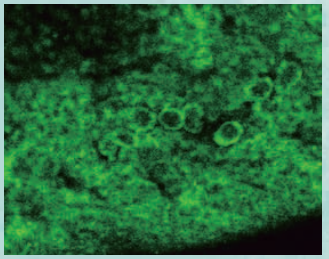
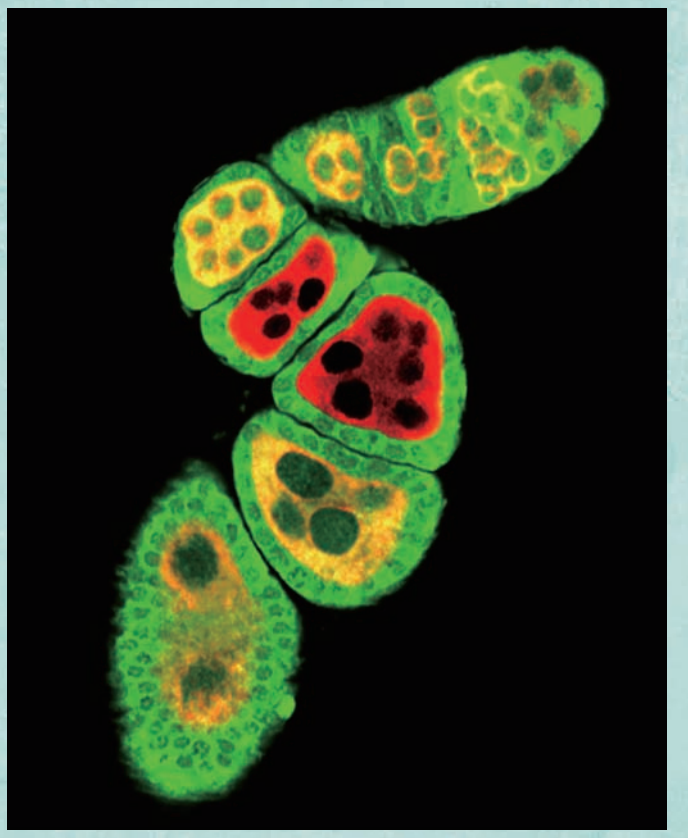
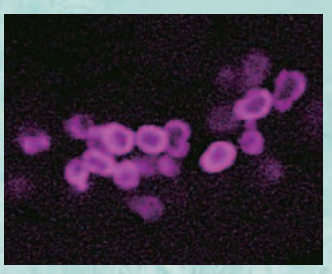
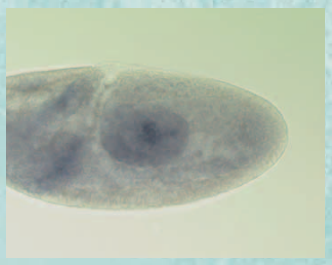




National Institute for Basic Biology

2011 ANNUAL REPORT

CONTENTS

The cover photographs are related to the study showing the essential role of *Sex lethal* (*Sxl*) gene in female germline development in *Drosophila* (Hashiyama, Hayashi and Kobayashi, Science, 2011). Ectopic expression of *Sxl* in male (XY) primordial germ cells (PGCs) was sufficient to induce them to enter oogenesis and remarkably produce functional eggs when transplanted into an female (XX) host. This observation provides powerful evidence that *Sxl* acts as a master gene to initiate female germline fate during sexual development. See page 20 of this report for details.

The Great East Japan Earthquake hit Northern Japan on March 11, 2011 and the subsequent collapse of nuclear power plants made us reexamine how to integrate science and technology with our culture. Though we did not suffer any direct damage to our personnel or facilities in Okazaki, many laboratories of basic biology lost their bioresources and facilities in the northern regions of Japan. We appreciated of the outpouring of help and support provided from colleagues of other countries proposing to share facilities and space to allow interrupted research to continue. Our institute also offered help and similar support to the researchers and students who suffered damage (as described on p. 5). In addition, we began preparation of a novel program of storing important bioresources as a defense against their loss or damage by future natural disasters. We are planning to prepare a special facility named IBBP Center (p. 5) with liquid-nitrogen tanks and freezers for protecting the bioresources and responding to the demands of researchers of nationwide universities and institutes. The program will start in 2012.

Our institute, the National Institute for Basic Biology (NIBB), has developed as a center of excellence in research, education, and inter-university collaboration in the various fields of basic biology since its foundation in 1977. In order to carry out our mission we have focused our efforts on five major activities, namely Promotion of Collaborative Research Projects, Promotion of Academic Research, Development of New Academic Fields, Cultivation of Future Researchers, and International Cooperation and Outreach. Our works and results for 2011 are shown in this report.

We welcomed 11 new members to the Advisory Committee for Programming and Management of our institute in April 2011 as shown in the list on page 3. Prof. Iguchi and Prof. Kondo were elected to be the chairperson and the vice-chairperson, respectively. Their term is 2 years.

In 2011 we welcomed several new colleagues, including 1 professor, 2 assistant professors and 10 NIBB research fellows, while 4 colleagues left the institute as shown on page 7. With the help of these new members, we produced a variety of high-ranked research as reported from page 8 to 73. The intensive activities of our supporting divisions are shown on the following pages 74–80.

In addition to our current research, education of the next generation of researchers is another important aim of NIBB. As a department of the Graduate University for Advanced Studies, we are engaged in education of graduate students. Our students joined the 2nd NIBB-EMBL PhD mini-symposium in November (page 81). We also continued the NIBB Internship Program and opened our laboratories to students and interns from abroad (page 85).

Owing to the large earthquake and the following confusion, many plans for our international collaborative activities were forced to be postponed or cancelled. Nevertheless, we were able to host the first NIBB-Princeton Symposium in Okazaki in November (page 83), and co-sponsor the third NIBB-TLL-MPIPZ joint symposium at Temasek Life Sciences Laboratory, Singapore in November (page 82). We deeply appreciate the efforts and support of the people involved in these activities.

Based on our endeavors, as shown in this booklet, we hope to develop joint activities in tight collaboration with external researchers and supporters. We hope you enjoy reading about the science being done at NIBB in the following pages. As always we appreciate your suggestions and comments on our activities.

Kiyotaka OKADA, D. Sci.
Director-General, NIBB
June 4, 2012

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 (OIIB) 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).

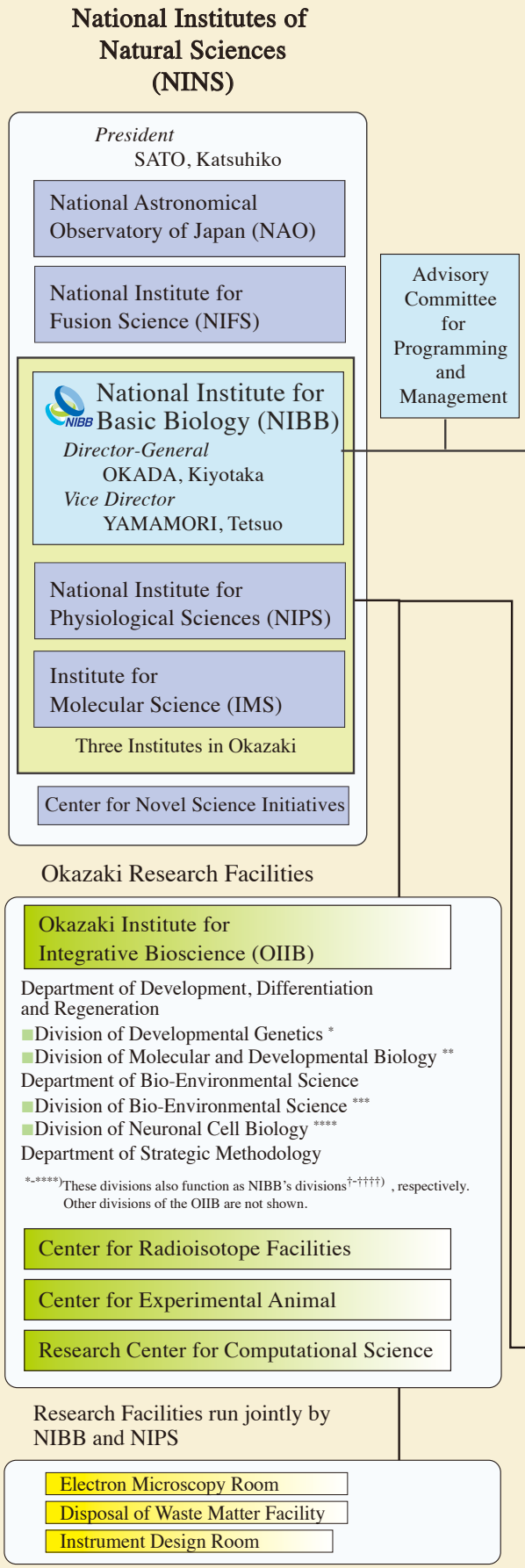
Policy, Decision Making, and Administration

The Director-General oversees the operation of NIBB, assisted by the Advisory Committee for Programming and Management. The Advisory Committee, comprised of professors within NIBB and an equal number of 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 Strategic Planning Department assists the Director-General with NIBB's evaluation procedures and in planning a long-range strategy for the institute. The Office of Public Relations and the Office of International Cooperation are central offices for public relations and the management of conferences and other extramural activities.

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, 2012

Research Units

- **Cell Biology**
  - Division of Cell Mechanisms
  - Division of Intercellular Signaling Biology
  - Laboratory of Neuronal Cell Biology††††
  - Laboratory of Cell Sociology
- **Developmental Biology**
  - Division of Morphogenesis
  - Division of Developmental Genetics †
  - Division of Molecular and Developmental Biology ††
  - Division of Embryology
  - Division of Germ Cell Biology
  - Laboratory of Molecular Genetics for Reproduction
  - Laboratory of Plant Organ Development
- **Neurobiology**
  - Division of Molecular Neurobiology
  - Division of Brain Biology
  - 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
- **Theoretical Biology**
  - Laboratory of Genome Informatics
- **Imaging Science**
  - Laboratory for Spatiotemporal Regulations

Strategic Planning Department

Research Support Units

- **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)**

This center is scheduled to open in July, 2012.

Other Research Support Sections

- Technical Division
- Office of Public Relations
- Office of International Cooperation

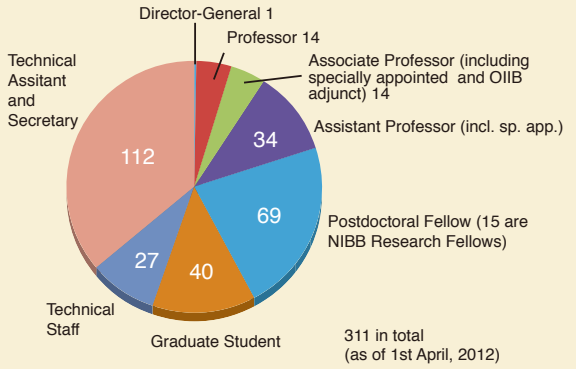
Okazaki Administration Office

Research and Research Support

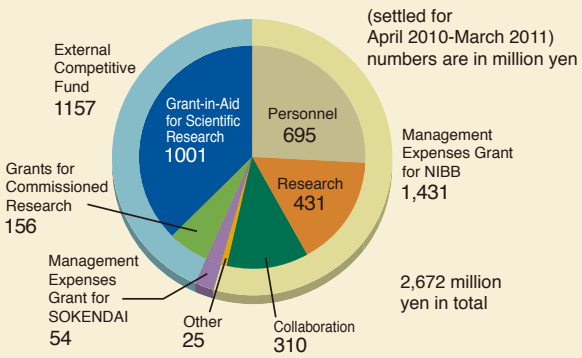
NIBB's research programs are conducted in research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research 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 Interuniversity Bio-Backup Project (IBBP Center) will be founded in 2012 to prevent loss of invaluable biological resources. 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 are one of the latter and are run by the technical staff of NIBB.

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

Members in NIBB



Financial Configuration of NIBB



Members of the Advisory Committee for Programming and Management* (as of April, 2012)

Chairperson	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
Vice-Chair	KONDO, Takao*	Professor, Nagoya University
Non-NIBB members	HIGASHIYAMA, Tetsuya**	Professor, Nagoya University
	MIZUSHIMA, Noboru*	Professor, Tokyo Medical and Dental University
	MORI, Ikue*	Professor, Nagoya University
	SHIMAMOTO, Ko*	Professor, Nara Institute of Science and Technology
	SIOMI, Haruhiko*	Professor, Keio University
	TAKABAYASHI, Junji*	Professor, Kyoto University
	TANAKA, Ayumi*	Professor, Hokkaido University
	UEMURA, Tadashi*	Professor, Kyoto University
	YAMAMOTO, Masayuki*	Director, Kazusa DNA Research Institute
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	KAWAGUCHI, Masayoshi*	Professor, National Institute for Basic Biology
	KOBAYASHI, Satoru	Professor, Okazaki Institute for Integrative Bioscience
	NISHIMURA, Mikio	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
	YAMAMORI, Tetsuo	Professor, National Institute for Basic Biology
	YOSHIDA, Shosei	Professor, National Institute for Basic Biology

* new member from April 2011
** new member from April 2012

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 in one to three years as group research by internal and external researchers with the purpose of developing pioneering research fields in biology and the "collaborative research projects for model organism/technology development" are for developing and establishing new model organisms and new research technology. For these projects, research expenses in addition to travel expenses are provided.

Shortly after the Great East Japan Earthquake in March 2011 we began to solicit special emergency collaborative research projects to allow researchers whose labs sufferred damage to stay at our institute to continue their work. Six projects from Tohoku University, Chiba University, and the University of Tokyo etc. were granted.

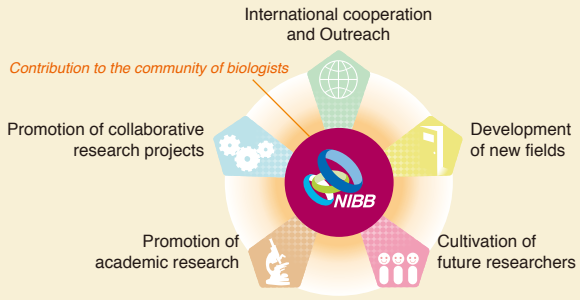
year	2008	2009	2010	2011
Priority collaborative research projects	0	1	4	6
Collaborative research projects for model organisms/ technology development	3	3	2	2
Individual collaborative research projects	49	54	68	88
NIBB workshops	5	3	3	6
Collaborative experiments using the large spectrograph	11	10	8	9
Collaborative experiments using the DSLM	–	–	7	8
Collaborative experiments using next generation DNA sequencers	–	–	11	45
Facility Use (Training Course Facility)	–	–	1	0
total	68	71	94	164

Collaborative Research Projects by Year

NIBB Core Research Facilities

The NIBB Core Research Facilities support research in NIBB and also act as an intellectual hub to promote collaboration among the researchers of 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. 69).

