of 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's interns visiting from foreign countries, and the dispatching of graduate students of SOKENDAI (the Graduate University for Advanced Studies) to international conferences, which aim to nurture the next generation of researchers in biology.

The main activities of the group in 2014

1) Coordination of international conferences and the International Practical Course

This group coordinated the following international conference and the International Practical Course hosted by NIBB:

The 8th NIBB International Practical Course and the 3rd NIBB-TLL-DBS/NUS Joint International Practical Course "Experimental Techniques using Medaka and *Xenopus* – The Merits of using both –" Okazaki, Japan, September 22-October 1, 2014 (p. 93)

The 62nd NIBB Conference "Force in Development" Okazaki, Japan, November 17-19, 2014 (p. 91)

2) Support for dispatching researchers to international conferences

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

The 5th NIBB-MPIPZ-TLL Joint Symposium "HORIZONS IN PLANT BIOLOGY" Max Planck Institute for Plant Breeding Research, Cologne, Germany, November 24-26, 2014 (p. 92)

EMBO Workshop "Wnt Signaling: Stem Cells, Development, Disease" Broome, Australia, October, 6-9, 2014

EMBO Conference "Stem Cells in Cancer and Regenerative Medicine" EMBL, Heidelberg, Germany, October, 9-12, 2014

NIBB Investigators' lab visits to EMBL, Heidelberg

3) Support of education related programs

This group supported the following student-related activities of NIBB:

NIBB Internship Program 2014 (p. 96)

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 also belong to NIBB core research facilities and are responsible for managing collaborative research projects and practical courses using their academic background, knowledge, and specialties. Based on these experiences, this group explores further promotion of information exchange and collaboration among universities and institutes, both academic and industrial, and supports the development and dissemination of new experimental equipment and methods.

In 2014, this group hosted or managed a total of 89 collaboration projects. Through these collaboration projects, several research papers were published. A noteworthy achievement was the study of a neural mechanism related to social familiarity using medaka, published in Science by Dr. Takeuchi of the University of Tokyo. The NIBB Collaborative Research Group supported mutant screening, laser cell ablation, establishment of transgenic lines and discussions through the Priority Collaborative Research Project from 2010 to 2013.

● Okuyama, T., Yokoi, S., Abe, H., Isoe, Y., Suehiro, Y., Imada, H., Tanaka, M., Kawasaki, T., Yuba, S., Taniguchi, Y., Kamei, Y., Okubo, K., Shimada, A., Naruse, K., Takeda, H., Oka, Y., Kubo, T., and Takeuchi, H. (2014). A neural mechanism underlying mating preferences for familiar individuals in medaka fish. Science *343*, 91-94.

Gender Equality Promotion Group

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 the period of childbirth and childcare, etc., aiming at promoting the employment of female researchers.

In 2014, this group contributed to the management of the research support system for the period of childbirth and childcare. This group organized an enlightenment lecture 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
	NODA, Chiyo
	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 Assistant:	*INABA, Kayo*

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 62nd NIBB Conference "Force in Development"

Organizers: Lance Davidson (Univ. Pittsburgh, USA), Toshihiko Fujimori (NIBB, Japan), Shigeo Hayashi (RIKEN CDB, Japan), Carl-Philipp Heisenberg (IST, Austria, Austria), Kenji Matsuno (Osaka Univ., Japan), Hiroyuki Takeda (Univ. Tokyo, Japan), Naoto Ueno (NIBB, Japan)

November 17 (Mon) - 19 (Wed), 2014

For the past 30 years, in the field of developmental biology, the primary focus has been on elucidating the role of genes on developmental regulation, with biologists pouring the bulk of their time and effort into the investigation of genes, proteins, and their signal transduction systems, that forms the central dogma. It is indisputable that genes and proteins are absolutely required components exerting fundamental biological actions, however, in recent years new light has been shed on the importance of physical forces generated by the shape-change and movement of cells and tissues, as well as the responses of cells and tissues to such forces in developmental processes making elucidation of these physical forces a rising hot-topic in the biological sciences.