The Functional Genomics Facility maintains a wide array of core research equipment, including cutting edge tools such as 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 a part of this undertaking (p. 84). The Spectrography and Bioimaging Facility manages research tools, such as confocal microscopes, the 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 large-scale biological data analysis, such as genomic sequence analysis, expression data analysis, and imaging data analysis. For this purpose, the fascility maintains high-performance computers with large-capacity storage systems.

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

The Great East Japan Earthquake caused massive damage to important biological resources. To prevent such loss, NIBB will establish the IBBP Center in 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources.

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), a research institute established in 1974 and funded by 21 mostly European countries, 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. Graduate students were sent to attend the PhD students symposium held in EMBL, Heidelberg in November, 2011 (p. 81).

NIBB formed an agreement with the Max Planck Institute for Plant Breeding Research (MPIPZ) in April 2009 to start a new initiative aimed at stimulating academic and scholarly exchange in the field of plant sciences. NIBB and MPIPZ work together to plan and promote joint research projects, collaborative symposia, training courses, and student exchange programs.

Collaborative programs have also been started with the Temasek Life Sciences Laboratory (TLL), of Singapore and Princeton University. The 3rd NIBB-TLL-MPIPZ Joint Symposium "Cell Cycle and Development" was held in November, 2011, at TLL (p. 82). The 1st NIBB-Princeton Symposium "Proteomics, Metabolomics, and Beyond" was held in November, 2011, at NIBB (p. 83).

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 58th conference "Gamete Stem Cells" had been planned to take place in July, 2011, but was postponed because of the earthquake. It will be held in July, 2012, in conjunction with the 60th conference under the title of "Germline". The 59th conference

"Neocortical Organization" was held March, 2012, and will appear in 2012 Annual Report.

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 6th course "Developmental Genetics of Medaka IV" was held jointly with TLL in November, 2011 (p. 84). Graduate students and young researchers from various areas including Taiwan, Norway, India, and Singapore, were provided with training in state-of-the-art research techniques. International conferences and courses are managed by the Office of International Cooperation (see page 79).

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. 74).

Strains of Japanese morning glory maintained in the center

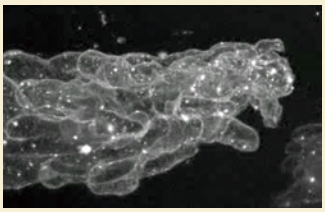
Outreach

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. We made a major revision of our English web page and opend a video gallery contaning an English language introduction of NIBB in 2011. NIBB also cooperates in the education of undergraduate and younger students through lectures and workshops. Outreach activities are managed by the Office of Public Relations (p. 79).

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 samples, and has developed an improved



A frame from a 3D movie of living *Amoeba proteus* first possible using DSLM due to its fast image taking capability.

model (p. 68). 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.

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 OBC8 "Speciation and Adaptation II: Environment and Epigenetics" was originally planned for March, 2011 but was postponed due to the earthquake and was held in March, 2012, which will appear in the 2012 Annual Report.

Cultivation of Future Researchers

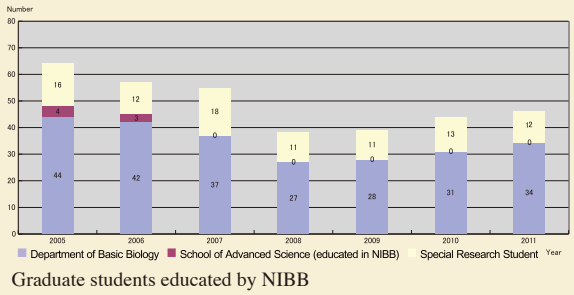
NIBB constitutes the Department of Basic Biology in the School of Life Science of the Graduate University for Advanced Studies (SOKENDAI). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree.

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 live an academic life and 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 are provided an opportunity to give oral and poster presentations at least once during their master's and doctoral program (p. 81).

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 (see page 85).



Graduate students educated by NIBB

Personnel changes in 2011*

Newly assigned in NIBB

Name	Position	Research Unit	Date
MATSUBAYASHI, Yoshikatsu	Professor	Division of Intercellular Signaling Biology	January 1
SHINOHARA, Hidefumi	Assistant Professor	Division of Intercellular Signaling Biology	February 1
TAKIZAWA, Kenji	NIBB Research Fellow	Division of Environmental Photobiology	February 1
OHTSUKA, Masanari	NIBB Research Fellow	Division of Brain Biology	February 1
CHAKRABORTY, Tapas	NIBB Research Fellow	Division of Molecular Environmental Endocrinology	February 1
KOYAMA, Hiroshi	Assistant Professor	Division of Embryology	April 1
YAMAMOTO, Yasuhiro	NIBB Research Fellow	Laboratory of Molecular Genetics for Reproduction	April 1
NAKAMURA, Yoshiaki	NIBB Research Fellow	Division of Germ Cell Biology	April 1
SATO, Yasufumi	NIBB Research Fellow	Division of Embryology	April 1
OSHIMA, Yusuke	NIBB Research Fellow	Laboratory for Spatiotemporal Regulations	April 1
KUBOYAMA, Kazuya	NIBB Research Fellow	Division of Molecular Neurobiology	May1
NAKAYASU, Tomohiro	NIBB Research Fellow	Laboratory of Neurophysiology	July 1
OTA, Ryoma	NIBB Research Fellow	Division of Developmental Genetics	December 1

Newly affiliated in other universities and institutes

Name	New Affiliation	Position	Date
OKUBO, Tadashi	Kitasato University	Lecturer	April 1
NAGAHAMA, Yoshitaka	Ehime University	Professor (specially appointed)	April 1
MATSUNAGA, Wataru	Nara Prefectural University	Assistant Professor	July 1
KAGEYAMA, Yuji	Kobe University	Associate Professor	October 1

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

Awardees in 2011

Name	Position	Award
OKAMOTO, Satoru KAWAGUCHI, Masayoshi	NIBB Research Fellow Professor	JSPP Plant and Cell Physiology Award for the Paper of Excellence (PCP Award)
TANAKA, Minoru	Associate Professor	The Commendation for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology
IGUCHI, Taisen	Professor	The Zoological Society of Japan Award
HIYAMA, Takeshi	Assistant Professor	Japan Neuroscience Society Young Investigator Award

Note: On the unit member lists from P. 8 all members who belonged to the unit during 2011 are listed irrespective of the length of the period they were members. Those appearing twice in the same list under different titles are those whose title was changed during 2011. The former title is indicated by an asterisk (*).

DIVISION OF CELL MECHANISMS

Professor
NISHIMURA, Mikio

Associate Professor
HAYASHI, Makoto

Assistant Professors:	*MANO, Shoji*
	YAMADA, Kenji
Technical Staff:	*KONDO, Maki*
NIBB Research Fellow:	*OIKAWA, Kazusato*
Postdoctoral Fellows:	*KANAI, Masatake*
	WATANABE, Etsuko
	TANAKA, Mina
	KAMIGAKI, Akane
	GOTO-YAMADA, Shino
Graduate Students:	*GOTO-YAMADA, Shino**
	NAKAI, Atsushi
	CUI, Songkui
	SHIBATA, Michitaro
Technical Assistants:	*NISHINA, Momoko*
	ARAKI, Masami
	SAITO, Miyuki
	NAKAYAMA, Tomomi
	HIKINO, Kazumi
	MATSUDA, Azusa
	YOSHINORI, Yumi
	YAMAGUCHI, Chinami
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 the 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 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.

Bioinfomatic 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 proposed that *PEX10* is essential for the maintenance of ER morphology and for biosynthesis of cuticular wax.

III. Identification of novel components essential for peroxisome biogenesis

To better understand peroxisome biogenesis, we isolated a number of *Arabidopsis* mutants having aberrant peroxisome morphology (*apem* mutants) based on them having a

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

different pattern of GFP fluorescence from the parent plant, GFP-PTS1, in which peroxisomes with normal sizes and numbers can be visualized with GFP.

Of these *apem* mutants, *APEM1* gene (whose defect causes the elongation of peroxisomes and mitochondria) encodes dynamin-related protein 3A, one member of the dynamin family. *APEM2* and *APEM4* (whose defects cause a decrease in the efficiency of protein transport) were revealed to encode proteins homologous to PEX13 and PEX12, respectively, and both proteins are components of the protein-translocation machinery on peroxisomal membranes.

In addition, we reported on characterization of other *apem* mutants, *apem3* and *apem9*. *APEM3* encodes Peroxisomal membrane protein 38, and its defect causes enlargement of peroxisomes. APEM9 is the plant-specific PEX that has a role in tethering the PEX1-PEX6 complex on peroxisomal membranes (Figure 1).

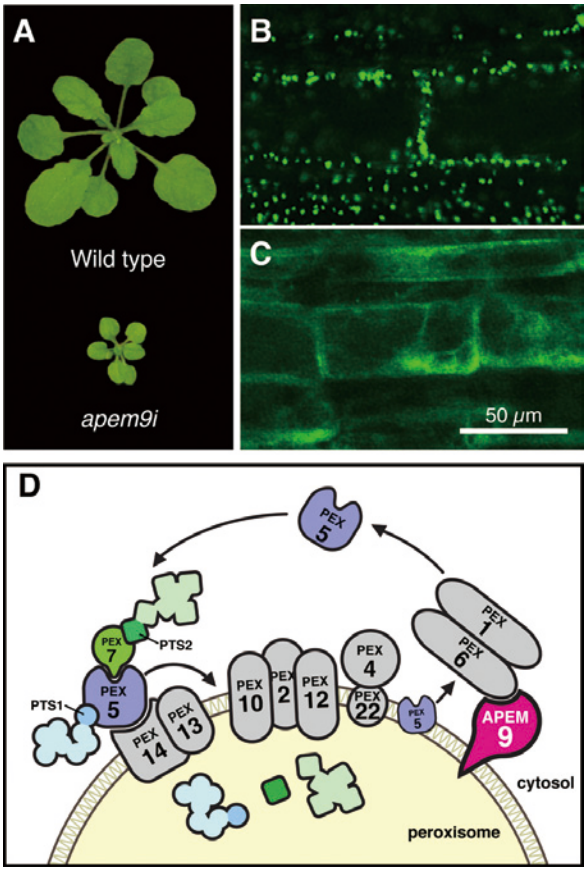


Figure 1. APEM9 is required for peroxisomal protein transport. (A) APEM9 knockdown mutants (*apem9i*) show growth defects. (B) Peroxisomal localization of GFP-PTS1 protein in wild type plants. (C) GFP-PTS1 protein is accumulated in the cytosol in *apem9i* mutants. (D) Working model of peroxisomal protein transport in plants, in which APEM9 tethers PEX1-PEX6 complex to the peroxisomal membrane.

We are currently analyzing the functions of other APEM proteins such as APEM10 and APEM11. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, and to address the mechanism at the molecular level.

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. Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid treatment. This suggests that ER bodies function in the defense against herbivores. ER bodies in seedlings include PYK10, a β-glucosidase with an ER retention signal. When plant cells are damaged, PYK10 forms large protein aggregates that include other β-glucosidases (BGLUs), GDSL lipase-like proteins (GLLs) and cytosolic jacalin-related lectins (JALs). The aggregate formation increases glucosidase activity, possibly producing toxic products (Figure 2). *Arabidopsis nai1* mutants have no ER bodies in the entire plant and do not accumulate PYK10. *NAI1* encodes a transcription factor that has a basic-helix-loop-helix (bHLH) domain 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. We found that the membrane protein of ER body 1 (MEB1) and MEB2 are integral membrane proteins of the ER body. NAI2 deficiency relocates MEB1 and MEB2 to the ER network. These findings indicate that NAI2 is a key factor that enables ER body formation. We are now investigating the function of NAI2 on ER body formation by heterologously expressing it in onion and tobacco cells.

V. Vacuoles responsible for programmed cell death in plants

The vacuolar processing enzyme (VPE) belongs to the cysteine protease family found in higher plants and animals. VPE is responsible for the maturation of various types of vacuolar proteins. We revealed a novel function of VPE in various instances of programmed cell death (PCD) in plants. VPE is identified as the proteinase that exhibits caspase-1

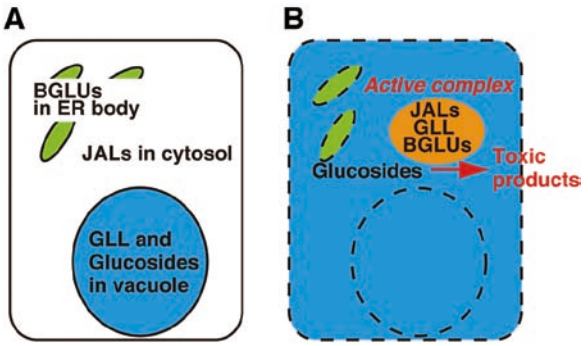


Figure 2. Activation model of β-glucosidases in ER bodies of *Arabidopsis*. (A) Healthy plants accumulate β-glucosidases (BGLUs) in the ER body, GDSL lipase-like proteins (GLLs) and glucosides in the vacuole, and jacalin-related lectins (JALs) in the cytosol. (B) When cell collapse occurs following herbivore or pathogen attack, β-glucosidases leak from the ER body and bind to JALs, which then form a large aggregate with increased glucosidase activity. This complex hydrolyzes glucosides to produce toxic compounds.

activity in plants. The plant hypersensitive response (HR), a type of defense strategy, constitutes well-organized PCD. No HR occurs on the tobacco mosaic virus-infected leaves of VPE-deficient tobacco plants. These results suggest that VPE is involved in vacuolar collapse, which triggers PCD.

Using inhibitors for caspase-3 and the proteasome (also known to affect animal cell death), we found that the activities of both are required for bacterium-induced cell death in plants. RNA interference-mediated silencing confirmed that one of the three *Arabidopsis* proteasome catalytic subunits, PBA1, is required for the fusion of the vacuolar and plasma membranes, which triggers PCD.

Plants evolve a death strategy mediated by vacuolar systems, which are not seen in animals. Interestingly, vacuoles are the key players in the plant-specific cell death system.

VI. 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. To clarify the roles of molecular chaperones on cell differentiation, we have purified and characterized chaperonin and HSP70s and analyzed their roles in the translocation of proteins into chloroplasts.

We found that HSP90 inhibitor induced 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 HSP90.2. 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. This data indicates 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.

VII. Update of The Plant Organelles Database 2 (PODB2) and release of Plant Organelles World

The Plant Organelles Database 2 (PODB2) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. PODB2 consists of four individual units: the organelles movie database, the organellome database, the functional analysis database, and external links. 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. The amount of included data is increasing day by day. We will add new content, which is dedicated to organelle movement and morphology in response to environmental stimuli, soon. It is expected that PODB2 will contribute to systems biology through the combination of the included data with other 'omics' data and computational analyses. In addition, we released a new website, Plant Organelles World, which is based on PODB2 as an educational tool to engage members of the non-scientific community. We expect that PODB2 and Plant Organelles World will enhance the understanding of plant organelles among researchers and the general public who want to explore plant biology.

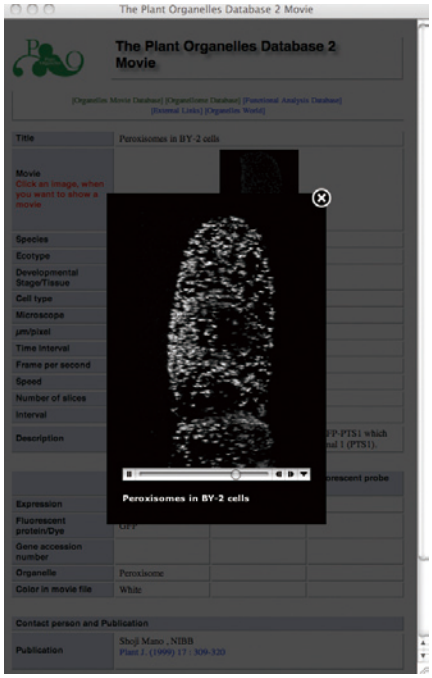


Figure 3. The graphical user interface of the organelles movie database in PODB2 (http://podb.nibb.ac.jp/Orgenellome).

Publication List

〔Original papers〕

- Goto, S., Mano, S., Nakamori C., and Nishimura, M. (2011). *Arabidopsis* ABERRANT PEROXISOME MORPHOLOGY 9 is a peroxin that recruits the PEX1-PEX6 complex to peroxisomes. Plant Cell *23*, 1573-1587.
- Hino, T., Tanaka, Y., Kawamukai, M., Nishimura, K., Mano, S., and Nakagawa, T. (2011). Two Sec13p homologs, AtSec13A and AtSec13B, redundantly contribute to formation of COPII transport vesicles in *Arabidopsis thaliana*. Biosci. Biotechnol. Biochem. *75*, 1848-1852.
- Mano, S., Miwa, T., Nishikawa, S., Mimura, T., and Nishimura, M. (2011). The Plant Organelles Database 2 (PODB2): An updated resource containing movie data of plant organelle dynamics. Plant Cell Physiol. *52*, 244-253.
- Mano, S., Nakamori, C., Fukao, Y., Araki, M., Matsuda, A., Kondo, M., and Nishimura, M. (2011). A defect of peroxisomal membrane protein 38 causes enlargement of peroxisomes. Plant Cell Physiol. *52*, 2157-2172.
- Masuda, S., Harada, J., Yokono, M., Yuzawa, Y., Shimojima, M., Murofushi, K., Tanaka, H., Masuda, H., Murakawa, M., Haraguchi, T., Kondo, M., Nishimura, M., Yuasa, H., Noguchi, M., Oh-oka, H., Tanaka, A., Tamiaki, H., and Ohta, H. (2011). A monogalactosyldiacylglycerol synthase found in the green sulfur bacterium *Chlorobaculum tepidum* reveals important roles for galactolipids in photosynthesis. Plant Cell *23*, 2644-2658.

〔Review Article〕

- Yamada, K., Hara-Nishimura, I., and Nishimura, M. (2011). Unique defense strategy by the endoplasmic reticulum body in plants. Plant Cell Physiol. *52*, 2039-2049.

DIVISION OF INTERCELLULAR SIGNALING BIOLOGY

Professor
MATSUBAYASHI, Yoshikatsu

Assistant Professor:	*SHINOHARA, Hidefumi*
Visiting Scientists:	*MORI, Ayaka*
	YOSHII, Tomoaki
Technical Assistants:	*OGAWA-OHNISHI, Mari*
	SUMIDA, Kumiko
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 "small post-translationally modified 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 Root meristem growth factor (RGF)

Root meristem growth factor (RGF) is a 13-amino-acid tyrosine-sulfated peptide involved in maintenance of the root stem cell niche in *Arabidopsis* identified by our group in 2010. RGF was identified in a search for sulfated peptides that recover root meristem defects of the loss-of-function mutant of tyrosylprotein sulfotransferase (*tpst-1*). TPST is a post-translational modification enzyme that catalyzes tyrosine sulfation of secreted peptides and proteins. This approach is based on the assumption that the severe short root phenotype of the *tpst-1* mutant reflects deficiencies in the biosynthesis of all the functional tyrosine-sulfated peptides, including undiscovered peptide signals. RGFs are produced from ≈100-amino-acid precursor peptides via post-translational sulfation and proteolytic processing. RGF family peptides are expressed mainly in the stem cell area and the innermost layer of central columella cells, and diffuse into the meristematic region. RGF peptides regulate root development by stabilizing PLETHORA transcription factor proteins which are specifically expressed in root meristem and mediate patterning of the root stem cell niche (Figure 1).

To gain more insight into RGF signaling, we have developed a positive screening system to identify *Arabidopsis* mutants with altered response to RGF peptides. Several mutants that are less sensitive to RGF have been identified and are currently being further analyzed.

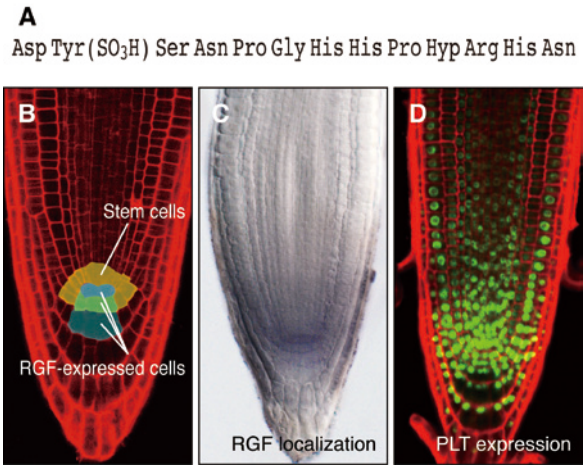


Figure 1. Mode of action of RGF peptide. (A) Structure of RGF1. (B) Expression domain of *RGF* genes. (C) Localization of RGF peptides visualized by anti-RGF1 antibody. (D) Expression pattern of PLETHORA (PLT2) transcription factor, a downstream target of RGF signaling.

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 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. Post-translational modification mechanisms

Post-translational modifications are known to affect peptide conformation through steric interactions with the peptide backbone, thereby modulating the binding ability and specificity of peptides for target receptor proteins. To date, the following types of post-translational modification have been identified in secreted peptide signals in plants: tyrosine sulfation and hydroxyproline arabinosylation (Figure 2).

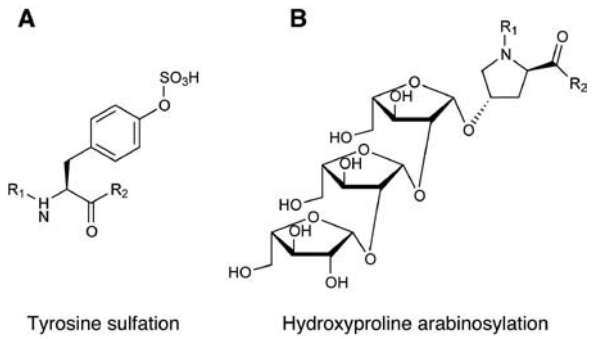


Figure 2. Post-translational modifications in secreted peptide signals in plants

2-1 Tyrosine sulfation

Arabidopsis tyrosylprotein sulfotransferase (AtTPST) is a Golgi-localized 62-kDa transmembrane protein identified by our group in 2009. AtTPST is expressed throughout the plant body, and the highest levels of expression are observed in the root apical meristem. A loss-of-function mutant of *AtTPST* (*tpst-1*) displayed a marked dwarf phenotype accompanied by stunted roots, loss of root stem cells, pale green leaves and early senescence, indicating the important roles of sulfated peptides in plant growth and development. Three known sulfated peptide signals, PSK, PSY and RGF, can almost fully restore root defects of *tpst-1* when added to the culture medium, but can not fully restore phenotypes in the above-ground parts of the plant. This observation suggests that as yet undiscovered sulfated peptides may regulate plant development. A search for novel sulfated peptide signals is now in progress.

2-2 Hydroxyproline arabinosylation

Hyp residues in several secreted peptide signals, such as CLV3 and CLE2 are further modified with an *O*-linked L-arabinose chain. This modification is physiologically important for these peptide signals. Biosynthesis of Hyp-bound β-1-2-linked triarabinoside involves two distinct arabinosyltransferases. The first is responsible for the formation of a β-linkage with the hydroxyproline (hydroxyproline arabinosyltransferase), and the second forms a β-1-2-linkage between arabinofuranose residues (arabinosyltransferase). Arabinosyltransferase has already been reported, but there have been no reports on hydroxyproline arabinosyltransferase (HPAT). We have established an *in vitro* assay system to detect HPAT activity and are currently attempting to purify this enzyme by affinity chromatography.

2-3 Chemical synthesis of arabinosylated peptides

Although extensive efforts have been devoted to the synthesis of various glycopeptides, a general and stereoselective synthetic route for arabinosylated peptides with β-1-2-linkage has yet to be developed. To address this issue we are attempting to synthesize β-1-2-linked tri-arabinosylated glycopeptides in a stereoselective manner.

III. 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.

3- 1 Receptor expression library

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 in the above section, 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.

3- 2 Structural basis for ligand recognition

The identification of ligand-binding sites within receptor ectodomains provides valuable structural information important for understanding the basis of ligand recognition and signal transduction. We are analyzing how large consecutive LRRs specifically recognize small peptide ligands by focusing on CLV1/BAM family receptor kinases as a model.

Publication List

〔Review articles〕

- Matsubayashi, Y. (2011). Post-translational modifications in secreted peptide hormones in plants. Plant Cell Physiol. *52*, 5-13.
- Matsubayashi, Y. (2011). Small post-translationally modified peptide signals in *Arabidopsis*. The Arabidopsis Book *9*, e0150.

LABORATORY OF NEURONAL CELL BIOLOGY

Associate Professor
SHIINA, Nobuyuki

Assistant Professor: *NAKAYAMA, Kei*
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 localized protein synthesis in dendrites 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. We are researching factors regulating mRNA transport and local translation, their target mRNAs, and the mechanisms 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.

I. Regulation of transport and local translation of dendritically-targeted mRNAs

Specific mRNAs are recruited into "RNA granules" in neuronal dendrites. RNA granules are macromolecular complexes composed mainly of mRNAs and ribosomes, and mediate the transport and local translation of their mRNA cargoes in response to synaptic stimulation (Figure 1).

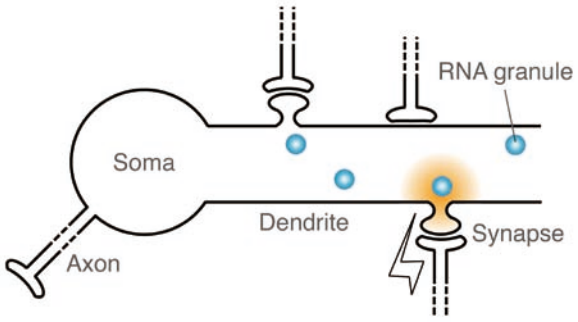


Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

We previously identified RNA granule protein 105 (RNG105), 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).

To understand the regulation of transport and local translation of dendritically-targeted mRNAs, we have identified RNG105 cargo mRNAs in neurons. The cargo mRNAs, e.g., that encode Na^+/K^+ ATPase subunit isoform protein (FXYD1), are recruited to RNA granules and transported to dendrites. Furthermore, translation of the mRNAs is upregulated by stimulation of neurons with brain-derived neurotrophic factor (BDNF) which mimics synaptic stimulation (Figure 2). We are currently investigating the molecular mechanism of the mRNA transport to dendrites and BDNF-stimulated translational upregulation of the cargo mRNAs.

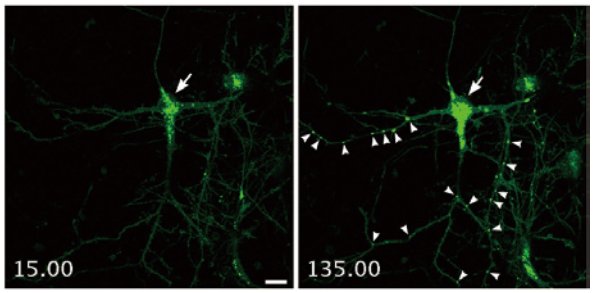


Figure 2. Translation of FXYD1-GFP is activated by BDNF in mouse cultured hippocampal neurons. Numbers indicate time (minute) after BDNF stimulation. FXYD1-GFP is increased in membrane compartments in the cell body (an arrow) and dendrites (arrowheads) after BDNF stimulation (right panel). Scale bar, 10 μm.

II. Molecular characterization of the RNA granule complex

RNA granules are abundantly formed in neurons, but not observed in many other types of cells. However, the formation of RNA granules is induced by stress such as oxidation in the cells. They are called "stress granules" and have common features with neuronal RNA granules, e.g., they are macromolecular complexes containing ribosomes and mRNAs, and repress translation of cargo mRNAs without stimulation. We previously found that RNG105 is a component of not only neuronal RNA granules but also stress granules and further that overexpression of RNG105 in fibroblastic cells induced the formation of granules which contain mRNAs.

To identify and characterize molecular components of RNA granules, we have performed mass spectrometric analysis of the RNG105-induced complex. A cultured cell line A6 transfected with RNG105-green fluorescent protein (RNG105-GFP) forms prominent granules in the cytoplasm (Figure 3). The transfected cells are subjected to immunoprecipitation with an anti-GFP antibody (Figure 3). Proteins co-precipitated with RNG105-GFP are then analyzed by mass spectroscopy, which revealed that several proteins responsible for RNA metabolism, membrane transport, posttranslational modification, etc. are contained in the complex. We are currently investigating the localization and function of the proteins in stress granules as well as in neuronal RNA granules.

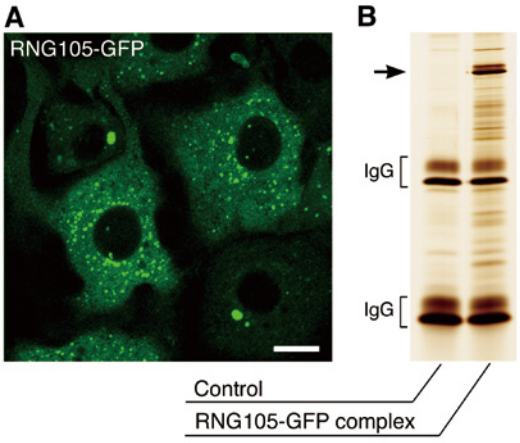


Figure 3. Identification of components of RNG105-induced granules. (A) A6 cells expressing RNG105-GFP. RNG105-GFP induced the formation of cytoplasmic granules. Scale bar, 10 μm. (B) SDS-PAGE of immunoprecipitants with an anti-GFP antibody from control A6 cells (left lane) and A6 cells expressing RNG105-GFP (right lane). The positions of RNG105-GFP (an arrow) and immunoglobulin (IgG) are indicated.