In order to capitalize on this opportunity, the 62nd NIBB Conference "Force in Development" was planned as an international convention. The meeting was held at the Okazaki Conference Center from November 17th to the 19th of 2014. With three keynote speakers, 21 lectures by invited researchers, Six sessions including seven short talks, and 44 poster presentations; the total number of participants was over 130 people. The Mecanobiology field has already existed for a long time, however, rather than cultured cells, the effects of force on multicellular systems, especially dynamics of cells and tissues which alter the physical environment of the embryo and how this impacts morphogenesis was the main theme of this conference. We are extremely grateful that top-class researchers from around the world were willing to gather in Okazaki during their tight schedules. This meeting, by bringing renowned researchers together, made it possible to deeply explore the theme of the conference, and we believe this will be seen as an epoch-making meeting for the future development of this field. In addition, with so many young researchers from home and abroad who participated in the poster presentations and discussions I feel that this specific area is growing exponentially. We are confident that these connections will become the foundation of future collaborative research and international cooperation.

The conference was possible because of three research groups, all of which are supported by the Grant-in-Aid for Scientific Research on Innovative Areas (MEXT, Japan): "From molecules and cells to organs: trans-hierarchical logic for higher-order pattern and structures" (Project leader: Hiroyuki Takeda), "Cross-talk between moving cells and microenvironment as a basis of emerging order in multicellular systems" (Project leader: Takaki Miyata, Nagoya University), and "Cell community in early mammalian development" (Project leader: Toshihiko Fujimori), I would like to express my gratitude for their generous support for this meeting.

(Naoto Ueno)

Speakers

Davidson, Lance (Univ. Pittsburgh), Eaton, Suzanne (MPI-CBG), Grill, Stephan (MPI-CBG), Heisenberg, Carl-Philipp (IST, Austria), Kiehart, Daniel (Duke Univ.), Lecuit, Thomas (IBDM), Leptin, Maria (EMBL Heidelberg), Munro, Edwin (Univ. Chicago), Plachta, Nicolas (EMBL/Monash Univ., Australia), Toyama, Yusuke (MBI, NUS/TLL) Vermot, Julien (IGBMC), Weber, Gregory (Rutgers Univ.) Fujimori, Toshihiko (NIBB), Hayashi, Shigeo (RIKEN CDB), Inoue, Yasuhiro (Kyoto Univ.), Matsumoto, Takeo (NITech), Matsuno, Kenji (Osaka Univ.), Miyata, Takaki (Nagoya Univ.), Ogura, Toshihiko (Tohoku Univ.), Sugimura, Kaoru (Kyoto Univ.), Takeda, Hiroyuki (Univ. Tokyo), Takeichi, Masatoshi (RIKEN CDB), Ueno, Naoto (NIBB), Wang, Yu-Chiun (RIKEN CDB)

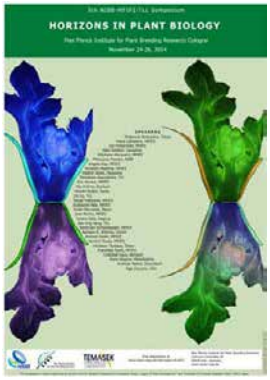
The 5th NIBB-MPIPZ-TLL Symposium
"Horizons in Plant Biology"

Organizers: George Coupland (MPIPZ), Mitsuyasu Hasebe (NIBB)
Venue: MaxPlanck Institute for Plant Breeding Research (MPIPZ), Cologne, Germany
November 24 (Mon) - 26 (Wed), 2014

The symposia jointly conducted by the National Institute for Basic Biology (NIBB), the Max Planck Institute for Plant Breeding Research (MPIPZ) and the Temasek Life Science Laboratory have been held four times since 2009. Accordingly, the 5th NIBB-MPIPZ-TLL joint symposium titled "Horizons in Plant Biology" was held from November 23rd to November 26th 2014, at the Max Planck Institute for Plant Breeding Research, Cologne, Germany with researchers gathered from Europe, Singapore, the USA and Japan. In the joint symposium, 18 researchers from Japan participated, and about 80 from Germany, Switzerland, the USA and Singapore. The oral presentations conducted in the symposium were comprised of 13 members of MPIPZ, 6 from Europe, 1 from the USA, 3 from TLL, 1 from NIBB and 6 from Japan's other universities. At this symposium one of the major academic trends in plant science, i.e., in addition to research in the laboratory, performing research that considers ecology and evolution under the divergent environmental conditions found in nature, really stood out. The rise of young researchers who have started taking leading roles in the plant science community was also noticeable with many presentations and proactive discussions from young scientists. For many of the participants it was also a wonderful opportunity to start new international collaborations. Finally NIBB concluded the proceedings with a review of the conference, as well as the ones previously held, and the announcement of our future directions as we step forward.

Some Japanese participants also had meetings with researchers in MPIPZ about collaborative research projects before and after the symposium and in the afternoon of the first day. In addition, some participants visited other universities in Germany to have discussions about individual collaborations.

(Mitsuyasu Hasebe)



Speakers

Cartolano, Maria (MPIPZ), Finkemeier, Iris (MPIPZ), Geldner, Niko (Univ. of Lausanne), Hacquard, Stéphane (MPIPZ), Hay, Angela (MPIPZ), Hayama, Ryosuke (MPIPZ), Kawashima, Tomokazu (Gregor Mendel Institute), Kemen, Eric (MPIPZ), Krämer, Ute (Ruhr-Univ. Bochum), Lu, Liu (TLL), Maekawa, Takaki (MPIPZ), Née, Guillaume (MPIPZ), Parker, Jane (MPIPZ), Schneeberger, Korbinian (MPIPZ), Seng, Gan Eng (TLL), Shimizu, Kentaro K. (Univ. of Zurich), Smith, Richard (MPIPZ), Tsuda, Kenichi (MPIPZ), Turck, Franziska (MPIPZ), Uauy, Cristobal (John Innes Centre), Wagner, Doris (Univ. of Philadelphia), Weber, Andreas (CEPLAS), Zuccaro, Alga (Köln University)
Betsuyaku, Shigeyuki (Tokyo Univ.), Hasebe, Mitsuyasu (NIBB), Ikeda, Hajime (Okayama Univ.), Kudoh, Hiroshi (Kyoto Univ.), Okuyama, Yudai (National Museum of Nature and Science), Sato, Yutaka (Nagoya Univ.), Tsukaya, Hirokazu (Univ. of Tokyo)

The 8th NIBB International Practical Course, The 3rd NIBB-TLL-DBS/NUS Joint International Practical Course "Experimental Techniques using Medaka and *Xenopus* - The Merits of using both -"

■Period: September 22 (Mon) - October 1 (Wed), 2014

■Participants: 16 (3 from Germany, 2 from Taiwan, one each from Hong Kong, India, Indonesia, Bangladesh, and USA, 6 from Japan)

■Venue: National Institute for Basic Biology, Japan

■Lecturers:

Dr. Toshinori HAYASHI (Tottori Univ.)
Dr. Yasuhiro KAMEI (NIBB)
Dr. Kei MIYAMOTO (Wellcome Trust/Cancer Research UK Gurdon Institute)
Dr. Hajime OGINO (Nagahama Institute of Bio-Science and Technology)
Dr. Hideaki TAKEUCHI (Univ. of Tokyo)
Dr. Thomas THUMBERGER (Heidelberg Univ.)
Dr. Christoph WINKLER (National Univ. of Singapore)
Dr. Hitoshi YOKOYAMA (Tohoku Univ.)
Dr. Takashi YOSHIMURA (Nagoya Univ./NIBB)

■Course Staff:

Dr. Satoshi ANSAI, Dr. Shin-ichi CHISADA, Dr. Hideki HANADA, Dr. Yasuhiro KAMEI, Dr. Akihiko KASHIWAGI, Dr. Keiko KASHIWAGI, Dr. Aiko KAWASUMI, Dr. Masato KINOSHITA, Dr. Kei MIYAMOTO, Dr. Kiyoshi NARUSE, Dr. Shigenori NONAKA, Dr. Tetsushi SAKUMA, Dr. Takao SASADO, Dr. Ken-ichi T. SUZUKI, Dr. Yusuke TAKEHANA, Dr. Marta TEPEREK, Dr. Thomas THUMBERGER, Dr. Hitoshi YOKOYAMA

■Contents of the course:

Gene knock-out using TALEN in *Xenopus*, Gene knock-out using CRISPR/CAS9 system in medaka, Gene knock-in using CRISPR/CAS9 system in medaka, *in vitro* fertilization of eggs and manipulation of embryos, Local gene induction with the infrared laser-evoked gene operator (IR-LEGO) method, Live imaging by 2-photon microscopy and Digital Scaning Light-sheet Microscopy (DSLM), Cryopreservation of sperm and artificial insemination for Medaka

The 8th NIBB International Practical Course & The 3rd NIBB-TLL-DBS / NUS Joint International Practical Course "Experimental Techniques using Medaka and *Xenopus* - The Merits of using both -" was held from September 22 to October 1st 2014. 16 participants from Taiwan, Hong Kong, India, Indonesia, Bangladesh, Germany, the United States, and Japan were selected from among the 29 applicants.

Unique Features of this NIBB International Practical Course were training with medaka and zebrafish, and concurrently training with *Xenopus*. Through these workshops important connections were made between the two communities of small fish and *Xenopus* researchers. The course was planned with the intention that participants should experience research using a variety of experimental systems.

In addition to the main project aims, there was also the sub-aim of displaying the rapid development of genome editing technology (such as mutants created by TALEN and CRISRPR-CAS9), which can be used in *Xenopus* and small fish. In response to these changes in technology and the research environment participants were allowed to use both medaka and *Xenopus* not only in live-imaging and training in important sperm freezing technologies for long-term storage of mutants, but also in the TALEN / CRISRPR-CAS9 genome editing system to produce knock-out and knock-in mutants, and the gene induction system by IR-LEGO. The results of the questionnaire show the course was very popular to those who participated. In addition to the standard training schedule, on every day except Sunday and on the last day, distinguished invited researchers in the field conducted seminars for participants. The seminars were extremely interesting because of both the cutting–edge research using small fish and amphibians, and the research using multiple model organisms concurrently.

I want to take this opportunity to dedicate a big thank you to the staff of the Office of International Cooperation. I would also like to thank the research groups that were co-sponsors of the course: National BioResource Projects (NBRP) Medaka, NBRP *Xenopus*, "Molecular Mechanisms Underlying Reconstruction of 3D Structures during Regeneration" (supported by MEXT, Japan), and "Enhancing Application of Innovative Optical Science and Technology by Making Ultimate Use of Advanced Light Sources" (supported by CREST/JST, Japan). Finally I would like to thank the staff in the Institute for Amphibian Biology, Hiroshima University, the Laboratory of BioResources, NIBB, and the Spectrography and Bioimaging Facility, NIBB, whose preparations made this training course possible.

(Kiyoshi Naruse)

The 8th Bio-Imaging Forum "Directions of Future Bio-Imaging" and the "Meeting for the Future Plan of Bio-Imaging Network among Universities and Institutes in Japan"

Organizers: Yasuhiro Kamei, Shigenori Nonaka, Naoto Ueno, Toshihiko Fujimori, Hiroshi Koyama, Kazuyoshi Murata (NIPS), Yoshitaka Kimori, Kagayaki Kato

Bio-Imaging Forum: March 4 (Tue), 2014 | The Meeting for the Future Plan: March 5 (Wed)-6 (Thu), 2014

The 8th Bio-Imaging Forum was held on the theme of "Directions of Future Bio-Imaging," and was immediately followed, with the same participants, by the "Meeting for the Future Plan of Bio-Imaging Network among Universities and Institutes in Japan", which was planned by the Department of Imaging Science, Center for Novel Science Initiative, NINS.