III. Knockout mice for RNA granule proteins

We previously generated RNG105 knockout mice. Neurons from the knockout mice exhibited reduced dendritic synapse formation and reduced dendritic arborization, which resulted in poor development of neuronal networks. The knockout neonates died soon after birth due to respiratory failure, which was associated with defects in fetal brainstem development. To investigate the role of RNG105 in higher brain functions, e.g., memory and learning, in adult mice, we have generated conditional RNG105 knockout mice using the Cre/loxP system. Alpha-CaMKII promoter is used to drive Cre recombinase since its promoter activity is low during embryonic stages but elevated after birth in the brain. However, even though the Cre/loxP system is used, we have not obtained adult RNG105 knockout mice. Therefore we are planning to generate drug-inducible RNG105 knockout mice.

RNG105 has one paralog, RNG140, 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 are going to investigate the role of RNG140 in higher brain functions in adult mice.

LABORATORY OF CELL STRUCTURE †

Associate Professor
OGAWA, Kazuo

Microtubules are polymers of α- and β-tubulin heterodimer. *In vitro*, we can induce microtubule formation at critical points where tubulins concentrate. *In vivo*, they are formed by γ-TuRC at the centrosome or may be formed by unknown protein(s) in the non-centrosome region. The faster growing end is defined as the plus-end of the microtubules. There are many plus-end tracking proteins of microtubules such as EB-1, CLIP-170, and dynactin et al. The minus-ends are considered to be the nucleation sites for microtubule polymerization. Microtubule nucleation at the non-centrosome region remained less clear.

Several antibodies were raised for answering this unsolved question and checked whether they stain the minus-end of microtubules by immunofluorescence microscopy. For simplicity, the primary cilia of three cell lines established in our laboratory were used instead of microtubules. If antibodies are able to react with the minus end of microtubules, they should bind to the base of primary cilia in the same way as γ-TuRC and antigens for those antibodies should exist there.

Finally one anti-serum successfully stained the bases of the primary cilia of cultured cells examined so far (Figure 1). I expect further interesting results will be forthcoming.

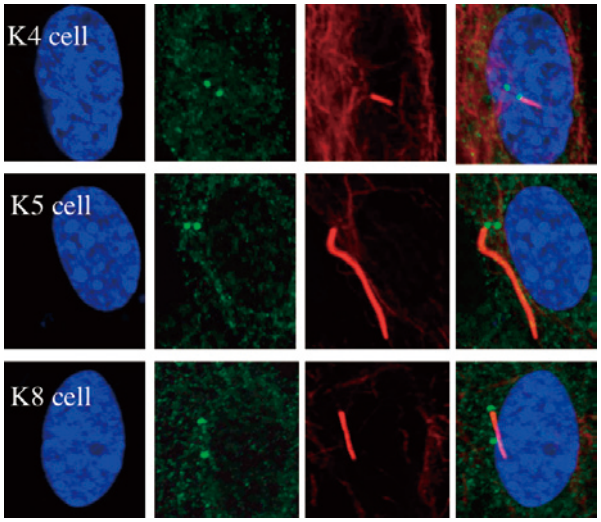


Figure 1. Antibodies stained the minus-end of the primary cilia. Green, candidate protein; red, acetylated tubulin (primary cilia); blue, DNA stained with DAPI.

†: This laboratory was closed on 31 March, 2012.

LABORATORY OF CELL SOCIOLOGY

Assistant Professor
HAMADA, Yoshio

Technical Assistants: *GONDA, Naoko*

Animal organs are made up of several types of cells, and organized in an orderly fashion wherein the proportion of each cell type is constantly maintained. This orderly cell arrangement and proportion are built up during organogenesis by cell-cell interactions. Since it has been postulated that *Notch* plays a role in cell fate decisions by mediating cell-cell interactions, we are endeavoring to discover the cellular and molecular mechanisms at work during organogenesis by studying the function of *Notch*.

Organogenesis of the mouse placenta occurs during early pregnancy, embryonic days 7-9, before the establishment of molecular transport mechanisms in the definitive placenta takes place. Trophoblasts not adjacent to the inner cell mass differentiate into trophoblast giant cells and lie at the outside, forming an interface with the maternal deciduas. The polar trophectoderm gives rise to the cells of the chorion as well as the ectoplacental cone; these produce the labyrinthine and spongiotrophoblast layers, respectively. While maternal red blood cells begin to perfuse into trophoblast cell layers and reach the labyrinthine layer by E9.5, the invasion of embryonic allantoic mesenchyme into the labyrinthine layer and the differentiation of fetal red blood and endothelial cells which line the fetal capillary take place around E9.5.

The *Notch2* null mutation results in embryonic lethality by embryonic day 11.5 due to the formation of poor maternal vascular beds. The mutant placenta shows a normal invasion of angiogenic allantoic mesenchyme followed by premature formation of fetal blood vessels in the mutant placentas as early as E9.0. However, the specification of trophoblast subtypes appears not to be drastically disturbed. Thus, in the developing mouse placenta, *Notch2* is likely not involved in cell fate decisions, but rather participates in the formation of circulatory systems in the labyrinth layer where the expression of *Notch2* is detected. Although inadequate formation of maternal vascular beds was partially restored by aggregating mutant diploid embryos with wild type tetraploid embryos, networks of maternal vascular beds appeared still compromised in the 4N chimeric placenta. These results indicate that *Notch2* promotes vasculogenesis.

How maternal vascular beds are formed in the developing mouse placenta has yet to be explored. The simplest way to form the beds among tightly adhered labyrinthine trophoblasts is through cell death. We studied a spatiotemporal appearance of dead cells in the developing placenta. While vasculogenesis does not occur in the presumptive labyrinth layer at E8.5, some dying cells were detected. At E9.5, extensive trophoblast cell death took place around newly forming maternal blood beds. In contrast to the wild type placenta, extensive cell death did not occur in the E9.5 mutant placenta (Fig. 1). It is likely that *Notch2* plays a role in vasculogenesis through being involved in the process of trophoblast cell death. We are now carrying out studies on how Notch2 participates in the cell death process and how the gene is activated in the trophoblast in cell culture.

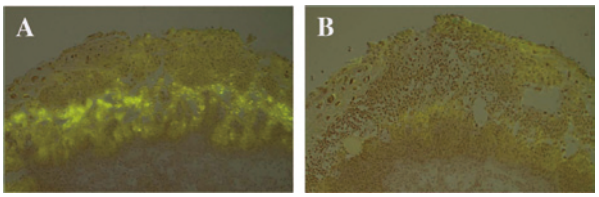


Figure 1. Programmed cell death in the developing mouse placenta. Dying trophoblast was visualized by staining with a fluorescent dye at E9.5. Wild type placenta showed extensive cell death around newly forming maternal blood beds which were surrounded by Notch2 expressing trophoblast (A), but cell death was scarce in the mutant placenta.

DIVISION OF MORPHOGENESIS

Professor
UENO, Naoto

Associate Professor
KINOSHITA, Noriyuki

Assistant Professors:	*TAKAHASHI, Hiroki*
	SUZUKI, Makoto
Technical Staff:	*TAKAGI, Chiyo*
NIBB Research Fellow:	*HASHIMOTO, Masakazu**
Postdoctoral Fellows:	*HASHIMOTO, Masakazu*
	MORITA, Hitoshi
	KAI, Masatake
Graduate Students:	*MORITA, Hitoshi**
	HARA, Yusuke
	TAKEDA, Taro
	MIYAGI, Asuka
	HAYASHI, Kentaro
Technical Assistants:	*YAMAMOTO, Takamasa*
	MURAKAMI, Michiyo
	SUZUKI, Atsuko
Secretaries:	*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. Biological significance of force for morphogenesis

Physical forces are a non-negligible environmental factor that can guide the morphogenesis of organisms. Such forces are generated by tissue-tissue interactions during early development where drastic tissue remodeling occurs. One good example is neural tube formation. In vertebrates, the neural tube that is the primordial organ of the central nervous system and is formed by the bending of the neural plate that is a flat sheet of neuroepithelial cells. The tissue remodeling is driven by cellular morphogenesis in which selected cells in the neural plate change their shapes from cuboidal to an elongated wedge-like shape. Recent studies have revealed that this cell shape change is controlled by cytoskeletal dynamics, namely the remodeling of F-actin and microtubules. On the other hand, we also know that such cell shape change is necessary but not sufficient to cause complete neural tube closure because an embryo with an absence of these cell shape changes will still display significant neural plate bending. We reasoned that other forces to bend the neural plate and contribute to the complete tube closure are generated by tissue(s) outside of the neural plate, possibly in the non-neural ectoderm. After a series of microscopic observations and experiments using *Xenopus laevis*, we have found that non-neural deep layer cells that underlie the non-neural ectoderm migrate actively toward the dorsal side dragging the overlying superficial layer cells and bringing them to the midline (Figure 1).

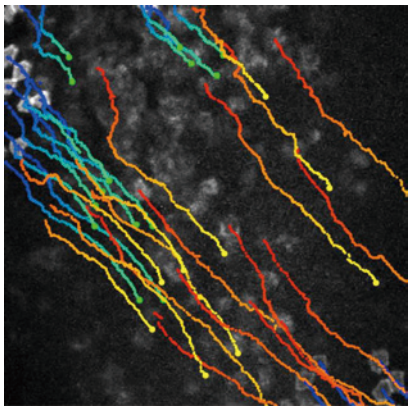


Figure 1. Tracking of cell movement of the non-neural ectoderm. Deep cells, labeled red/orange, migrate faster than superficial cells, labeled blue/green. (observation with DSLM).

This has been confirmed by the observation that disruption of deep cell migration by inhibiting the cell-substrate interaction between mesoderm and deep cells, or cell-cell interaction between deep cells and superficial cells, both of which are the basis of traction forces, caused neural tube closure defects. This study highlights the importance of physical force during complex organ formation (Figure 2).

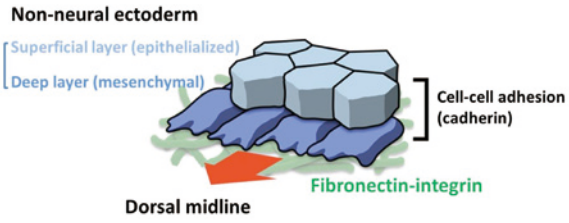


Figure 2. Model for the coordinated movement of non-neural ectoderm. Deep cells (dark blue) migrate on the fibronectin ahead of superficial cells and therefore the superficial cells are dragged toward the midline. This also causes a stretching force for passive shape change of superficial cells.

Another example is the axial mesoderm, which elongates along the anterior-posterior axis during gastrulation cell movements by which rearrangements of the three germ layers is driven. The axial mesoderm is led by the anteriorly precedent tissue Leading Edge Mesoderm (LEM). When surgically isolated the LEM migrates fairly rapidly toward the predetermined anterior side, while the following axial mesoderm shows little directed tissue migration. We hypothesized that the LEM generates traction force on the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

following axial mesoderm. To prove the biological significance of the force generated by the LEM, we have estimated the magnitude of the traction force by measuring displacement of a glass needle with a known spring constant by the anterior movement of the axial mesoderm. Furthermore, we have been examining whether the artificial application of force, for example by tissue-stretching, can cause biological outputs such as intracellular relocalization of signaling molecules or formation of cell polarity.

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

During gastrulation, dorsal mesoderm cells migrate toward the midline and align along the anteroposterior axis to form the notochord. In this process, cells show polarized morphological change and coordinated cell migration. To achieve this systematic cell movement, cell-to-cell interaction must be tightly regulated. We focus on one of the cell adhesion proteins, paraxial protocadherin (PAPC). PAPC has been shown to be involved in gastrulation cell movements during early embryogenesis. Using *Xenopus* embryos, we found that both knock down and overexpression of PAPC in the dorsal mesoderm impair mesoderm cell movement. PAPC knock down reduced cell-cell interaction and cells migrate disorderly. PAPC overexpression inhibited cell migration toward the midline. This suggests that PAPC is essential at the early stage and then must be decreased. It has been reported that the PAPC gene is first expressed in the dorsal marginal zone at the early gastrula stage and subsequently down-regulated in *Xenopus* and zebrafish. We found that PAPC is also regulated in the same way at the protein level (Figure 3). PAPC is degraded via lysosome and excluded from the plasma membrane in the axial mesoderm at the late gastrula stage. PAPC is phosphorylated by GSK3, providing a signal for poly-ubiquitination by β-TrCP. Localization of PAPC to the plasma membrane at the early gastrula stage is mediated by a novel deubiquitinating enzyme XTI3. Taken together, our findings suggest a novel mechanism of regulation of a cell adhesion protein by the ubiquitin system, which plays a crucial role in vertebrate embryogenesis.

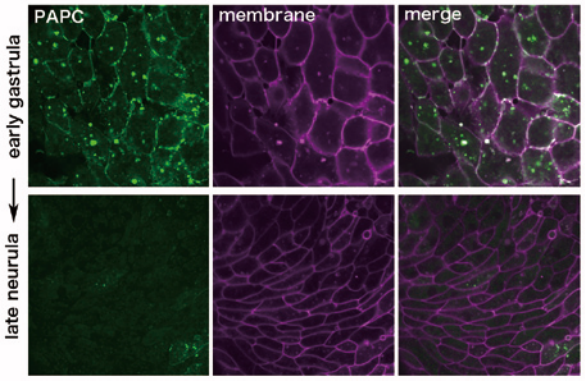


Figure 3. The dynamic regulation of PAPC stability during convergent extension. PAPC-GFP is expressed with membrane-tethered RFP in dorsal mesoderm cells. At the early gastrula stage, PAPC localized at the cell membrane and vesicles, but was drastically reduced later at the neurula stage.

III. Cellular morphogenesis during development

For the morphogenesis of organs, cellular morphogenesis as well as cellular behaviors play critical roles, as discussed above for neural tube formation of *Xenopus laevis*. Unlike *Xenopus* and amniote such as chicks and mammals, however, some of the teleost fish display a unique way of forming the neural tube in that rather than bending of a neuroepithelial sheet, they adopt drastic cell rearrangement of neural cell progenitors. During zebrafish neurulation, for example, highly motile cells converge to the midline, elongate their shape mediolaterally, and interdigitate with contralateral cells to form the neural rod in a highly coordinated manner. To date, although non-canonical, the Wnt/PCP pathway and several cadherins have been reported to function in this process, how the cytoskeleton controls these cellular behaviors is poorly understood.