Both the forum and the meeting were managed by the Collaborative Research Group, Research Enhancement Strategy Office, NIBB. The forum and the meeting were seamlessly planned by the same organizers who undertook detailed discussions prior to the events. The aim of the forum was to promote the exchange of information on the newest technologies of obtaining and analyzing biological images, and that of the meeting was to grasp the present situation of various imaging facilities in Japan and to discuss the collaborations of these facilities and the future directions of bio-imaging, with the prospect of promoting collaborative use of the Spectroscopy and Bioimaging Facility, NIBB.

The Forum welcomed ninety participants and included nine lectures on a wide variety of bio-image acquisition methods, ranging from the newest techniques of electron and optical microscopy to macro bio-imaging methods such as PET and MRI, and also on the image analysis methods (Bio-Image Informatics) of acquired images. These lectures were a part of the imaging facilities' introduction to the later meeting during which they served as materials of discussion. The meeting started with self-introductions by the twenty imaging facilities on their missions, present status, problems and prospects for collaboration. Dr. Kota Miura of the Advanced Light Microscopy Facility, EMBL Heidelberg was invited to report the present status and the future directions of the network of bio-imaging facilities in Europe. Further discussions resulted in common understanding on the present status and problems of imaging facilities and as the first step of the collaboration we agreed to open a portal web site of bio-imaging facilities and to continue to hold similar meetings to promote formation of networks in the imaging science field through the collaboration of the Spectroscopy and Bioimaging Facility and the Department of Imaging Science.

The organizers appreciate the precious information brought by the participants, and the Spectroscopy and Bioimaging Facility benefitted much in enhancing collaborative programs and preparing for improvement of its equipment. The forum and the meeting served an important role in promoting NINS as a hub of the worldwide scientific community working in the field of imaging science.

(Yasuhiro Kamei)

The NIBB Genome Informatics Training Course

NIBB organizes a series of training courses on up-to-date research techniques for researchers from mainly Japanese universities and institutions. In 2014 we held two of these training courses on Genome Informatics. The two-day programs offer lectures and hands-on tutorials to introduce basic knowledge and skills to deal with genomic scale large data such as those obtained 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"

September 17 (Thu) -19 (Fri), 2014

- Organizer: Dr. Shuji Shigenobu (NIBB Core Research Facilities)
- Lecturers: Dr. Shuji Shigenobu, Dr. Masanao Sato, Dr. Ikuo Uchiyama, Dr. Katsushi Yamaguchi, Dr. Taro Maeda
- Participants: 20 (including 2 from NIBB)
- Program:
  1. Overview: Transcriptome data analysis
  2. Introduction to statistics
  3. Introduction to "R"
  4. Basic format and tools of NGS
  5. RNA-seq 1: Basics
  6. RNA-seq 2: Genome-based analysis
  7. RNA-seq 3: de novo assembly
  8. Multivariate statistics
  9. Practical exercises

"Introduction to Next-generation DNA Sequence Data Analysis"

September 19 (Thu) -20 (Fri), 2013

- 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. Overview
  2. UNIX for beginners
  3. NGS basic data formats
  4. NGS basic tools
  5. Introduction to statistics
  6. Basics of RNA-seq
  7. RSA-seq genome base
  8. Multivariate statistics
  9. Introduction to "R"
  10. RNA-seq de novo
  11. Practical exercises

The 2nd NIBB Bioimage Analysis Training Course

This course was held as a workshop of the Department of Imaging Science, the Center for Novel Science Initiative, NINS. The aim of the course was to deliver enough basic knowledge to attendees that they would be able to descriminate the difficulty of the problems facing them with image analysis; giving the ability to solve easy problems alone, while allowing communication with experts regarding more challenging analyses. The course therefore was centered on basic methods of image analyses, basic techniques of ImageJ, and writing of simple macros of ImageJ. At the end of the course, lecturers and participants discussed real problems some of the participants are facing. During the practice using PCs seven volunteers, some from NIBB and others from outside, supported participants. There was also an introduction of NIBB as a collaborative research institute by the vice-director Prof. Ueno during the get-together. Answers to questionnaires on the course were mostly favorable.