We analyzed non-muscle myosin II (NMII) activity focusing on its regulatory light chain (MRLC) during zebrafish neurulation. Our analysis revealed that activated MRLCs were enriched in the cortex of highly migratory neural cells, suggesting that NMII functions in zebrafish neurulation. Consistent with this, inhibition of NMII suppressed cell elongation and interdigitation. Then we performed live-imaging analysis with GFP-tagged MRLC and found that its mutants that mimic the active forms were accumulated in the cell cortex as foci. These foci were dynamically formed and coalesced during cell elongation and interdigitation. We further found that these positioned proximal to cell protrusion and partially colocalized with adherens junction and tight junction proteins. These data suggest that NMII containing phosphorylated MRLC controls local cell-cell adhesion as intercellular linkage, which positively regulates highly coordinated cell shape changes in zebrafish neurulation.

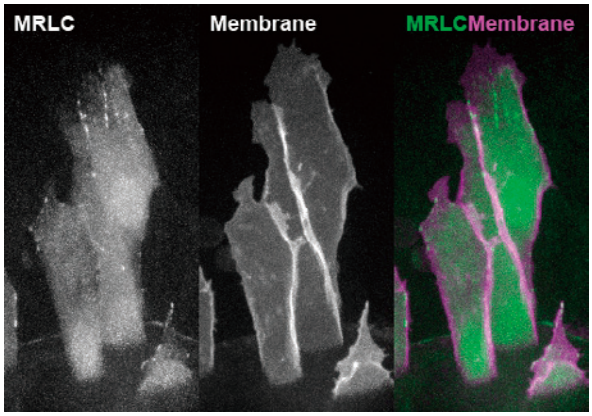


Figure 4. By injecting in vitro transcribed mRNA, we observed active MRLC-GFP foci at the single cell level. During neural cells undergoing convergence and cell elongation processes, these foci were dynamically formed in the cell cortex and coalesced, sometimes resulting filamentous structures, which seems more stable than cell protrusive activity in the distal region. (Left) MRLC-GFP, (middle) membrane-RFP, (right) merge images of elongating neural cell to medial side of the neural plate.

IV. Notochord and evolution of chordates

The early embryogenesis of amphioxus, up to the late neurula stage, provides us with useful suggestions about chordate origins and evolution. Its cleavage pattern, resulting in the formation of hollow blastula, resembles those of sea cucumbers (echinoderms) and acorn worms (hemichordates). In contrast to the mode of non-chordate deuterostome gastrulation, in which the archenteron invaginates into a wide blastocoelic space and causing the embryo to become cup-shaped, with a deepened archenteron. This mode of gastrulation is seen in ascidian embryos as well. By the late gastrula stage, the embryo has become ovoid and slightly flattened, and the neural plate is formed from the flattened dorsal side of the embryo. As in vertebrates, neurulation begins with enclosure of the neural plate.

During the period of neural tube formation, the notochord develops from the adjacent chordamesodermal plate that constitutes the roof of the archenteron (Figure 5). Specifically, the notochord is formed by pounching off from the archenteron. Interestingly, *Brachyury* is expressed not only in the region where the notochord pouches off, but also in the region where the somite pouches off. Furthermore, the amphioxus notochord has properties of muscle tissue. The morphogenetic movement of the notochord (and somites) in cephalochordate embryos looks like a continuation of the archenteron invagination. In other words, amphioxus might have recruited the secondary *Brachyury* expression for this second invagination-like morphogenetic movement.

By investigating the cellular morphogenesis and remodeling of tissues underlying amphioxus development and by gaining a comparative developmental biology view of the events, specifically relevant to notochord development, we aim to obtain new insights into the evolutionary changes that took place in a branch of the bilaterian lineage and gave rise to the chordates more than 550 million years ago.

Figure 5. Amphioxus embryos (Branchiostoma floridae). Side views. Anterior at left. (A) Mid-neurula stage embryo. (B) Early larval stage embryo. The notochord runs through the dorsal side by reaching to the anterior tip of the body. Embryos were stained with Alexa 488 phalloidine (green) and CellMask (red and magenta).

Publication List

〔Original papers〕

- Endo, T., Ueno, K., Yonezawa, K., Mineta, K., Hotta, K., Satou, Y., Yamada, L., Ogasawara, M., Takahashi, H., Nakajima, A., Nakachi, M., Nomura, M., Yaguchi, J., Sasakura, Y., Yamasaki, C., Sera, M., Yoshizawa, A., Imanishi, T., Taniguchi, H., and Inaba, K. (2011). CIPRO 2.5: *Ciona intestinalis* protein database, a unique integrated repository of large-scale omics data, bioinformatic analyses and curated annotation, with user rating and reviewing functionality. Nucleic Acids Res. *39*, D807-D814.
- Takebayashi-Suzuki, K., Kitayama, A., Terasaka-Iioka, C., Ueno, N., and Suzuki, A. (2011). The forkhead transcription factor FoxB1 regulates the dorsal-ventral and anterior-posterior patterning of the ectoderm during early *Xenopus* embryogenesis. Dev Biol. *360*, 11-29.
- Tao, H., Manak, J. R., Sowers, L., Mei, X., Kiyonari, H., Abe, T., Dahdaleh, N. S., Yang, T., Wu, S., Chen, S., Fox, M. H., Gurnett, C., Montine, T., Bird, T., Shaffer, L. G., Rosenfeld, J. A., McConnell, J., Madan-Khetarpal, S., Berry-Kravis, E., Griesbach, H., Saneto, R. P., Scott, M. P., Antic, D., Reed, J., Boland, R., Ehaideb, S. N., El-Shanti, H., Mahajan, V. B., Ferguson, P. J., Axelrod, J. D., Lehesjoki, A. E., Fritzsch, B., Slusarski, D. C., Wemmie, J., Ueno, N., and Bassuk, A. G. (2011). Mutations in prickle orthologs cause seizures in flies, mice, and humans. Am. J. Hum. Genet. *88*, 138-149.
- Yamada, S., Ueno, N., Satoh, N., and Takahashi, H. (2011). *Ciona intestinalis* Noto4 contains a phosphotyrosine interaction domain and is involved in the midline intercalation of notochord cells. Int. J. Dev. Biol. *55*, 11-18.

DIVISION OF DEVELOPMENTAL GENETICS

Professor
KOBAYASHI, Satoru

Assistant Professors:	*HAYASHI, Yoshiki*
	SATO, Masanao
Assistant Professor (Specially Appointed):	
	KAGEYAMA, Yuji
Technical Staff:	*NODA, Chiyo*
NIBB Research Fellows:	*HASHIYAMA, Kazuya*
	OTA, Ryoma
Postdoctoral Fellows:	*FUJISAWA, Chiemi*
	INAGAKI, Sachi
Graduate Students:	*KUBO, Satoru*
	SUGIYAMA, Arisa
	SHINOZUKA, Yuko
Visiting Scientists:	*FUJISAWA, Toshitaka*
	OHARA, Yuya
Technical Assistants:	*SATO, Kaori*
	ISHIHARA, Hitomi
	NIIMI, Kaori
	YAMAMOTO, Manami
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, germline sex determination and GSC niche function 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. Here, we describe a genome-wide survey of maternal transcripts that encode for transcription factors and are enriched in the germ plasm. We isolated pole cells (primordial germ cells; PGCs) from blastodermal embryos by fluorescence-activated cell sorting (FACS) and then used these isolated cells in a microarray analysis. Among the 835 genes in the Gene Ontology (GO) category "transcription regulator activity" listed in FlyBase, 68 were found to be predominantly expressed in PGCs as compared to whole embryos. As the early PGCs are known to be transcriptionally quiescent, the listed transcripts are predicted to be maternal in origin. Our *in situ* hybridization analysis revealed that 27 of the 68 transcripts were enriched in the germ plasm. Among the 27 transcripts, six were found to be required for germline-specific gene expression of *vasa* and/or *nanos* by knockdown experiments using RNA interference (RNAi).

Among the 6 transcripts, we focused on *ovo*. The *ovo* gene encodes for 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 PGCs during embryogenesis. To understand its function, we over-expressed the Ovo-A repressor only in 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, which thereby causes sterility in both female and male adults. Thus, maternal Ovo-B has an essential role in germline development in both sexes.

II. Mechanism regulating sex determination of PGCs

"Sex reversal" leads to infertility, because the soma and germ cells become incompatible; male gonads are not able to deliver eggs, and vice versa. Various genetic conditions result in sex change in the soma, but in these cases germ cells retain their original gender, and how sex is determined in germ cells has remained unclear. It is widely accepted in mammals and *Drosophila* that male sexual development is imposed in PGCs by the sex of the gonadal soma, and that PGCs assume a female fate in the absence of a masculinizing environment. How PGCs initiate female development, however, is a long-standing question in reproductive and developmental biology.

In the soma, sex determination is controlled by the *Sex lethal (Sxl)* gene, which is first expressed at the blastodermal stage. *Sxl* encodes an RNA binding protein involved in alternative splicing and translation. In the soma of female (XX) embryos, it functions through *transformer* (*tra*) and *transformer-2* (*tra-2*), which in turn regulate alternative splicing of the *doublesex* (*Dsx*) gene to produce a female-specific form of Dsx. In male (XY) embryos, this pathway is turned off and a male-specific form of Dsx is produced by default. These Dsx proteins determine the sexual identity of somatic tissues. Previous reports, however, suggested that *Sxl* does not induce female sexual development in the germline, as it does in the soma. Although *Sxl* is autonomously required for female sexual development, constitutive mutations in *Sxl* (Sxl^{M}) that cause XY animals to undergo sexual transformation from male to female does not necessarily interfere with male germline development. Moreover, *tra*, *tra-2*, and *dsx* are not required for female germline development. Finally, female-specific *Sxl* expression has been detected later in gametogenesis, but not in early germline development.

Contrary to the previous observations, we found that *Sxl* was expressed in XX, but not XY PGCs, during their migration to the gonads. Furthermore, we found that the Sxl^{M1} mutation does not result in *Sxl* expression in XY PGCs,

as early as in XX PGCs. To determine whether *Sxl* induces female development in XY PGCs, we then induced *Sxl* expression in XY PGCs using *nanos-Gal4* and *UAS-Sxl*. We transplanted three types of XY PGCs (Figure 1), each characterized by a different duration of *Sxl* expression: (i) XY PGCs in which *Sxl* was expressed from stage 9 until stage 16/17 using maternal *nanos-Gal4* (XY-mSxl), (ii) XY PGCs in which *Sxl* was expressed from stage 15/16 onward using zygotic *nanos-Gal4* (XY-zSxl), and (iii) XY PGCs in which *Sxl* was expressed from stage 9 onward using both maternal and zygotic *nanos-Gal4* (XY-mzSxl). We found that XY-mzSxl and XY-mSxl PGCs entered the oogenic pathway and produced mature oocytes in XX females. These oocytes contributed to progeny production. In contrast, XY-zSxl PGCs did not enter the oogenic pathway. These observations demonstrate that *Sxl* expression in XY PGCs during embryogenesis induces functional egg differentiation in the female soma.

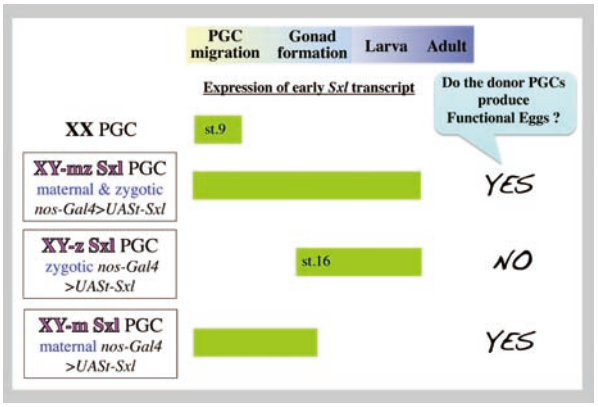


Figure 1. Expression of *Sxl* is sufficient to induce female fate in XY PGCs.

In contrast, *Sxl*-specific double-stranded RNA (*UAS-Sxl*^{RNAi}) under the control of maternal *nanos-Gal4* was then used to reduce *Sxl* activity in XX PGCs during embryogenesis. Introducing *UAS-Sxl*^{RNAi} resulted in tumorous and agametic phenotypes in female adults, indicating that the XX germline lost female characteristics.

Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. XX PGCs initiate female sexual identity based on their *Sxl* expression, while, lacking *Sxl* expression in XY PGCs, male sexual fate occurs primarily by a signal from gonadal soma (Figure 2). One remarkable example of germline-autonomous regulation of sexual dimorphism has been reported in a primitive animal, cnidarian *Hydra*. It has long been known that sex of the germline is not influenced by the surrounding soma, and the germline, rather than soma, determines the phenotypic sex of the polyp. Thus, we speculate that germline-autonomous regulation of sex is a primitive trait conserved throughout the evolution of animals.

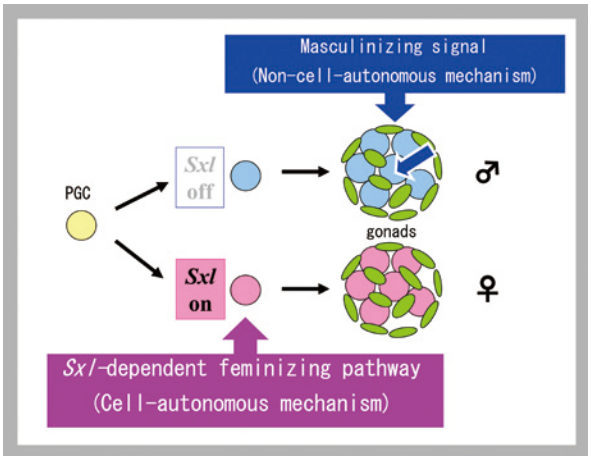


Figure 2. A model for the regulation of sexual dimorphism in pole cells.

III. The role of heparan sulfate proteoglycans in the germline-stem-cell niche

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 germline-stem-cell (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 TGF-beta 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, 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 GSC niche.

Heparan sulfate proteoglycans (HSPGs) are a group of glycoproteins, which are expressed on the cell surface and/or in the extracellular matrix. Recent studies have revealed that HSPGs play critical roles in regulating signaling pathways during development by controlling extracellular ligand distribution. For example, one of the *Drosophila* glypicans, *dally* controls distribution of TGF-beta ligand, or Dpp, and thereby establishing a Dpp gradient during wing development.

We recently identified *Drosophila* glypicans, *dally* and *dally-like* as important components of GSC niche in both sexes. Mutations for these glypicans caused a significant reduction in GSC number, due to the failure of proper activation of the signaling pathway for GSC maintenance. Conversely, ectopic expression of *dally* in female gonads caused an increase in GSC number. These results strongly suggest that these glypicans define GSC niche by regulating distribution of niche signals.

To address this possibility, we have been trying hard to visualize niche signals in the GSC niche. By modifying

protocols for antibody staining and generating new antibodies, we succeeded in visualization of niche signal distribution. We found that Dpp distribution was significantly expanded when *dally* was ectopically expressed in female gonads, while Dpp-producing cells were unaffected (Figure 3). The above results support our model that glypicans define the GSC niche by regulating extracellular distribution of niche signals.

We also succeeded in detecting spatial distribution of JAK/STAT ligand, Upd (Figure 3). Upd is known to act as a morphogen for proper formation of egg chambers, as well as a niche signal in male GSC maintenance. We found that *dally* was able to affect Upd distribution within the egg chambers. This is the first evidence supporting the role of glypican in regulating extracellular distribution of Upd. Experiments testing whether glypicans regulate Upd distribution in male GSC niche are now ongoing.

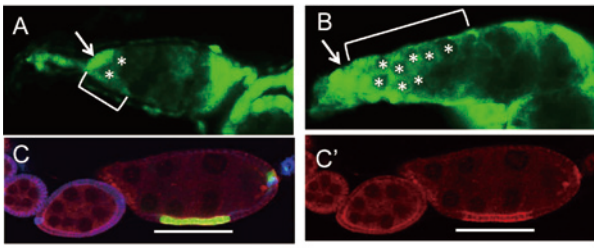


Figure 3. Glypican regulates spatial distribution of niche signals.
(A,B) Dpp distribution in distal tip region (germarium) of normal ovary (A) and of ovary expressing *dally* in the somatic cells throughout germarium (B). Green signal indicates Dpp protein (Blackets). Dpp distribution is expanded in *dally*-expressing ovary, compared to that observed in normal ovary. Arrows show niche cells, which are the source of Dpp. Asterisks indicate GSCs. (C, C') Distribution of Upd in egg chamber. Upd (red signal) is ectopically accumulated in *dally*-expressing clone (green, Bar).

Publication List

〔Original papers〕

- Hashiyama, K., Hayashi, Y., and Kobayashi, S. (2011). *Drosophila Sex lethal* gene initiates female development in germline progenitors. Science *333*, 885-888.
- Mukai, M., Kato, K., Hira, S., Nakamura, K., Kita, H., and Kobayashi, S. (2011). Innexin2 gap junctions in somatic support cells are required for cyst formation and for egg chamber formation in *Drosophila*. Mech. Dev. *128*, 510-523.

Division of Molecular and Developmental Biology

Professor
TAKADA, Shinji

Professor:	*TAKADA, Shinji*
Assistant Professors:	*OKUBO, Tadashi*
	YABE, Taijiro
Technical Staff:	*UTSUMI, Hideko*
NIBB Research Fellow:	*CHEN, Qiuhong*
Postdoctoral Fellows:	*KAMETANI, Yoshiko*
	TAKAHASHI, Jun
Visiting Scientist:	*TAKADA, Ritsuko*
Graduate Students:	*ISHITANI, Shizuka*
	TSUNOKUNI, Hiroyuki
	WANGLAR, Chimwar
	SHINOZUKA, Takuma
	KAMAGATA, Takanori
Technical Assistants:	*TAKASHIRO, Kayoko*
	TOMIDA, Sanai
	OHHARA, Kaori
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 mechanism 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 mechanism 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 periodical structures. The molecular mechanism underlying this conversion and morphological segmentation, however, is not yet fully understood. Thus, another goal of our current studies is to reveal the molecular mechanism of *this other and unique mode of patterning* that underlies the periodical and sequential sub-division in the development of somites and pharyngeal arches.

I. Lipid modification and extracellular trafficking of Wnt proteins

The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis. Most Wnt proteins transmit their signals locally in most of the cases where they function, presumably since their secretion and transport are under tight control. One important step regulating the extracellular transport of various secreted signal proteins involves post-translational modification with lipid moieties. We found that murine Wnt-3a is modified with a mono-unsaturated fatty acid, palmitoleic acid, at a conserved Ser residue (Figure 1). Wnt-3a defective in this modification is not secreted from cells in culture or in Xenopus embryos, but is retained in the endoplasmic reticulum (ER). Furthermore, Porcupine (Porcn), a protein with structural similarities to membrane-bound O-acyltransferases, is required for this Ser-dependent modification, as well as for Wnt-3a transport from the ER for secretion. These results strongly suggest that the Wnt protein requires a particular lipid modification for proper intracellular transport during the secretory process. We expect that the discovery of this unexpected lipid modification might provide a clue regarding the higher-order structure of secreted Wnt proteins and their gradient formation.

Figure 1. Post-translational modification of Wnt proteins: Several secreted signal proteins are specifically modified. These modifications are required for proper secretion, extracellular trafficking, and binding to their receptors. Wnt proteins are lipidated, glycosylated, and sulfated. We have shown that one type of lipidation, palmitoleoylation, is required for proper secerion of several Wnt proteins and is dependent on an acyl-transferase, Porcupine. In this figure, a schematic representation of mouse Wnt3a, which is lipidated at Cys77 and Ser209 and glycosylated at Asn87/298, is shown. In addition, the positions of Tyrosine residues sulfated in Wnt5a/11 are also indicated. The conserved cysteines are shown with vertical bars.

On the other hand, recent evidence suggest that this Ser-dependent lipidation may not always be required for secretion of Wnt proteins. In the case of Wingless, the *Drosophila* ortholog of Wnt1, this Ser residue is not required for secretion from *Drosophila* S2 cells, but for the function of this protein, suggesting that the role of the Ser-dependent lipidation may differ for different Wnt proteins or in different cellular contexts. To reveal the role of the Ser-dependent lipidation in different contexts, we examined the role of Porcn in zebrafish embryos. *Porcn*-deficient zebrafish embryos only exhibited defects in convergent-extension (CE) movement, which requires non-canonical Wnt signaling. Surprisingly, these embryos showed no sign of reduction in canonical Wnt signaling. Consistent with this array of phenotypes, intracellular trafficking of zebrafish Wnt5b and mouse Wnt5a, but not zebrafish and mouse Wnt3a, was specifically impaired in *Porcn*-deficient embryos. These results indicate that *Porcn* is required for proper function and

trafficking of at least one Wnt protein during early developmental stages of zebrafish, but decrease of Porcn does not equivalently affect the trafficking and lipidation of different Wnt proteins in these embryos. Together, the mechanism of trafficking and modification of Wnt proteins appear to be inconsistent between different types of Wnts. We are currently examining the molecular mechanism underlying the variability of Wnt modification.

In addition to the study of the secretory process of Wnt proteins, we are also examining the extracellular transport of Wnt proteins during embryogenesis using frog and mouse embryos.

II. Molecular mechanism of somite development.

The molecular mechanism underlying the periodical formation of somites is coupled to an internal oscillator, referred to as the "segmentation clock," which has been evidenced by the cyclic expression of genes in the presomitic mesoderm (PSM). For example, *hairy/Enhancer of split* (*Espl*)-related bHLH genes, including *her1* and *her7* in zebrafish, are expressed in a dynamic pattern of stripes across the PSM in a posterior to anterior direction. The oscillating and anteriorly propagating wave of gene expression, which is maintained in the posterior PSM, becomes fixed to cause segmentation in the anterior PSM. Prior to morphological segmentation, a segmental pre-pattern, characterized by the periodical borders between neighboring somites and by the rostro-caudal within a somite, is established in the anterior PSM. We have already shown that a gene identified by our *in situ* hybridization screening, *ripply1,* is required for the maintenance of the rostro-caudal patterning in zebrafish embryos. Ripply proteins have been shown to associate with the transcriptional co-repressor Groucho/TLE and the T-box transcription factors through two distinct amino acid sequences, one termed the WRPW motif, which is a highly conserved 4-amino acid stretch in the N-terminal half, and the other, the Ripply homology (RH) domain, a conserved ~50-amino acid stretch that interacts with the T-domain of the T-box proteins. Therefore, Ripply is able to recruit the Groucho/TLE co-repressor to T-box proteins and control their intrinsic transcriptional properties.

Recently, we also showed that Ripply1 and another structurally related-protein, Ripply2, are required for the positioning of intersomitic boundaries in mice. The positions of somite boundaries are defined by the anterior limit of the Tbx6 protein domain, which is regulated by degradation of Tbx6 proteins. In addition, a transcription factor, Mesp2, is required for this degradation although it is uncertain how Mesp2 induces this degradation. We showed that expression of mouse *Ripply1* and *Ripply2* is dependent on Mesp2 and *Ripply1/2*-deficient mouse embryos exhibit anterior expansion of the Tbx6 protein domain, suggesting that *Ripply1/2* expression by *Mesp2* is important in the establishment of somite boundaries by regulating Tbx6 degradation. To compare the mechanism of somite segmentation between different species, we are also examining the mechanism of the formation of intersomitic boundaries in zebrafish.

III. The role of Ripply3 in the development of pharyngeal arches.

The pharyngeal apparatus is another example of segmental structure in the vertebrate embryo. The pharyngeal apparatus is a transient structure formed ventrolateral to the hindbrain in vertebrate embryos. This structure consists of bilaterally segmented arches, and ectodermal grooves and endodermal pouches, both of which are formed between the arches. Components of the pharyngeal apparatus give rise to distinct tissues in later stages of development. For instance, the pharyngeal arteries and neural crest cells in the caudal pharyngeal arches contribute to cardiovascular development, while the endodermal cells located in the caudal pouches give rise to several organs, including the thymus and parathyroid gland. Thus, pharyngeal development is a key process in the generation of these organs.

Chromosome 22q11 deletion syndrome (22q11DS), which includes the DiGeorge syndrome (DGS), is characterized by the abnormal development of the pharyngeal apparatus in the form of thymic hypoplasia or aplasia, hypocalcemia arising from parathyroid hypoplasia, and defective cardiac outflow. A number of mouse genetic studies and mutational analyses in human patients have indicated that *Tbx1*, which encodes a member of the T-box family of transcription factors, is the most likely gene responsible for the phenotype of 22q11DS. During murine pharyngeal development, *Tbx1* is first expressed in the mesoderm at E7.5. Between E8.5 to 11.5, *Tbx1* also becomes located in the pharyngeal endoderm, ectoderm, and core mesoderm, but not in the neural crest cells. Cell type-specific inactivation and analysis of downstream targets of *Tbx1* in mice indicate that *Tbx1* plays multiple roles in endoderm, mesoderm, and ectoderm cells during pharyngeal development. However, the molecular mechanisms underlying the cell type-specific roles of Tbx1 have not been fully elucidated.

Interestingly, another member of the Ripply family, Ripply3, is expressed in the pharyngeal endoderm cells. Because we found that both *Ripply3* and *Tbx1* were strongly expressed in the pharyngeal endoderm and endoderm, we then examined the role of Ripply3 by generating *Ripply3*-deficient mice, as well as its relationship with Tbx1. We show that Ripply3 can modulate Tbx1 activity in *in vitro* luciferase reporter assays. Furthermore, *Ripply3*-knock out mouse showed hypotrophy of the caudal pharyngeal apparatus (Figure 2). Ripply3 represses Tbx1-induced expression of *Pax9* in *in vitro* luciferase assays, and *Ripply3*-deficient embryos exhibit up-regulated *Pax9* expression. Thus, our results showed that *Ripply3* most likely plays its role in pharyngeal development by regulating Tbx1 activity.

More precise analysis revealed that the development of pharyngeal derivatives was impaired in *Ripply3* mutant mice, including ectopic formation of the thymus and the parathyroid gland, as well as cardiovascular malformation. For instance, the disrupted development of the caudal pharyngeal arches seems to have resulted in two distinct cardiovascular defects in the *Ripply3*$^{-/-}$ embryos. One involved an abnormality in heart development. The severe

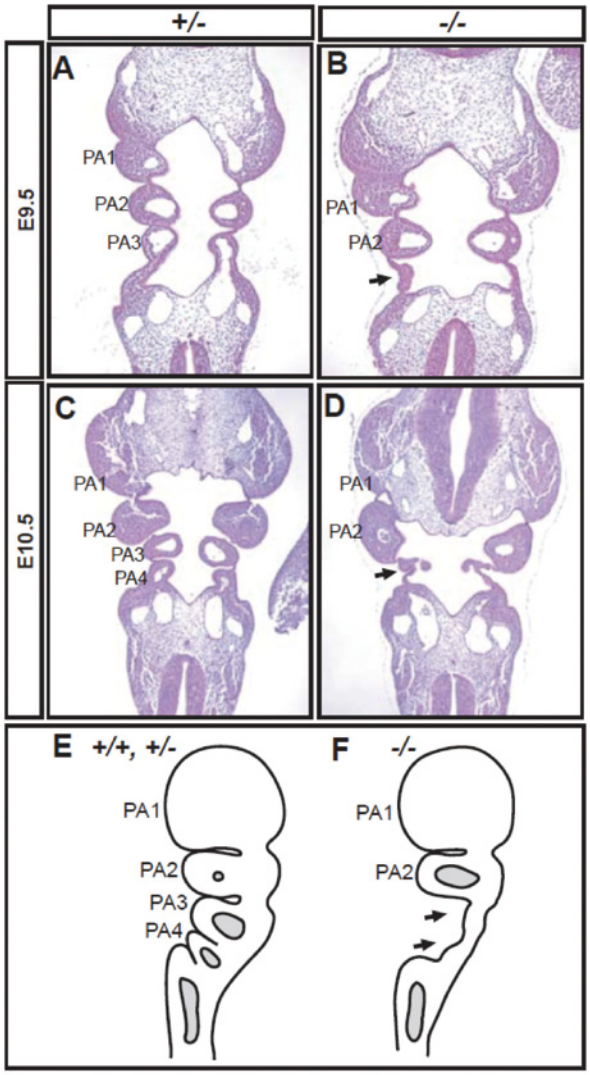


Figure 2. *Ripply3* is required for proper development of the third and fourth pharyngeal arches: (A-D) Hematoxylin-eosin-stained coronal sections of *Ripply3* $^{+/-}$ (A, C) and *Ripply3* $^{-/-}$ (B, D) embryos at E9.5 (A,B) and E10.5 (C,D). (E, F) Schematic images illustrating phenotypes of *Ripply3* $^{+/-}$ (E) and *Ripply3* $^{-/-}$ (F) embryos.

reduction in neural crest cell number in the caudal pharyngeal arches appears to have led to the abnormal development of the outflow tract, including hypotrophy of the aorta and incomplete formation of the ventricular septum. The other defect involved the loss of the 3rd and 4th PAAs. This defect appeared to result in abnormal development of the vascular system including deletion of the aortic arch and misshapen major blood vessels. Interestingly, the second PAAs, which normally disappear after E10.5, persisted until birth in the *Ripply3*$^{-/-}$ embryos (Figure 3). Persistence of the 1st and 2nd PAAs has also been reported in *Endothelin-1*-deficient mouse embryos, where the 4th PAAs are also poorly developed, suggesting that the formation of PAA proximal to the heart may be a pre-requisite for the regression of the more distal ones. We speculate that development of the proximal PAAs resulted in a decrease in blood flow running through the existing distal PAAs, which in turn decreased the mechanical stress caused by blood flow, making it virtually impossible for the distal PAAs to be maintained.