(Yasuhiro Kamei)

December 10 (Wed) -12 (Fri), 2014

- Organizers: Dr. Yoshitaka Kimori, Dr. Kagayaki Kato, Dr. Yasuhiro Kamei, Dr. Shigenori Nonaka, Dr. Takashi Murata, Dr. Hiroshi Koyama
- Supervisors: Dr. Naoto Ueno, Dr. Toshihiko Fujimori
- Participants: 21 (including 2 from NIBB)
- Program:
  - Basis of image data processing and analysis (lecture and practice)
  - Usage of ImageJ software and its macros (lecture and practice)
  - Quantitative analysis of images (lecture and practice)
  - Selection of microscopes and important points in obtaining images (lecture)

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 2014 there were 12 applicants, out of which seven interns were selected. These interns were from universities located in 4 countries (India, China, Hungary, and Germany) and spent periods ranging from one to twelve weeks experiencing life as a member of a research team.

Report from a participant
Dilukshi Chinthani Perera
Shanghai Jiao Tong University, China

I was an intern student at Professor Minoru Tanaka's lab from 4th of August 2014 to 15th of August 2014. My main area of study was Gonadal sex differentiation in Medaka by observing Germ cells.

Germ cells are a type of biological cell that involves reproduction. In many animals the germ cells originate in the primitive streak and migrate via the gut of the embryo to the developing gonads. There, they undergo cell division of two types, mitosis and meiosis, followed by cellular differentiation into mature gametes, either eggs or sperm.

Germ cells play an essential role in Sex differentiation in Medaka. They are required for ovarian formation and also Germ cell deficient gonads develop a testis-like structure.

In medaka the sex-determining gene *dmY/dmrt1y* has been identified. My studies were mainly focused on Genotypic Sex Determination (GSD), and two important genes *SDiG* and *figla*. At the beginning of the experiment I had to dissect Medaka larva to extract gonads which was a very interesting activity. I enjoyed collecting fish eggs at Myodaiji. Performing micro surgery was where I used my skills as a medical student who had assisted in surgery at a hospital. It was also an opportunity to exchange knowledge with the PhD students at the lab. Therefore, my overall experience was invaluable. I certainly had a very useful time with Professor Tanaka and his team.

INDEX

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

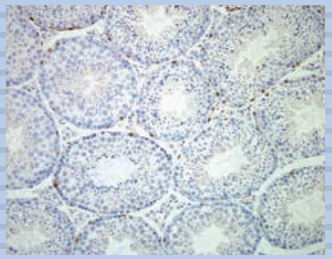
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

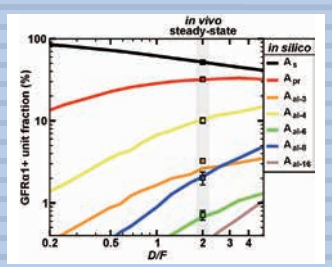
From Higashi Okazaki Station to Each Area

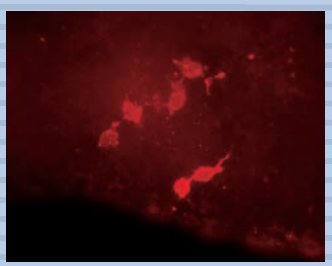
Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

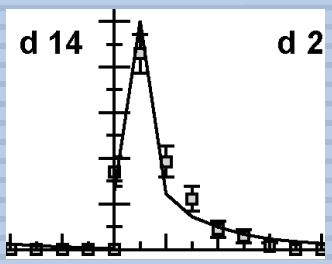
By car

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









National Institutes of Natural Sciences
National Institute for Basic Biology

38 Nishigonaka, Myodaiji, Okazaki, Aichi 444-8585 Japan
tel: +81 564-55-7000 fax: +81 564-53-7400
http://www.nibb.ac.jp/en/
Issued in July 2015
Edited by the Research Enhancement Strategy Office, NIBB