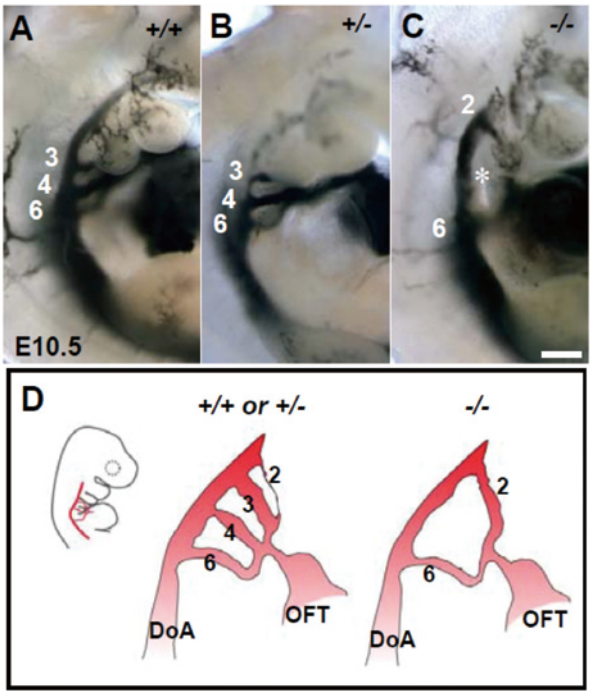


Figure 3. Cardiovascular defects in the Ripply3-deficient mice: (A-C) Typical morphology of pharyngeal arch arteries (PAAs) in the wild-type (A), heterozygous (B), and homozygous mutant (C) mice at E10.5 as visualized by intracardiac ink injection. The asterisk indicates the absence of the 3rd and 4th PAAs. (D) Schematic representation of PAA pattern.

Publication List

〔Original paper〕

- Okubo, T., Kawamura, A., Takahashi, J., Yagi, H., Morishima, M., Matsuoka, R., and Takada, S. (2011). Ripply3, a Tbx1 repressor, is required for development of the pharyngeal apparatus and its derivatives in mice. Development *138*, 339-348.

DIVISION OF EMBRYOLOGY

Professor
FUJIMORI, Toshihiko

Assistant Professors:	*TOYOOKA, Yayoi*
	KOYAMA, Hiroshi
Technical Staff:	*OKA, Sanae*
NIBB Research Fellows:	*KOMATSU, Kouji*
	SATO, Yasufumi
Postdoctoral Fellow:	*KOBAYAKAWA, Satoru*
Visiting Scientist:	*SHI, Dongbo*
Technical Assistants:	*HIRAO, Mayumi*
	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 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, 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 mouse lines for live imaging

Currently, it is common to observe the behavior of cells and gene expression in living embryos to understand the mechanisms underlying embryonic development and morphogenesis. Progress in GFP technologies, genetic engineering and embryonic manipulation concomitantly with the progress in optics and microscope technologies, including development of highly sensitive photo-detectors, has allowed us to observe developing embryos live, even in mammalian species. We have previously established a transgenic mouse line ubiquitously expressing EGFP (Enhanced Green Fluorescent Protein) fused with histone H2B by homologous recombination in the Rosa26 locus, and embryos obtained from this mouse line have been used for timelapse live imaging to analyze cell behavior. It would be useful to visualize nuclei with other colors of fluorescent proteins and visualize cell shapes, cytoskeleton or other organelles in a similar way to observe cells and cell behaviors in living embryos. Prof. Fujimori, as a visiting scientist, has been participating in a project producing a series of mouse reporter lines to express fluorescent markers, which is taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CDB. This year we have established 17 lines of these mice. In each mouse, cDNA encoding fusion protein with fluorescent protein and a localization sequence was inserted into Rosa26 locus. The sequence for the fusion protein was following stop sequences that are surrounded by loxP sites on both sides. These loxP sites can be recognized by an enzyme called Cre recombinase that catalyzes recombination between two loxP sites to remove the stop sequences. Thus, when Cre recombinase is activated in a spatial-temporally specific manner, the following reporter fluorescent proteins are

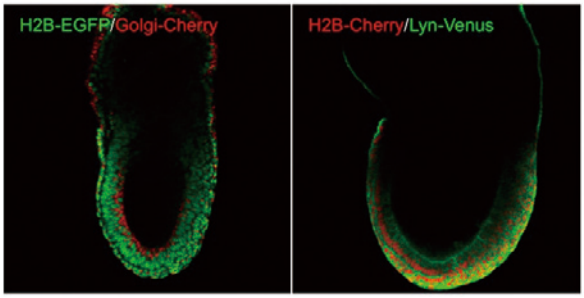


Figure 1. Examples of 7.5 day embryos obtained after crossing of two reporter mouse lines. Nuclei and golgi apparatus and cell membrane and nuclei are visualized with green and red fluorescent proteins genetically introduced as transgenes.

expressed in a specific way. And once this irreversible reaction is induced in the germ line, the derived offspring possess the transgene without the stop sequence and express the reporter fusion protein ubiquitously. These mice will provide useful materials for live imaging of cells in early embryos as well as cells in the tissues of developing and adult animals. In addition to the establishment of mouse lines, we have been trying to improve optical systems for live imaging, and we could improve systems to culture in better conditions during live imaging. The culture conditions of peri-implantation mouse embryos have also improved. We are planning to observe and reveal cell shape, morphogenesis, cell lineage, gene expression and cell differentiation by combining these techniques.

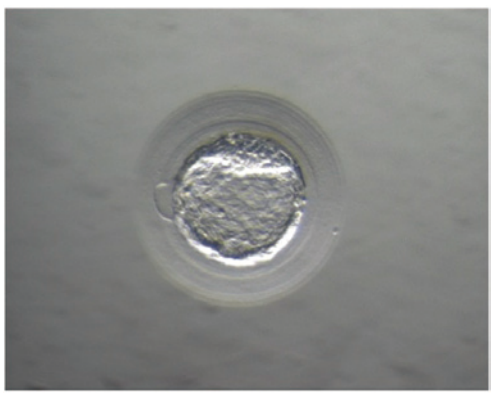


Figure 2. A 4.5day rabbit embryo. The shape is very similar to other mammalian blastocysts at this stage. However, development from slightly after this stage is different from corresponding stages of mouse embryo development.

II. Studying early development of rabbit as a new model of mammalian embryogenesis.

Mice have been the main experimental animal used for the study of mammalian developmental biology because genetics and genetic engineering including transgenesis and targeted mutagenesis are applicable for mice. We also have been mainly studying mouse embryogenesis. However, rodent embryos do not necessarily provide ideas common in mammalian embryogenesis because the styles of early embryonic development differ between species, and rodent embryos do not provide a typical style when compared with other animals. We searched for animals that are suitable for the study of early embryogenesis comparing possible candidates, and decided to use rabbits. We started examination of morphological changes and gene expression during early stages of development until the peri-implantation stage. We will focus especially on the formation of body axes, and compare with corresponding stages in mice.

III. Formation, maintenance of ell polarity and tissue morphogenesis in the mouse oviduct

The oviducts (fallopian tubes) are tubes connecting the periovarian spaces and the uterine horns. The ova released from the ovary are transported through the oviduct, wherefertilization occurs with the spermatozoon moving from the uterus. We are focusing on the region of the oviduct close to the opening to the ovary. In this region many multi-ciliated cells exist. The cilia on the surface of ovarian epithelial cells were observed with a high-speed CCD camera and these movements were recorded. These cilia moved in one direction along the ovary-uterus axis. This directional movement of multi-cilia might play a major role in the transportation of ovum from periovarian space, although muscle contractions also play roles in the region close to the uterus. The importance of the directional ciliary beating for the transport of ova is supported by the experiments shown in the figure 3. An ovum was put at the opening end of a longitudinally opened oviduct, and this ovum was moved along the fold running parallel to the ovary-uterus axis of the oviduct without muscle contraction. This suggested that the ovum was transported by the directionally beating cilia. This directional beating of cilia was based on the polarity of the microtubule assembly in the cilia. Skeletal microtubules in the cilia are arranged in a “9 +2”array, and the central two bundles are aligned facing the same direction in each cell. This suggests that oviduct epithelial cells possess polarity along the cell surface parallel to the longitudinal axis of the oviduct; this type of cellular polarity is called “Planner Cell Polarity (PCP)”. We have been studying how PCP is established during development, and how this polarity is maintained for a long period in later stages. Ciliary movement is one good indicator of PCP, therefore we established a system to analyze ciliary beats with automated image processing, and found ciliary beat frequency was slightly different between estrus cycles.

We are now focusing on several genes involved in PCP formation. Molecular basis of PCP formation has been genetically studied particularly using fruit fly, and several genes have been shown to be involved in the regulation of PCP formation. Homologues of these genes are known also in mammalian species, and some of them have shown to be playing similar roles. Disruption of functions of some of these genes resulted in abnormal PCP formation in skin, inner ear and early embryos of mice. We are also focusing on some of the PCP regulators, and are trying to understand how oviduct epithelial cells establish and maintain polarity. We would like to reveal mechanisms including the molecular functions, cellar shape, tissue morphology and involvement of mechanical forces in this system.

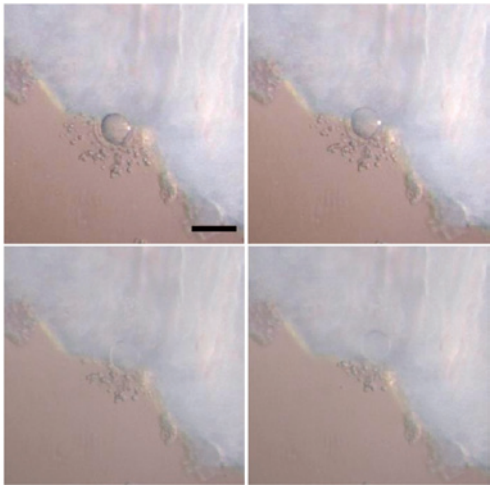


Figure 3. Transport of an ovum via the movements of cilia on the oviduct epithelial cells. Timelaplse images of movement of an ova on the longitudinally opened preparation of the mouse oviduct.

Publication List

〔Original papers〕

- Abe, T., Kiyonari, H., Shioi, G., Inoue, K., Nakao, K., Aizawa, S., and Fujimori, T. (2011). Establishment of conditional reporter mouse lines at ROSA26 locus for live cell imaging. Genesis *49*, 579-590.
- Nakagawa, T., Izumino, K., Ishii, Y., Oya, T., Hamashima, T., Jie, S., Tomoda, F., Fujimori, T., Nabeshima, Y., Inoue, H., and Sasahara, M. (2011). Roles of PDGF receptor-beta in the structure and function of postnatal kidney glomerulus. Nephrol Dial. Transplant. *26*, 458-468.
- Shi, D., Komatsu, K., Uemura, T., and Fujimori, T. (2011). Analysis of ciliary beat frequency and ovum transport ability in the mouse oviduct. Genes to Cells *16*, 282-290.
- Shioi, G., Kiyonari, H., Abe, T., Nakao, K., Fujimori, T., Jang, C., Huang, C., Akiyama, H., Behringer, R.R., and Aizawa, S. (2011). A mouse reporter line to conditionally mark nuclei and cell membranes for in vivo live-imaging. Genesis *49*, 570-578.

DIVISION OF GERM CELL BIOLOGY

Professor
YOSHIDA, Shosei

Assistant Professors:	*HARA, Kenshiro* *KITADATE, Yu*
NIBB Research Fellow:	*NAKAMURA, Yoshiaki*
Technical Staff:	*MIZUGUCHI-TAKASE, Hiroko*
Graduate Students:	*IKAMI, Kanako* *TOKUE, Moe*
Technical Assistants:	*ICHIKAWA, Rie* *INADA, Kana* *MARUYAMA, Ayumi* *SUGIMOTO, Ryo*
Secretary:	*KUBOKI, Yuko*

Mammalian testes produce numerous sperm for a long period in a constant manner, which is supported by the robust behaviors of the stem cell population. Decades of research, including morphological examinations, post-transplantation repopulation, and in vitro culture, have made it one of the most intensively studied mammalian tissue stem cell systems. However, the nature of the stem cells and their control, as well as their niche, remains largely unknown.

The Division of Germ Cell Biology aims to fully understand the mouse sperm stem cell system in vivo, i.e., in the context of testicular tissue architectures. We have revealed a number of characteristics of this potent stem cell system, including: 1) Cells believed to have irreversibly committed for differentiation still retain the self-renewing potential and can contribute to stem cell pool maintenance ("potential stem cells"). 2) "Reversion" from potential stem cells occurs at a higher frequency when testicular tissue is damaged and regeneration is induced. 3) The undifferentiated spermatogonia (A_{undiff}) population that includes both "actual" and "potential" stem cells localized to vasculature (vascular-associated niche). 4) Stem cells turn over frequently and stochastically even under steady-state situations. (Nakagawa et al., Dev. Cell 2007, Science 2010; Yoshida et al., Science 2007; Klein et al., Cell Stem Cell 2010)

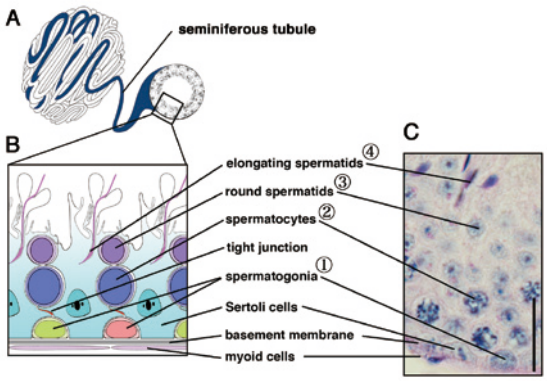


Figure 1. Architecture of seminiferous tubules and seminiferous epithelium. The seminiferous epithelium exhibits a stratified organization of the differentiating germ cells (numbers in circle), which are nourished by Sertoli cells. Bar, 20 μm.

In 2011, we revealed the molecular mechanisms underlying the temporal regulation of differentiation and self-renewal of stem cells (A_{undiff}) in mouse seminiferous epithelium, which are comprised of germ cells –both stem and differentiating cell types- and supporting somatic cells. Our study proposed that local retinoic acid (RA) metabolism plays important roles for the timed stem cell differentiation and coordination with somatic cells that support the differentiation process to spermatozoa (Sugimoto et al., Mech. Dev. 2011).

I. Architecture and cyclic dynamics of seminiferous epithelium

Spermatogenesis occurs inside the seminiferous tubule of the testis, where differentiating germ cells and supporting somatic Sertoli cells comprise a composite epithelium, termed seminiferous epithelium (Fig. 1). Sertoli cells are huge cells that form typical epithelium. All the stages of germ cells (from stem cells to spermatozoa) are nourished by Sertoli cells in a striated manner. This stratification is established as a result of the periodic differentiation of A_{undiff} into A_1 spermatogonia with an interval of 8.6 days, which is followed by programmed differentiating process toward spermatozoa that takes 35 days (Fig. 2). Consequently, the resultant combination of the differentiating germ cell observed at a particular region appears in an 8.6-day cyclic manner, known as 'seminiferous epithelial cycle'. In mice, this cycle is divided into stages I to XII, while A_{undiff}-to-A_1 differentiation occurs at stage VII to VIII.

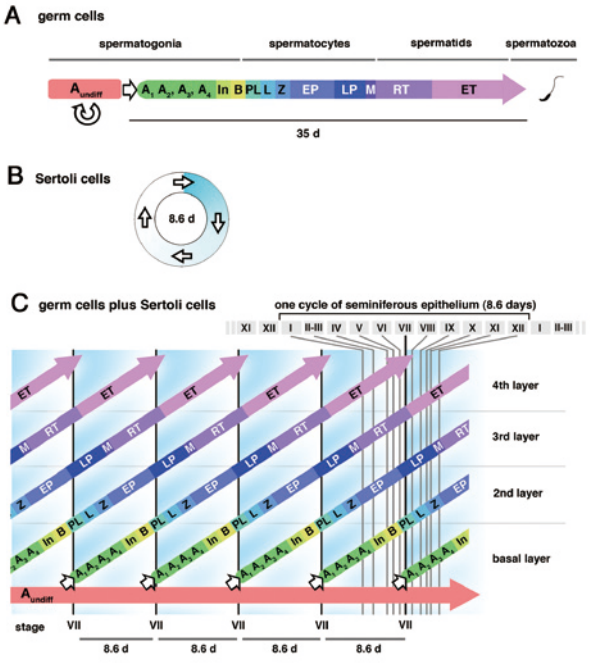


Figure 2. Coordination of periodical events in mouse seminiferous epithelium. (A) Germ cell differentiation, which takes 35 days from A_{undiff} to spermatozoa. (B) 8.6-day cycle of gene expression in Sertoli cells. (C) Coordination of germ cell differentiation and Sertoli cell cycle.

Sertoli cells, while quiescent in their cell cycle, cyclically change their gene expression so that they can nourish the appropriate stages of germ cells. It is an interesting question but remains a mystery how this periodic differentiating of A_{undiff} is achieved and the cycle of Sertoli cells is coordinated with germ cell differentiation.

II. Role of RA and regulation of RA metabolism

It has been known that RA plays important roles in both differentiation of A_{undiff} and control of the seminiferous epithelial cycle. This notion was primarily derived from observations made after RA signaling blockade by deficiency of the dietary vitamin A (VA) -the only precursor of RA- and by disruption of retinoic acid receptor genes. However, results of these and other preceding studies were somewhat puzzling and it remained a challenge to reveal how RA is involved in the genesis of the coordinated cycling of the seminiferous epithelium.

We revisited the classical VAD model and discovered that artificial elevation of RA signaling elicits not only differentiation of A_{undiff} into A_1 spermatogonia but also a reset of the cycle of Sertoli cells' function to the appropriate stage that supports the A_1 spermatogonia in transition between A_{undiff} and A_1 (stage VII). Then we asked how the RA-related metabolism is regulated in the seminiferous epithelium based on the expression of genes encoding enzymes involved in the synthesis of RA from VA and inactivation of RA. Based on the data from a previous study of Manuel Mark's group and our own analyses, it was strongly suggested that the modes of RA metabolism dynamically shift in accordance with the seminiferous epithelial cycle, with a prominent increase of RA concentration across stage VII (Fig. 3). This is also supported by the fact that artificially increased RA induced A_{undiff}-to-A_1 differentiation and reset Sertoli cells to stage VII.

Interestingly, RA metabolism involves multiple enzymes (Fig. 3A) that are expressed separately among different cell types including meiotic and haploid germ cells and Sertoli cells. Given that RA and its precursors are able to translocate across adjacent cells rather freely, the different cell types seem to cooperate in the regulation of RA metabolism. Then, the next question was raised: How is coordinated expression of these genes among different cell types achieved? We discovered that RA metabolism-related genes expressed in Sertoli cells are regulated by RA signaling, forming positive and negative feedback loops; while those expressed in meiotic and haploid germ cells hardly respond to RA signaling: Perhaps a differentiation step-related mechanism controls their expression. Thus, control of RA metabolism-related genes show a good contrast between Sertoli cells and germ cells.

III. Model for the seminiferous epithelial cycle

Based on these observations, we have proposed a model that can explain the coordinated expression of RA metabolism-related genes that appears to cause the cyclic change of the RA concentration (Fig. 4, 5). Importantly, this model suggests that meiotic and haploid germ cells regulate the local RA metabolism to increase and decrease the RA

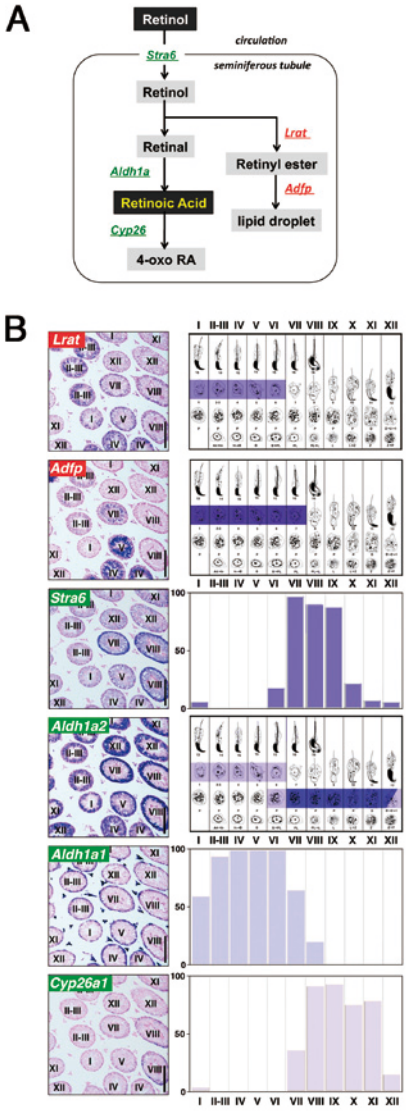


Figure 3. RA metabolism-related genes and their cyclic expression. (A) Multiple genes act together in the RA metabolic pathways, starting from retinol (VA). (B) These genes are expressed in different cell types and in different stages of the seminiferous epithelial cycle.

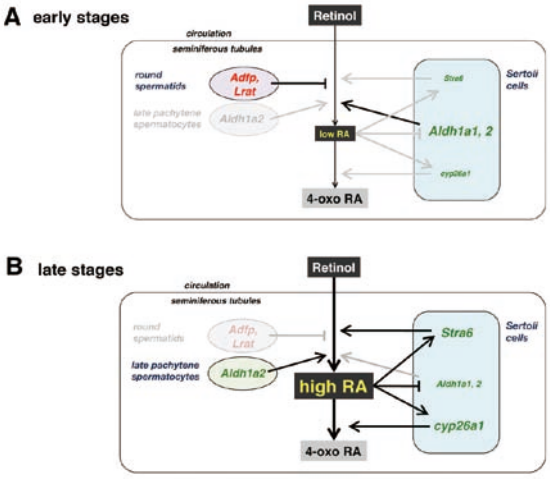


Figure 4. Regulation of RA metabolism-related gene expression. Expression patterns of these genes (Fig.3) in early (A) and late (B) stages are explained as a result of feedback regulation in Sertoli cells but not in germ cells.

concentration, respectively (Fig. 4). Given that RA signaling causes the induction of A_{undiff}-to-A_1 differentiation and reset of Sertoli cell's cycle, it can be said that these differentiating germ cells play a central role in the coordination of the seminiferous epithelial cycle by sending 'go' and 'wait' signals to the A_{undiff} and Sertoli cells (Fig. 5). Therefore, the differentiation program of germ cells appears to determine the timing of stem cell differentiation and makes the local environment appropriate for germ cell differentiation.

This model nicely explains the beautiful orchestration between germ and Sertoli cells in the seminiferous epithelium. Generally speaking, regulation of stem cell differentiation by their differentiating progeny that modulate the local environment may be a common strategy of stem cell control in other systems.

IV. Perspectives and ongoing research

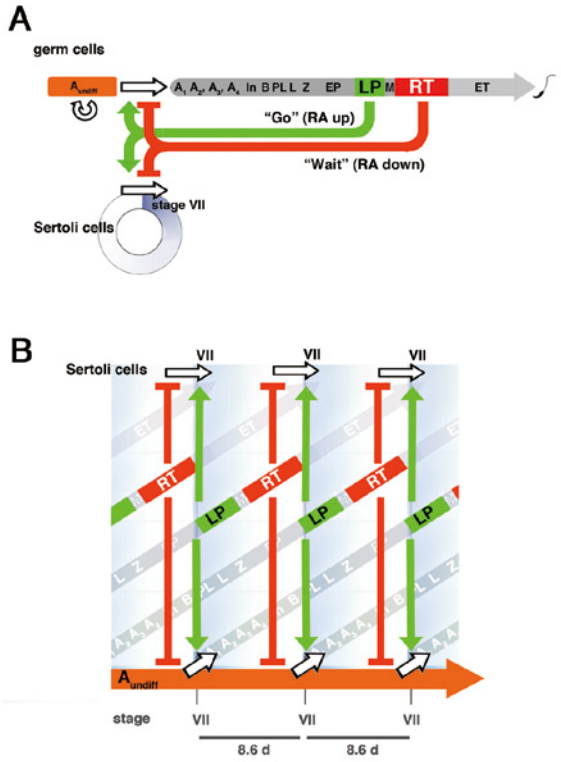


Figure 5. Coordination of germ cell differentiation and Sertoli cells' cycle. Particular stages of meiotic cells (LP) and haploid cells (RT) send the *'go'* and *'wait'* signals to A_{undiff} and Sertoli cells, by regulating the local RA metabolism to increase and decrease the RA concentration, respectively (A). These signals will occur reciprocally and periodically to maintain the seminiferous epithelial cycle. All the figures are reproduced from Sugimoto *et al*., Mech. Dev. (2011) with permission.

The suggested model for the role of RA in the regulated stem cell differentiation warrants farther evaluation: While the central role of RA is in no doubt, it is also clear that RA solely cannot regulate the very complex and highly orchestrated events that occur in seminiferous epithelial cycle. In addition, stem cells should behave in response to the cyclically changing microenvironment. We hope that our model and future investigations will clarify the details of the testicular environment and the stem cell response.

Besides the seminiferous epithelial cycle, we are also investigating a number of yet-to-be elucidated problems in spermatogenesis, especially with regard to its stem cells. In addition to temporal regulation described here, the spatial regulation of stem cells is also a very important issue. We previously observed that A_{undiff} preferentially localize to the vasculature-proximal region, and dispatch this region when they differentiate into A_1 at stage VII (Yoshida et al., Science 2007), which is likely to occur in response to RA signaling. We are also investigating the cellular and molecular nature of this 'vasculature-associated niche'. We are also investigating the behavior of A_{undiff} taking advantages of live imaging that we have developed (Yoshida et al., Science 2007, Nakagawa et al., Science 2010). We hope that these studies will throw light to a better understanding the mouse sperm stem cell system.

Publication List

〔Original paper〕

- Sato, T., Aiyama, Y., Ishii-Inagaki, M., Hara, K., Tsunekawa, N., Harikae, K., Uemura-Kamata, M., Shinomura, M., Zhu, X. B., Maeda, S., Kuwahara-Otani, S., Kudo, A., Kawakami, H., Kanai-Azuma, M., Fujiwara, M., Miyamae, Y., Yoshida, S., Seki, M., Kurohmaru, M., and Kanai, Y. (2011). Cyclical and patch-like GDNF distribution along the basal surface of sertoli cells in mouse and hamster testes. PLoS ONE 6, e28367.

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

- Gely-Pernot, A., Raverdeau, M., Célébi, C., Dennefeld, C., Feret, B., Klopfenstein, M., Yoshida, S., Ghyselinck, N.B., and Mark, M. Spermatogonia differentiation requires retinoic acid receptor γ. Reproduction 2011 Nov. 1.
- Sugimoto, R., Nabeshima, Y., and Yoshida, S. Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium. Mechanisms of Development 2011 Dec. 19.

〔Review articles〕

- Spradling, A., Fuller, M.T., Braun, R.E., and Yoshida, S. (2011). Germline stem cells. In Cold Spring Harbor Perspectives in Biology Collection, "Germ Cells", Cold Spring Harbor Laboratory Press, 1-20.
- Yoshida, S. (2011). Stem cell niche system in mouse spermatogenesis. In Stem Cell Biology and Regenerative Medicine Series, "Male Germline Stem Cells: Developmental and Regenerative Potential", Humana Press, 159-175.

DIVISION OF REPRODUCTIVE BIOLOGY †

Professor (Specially appointed)
NAGAHAMA, Yoshitaka

Postdoctoral Fellows: *GUAN, Guijun*
SAKAI, Fumie
SHIBATA, Yasushi
NAKAMOTO, Masatoshi
Secretary: *SHIMADA, Yu*

Fish exhibit a range of gonadal forms from gonochorism to several types of hermaphroditism, thus providing an excellent animal model to study the molecular mechanisms of sex determination, gonadal sex differentiation and gametogenesis in vertebrates. Our research focuses on (1) the identification of regulators involved in sex determination, gonadal sex differentiation, sexual plasticity, and gametogenesis (oocyte maturation and ovulation), and (2) the mechanisms of synthesis and action of these regulators.

Molecular mechanisms of sex determination, gonadal sex differentiation and sex change

We previously identified *DMY* (*DM*-domain gene on the *Y* chromosome) as the sex-determining gene of the medaka (*Oryzias latipes*), the first in non-mammalian vertebrates. Recently, we developed a gene-specific transgenic RNA interference (RNAi) technology for the analysis of loss-of-function phenotypes that develop over long periods of time, and used it to knock down the *dmy* gene in genetically male (XY) fish. Knockdown of *dmy* strongly downregulated the expression of the only other male-associated genes (*gsdf*, *sox9a2* and *dmrt1*), and upregulated the expression of female-associated genes (*foxl2* and *Rspo1*) in XY gonads during the early stages of sexual differentiation. This shift in the gene expression pattern resulted in a complete male-to-female sex-reversal with a typical female pattern of secondary sex characteristics, producing fertile eggs. Importantly, we were able to continue a trans-generational knockdown effect on *dmy* until at least the F3 generation. In order to rescue the effect of *dmy* knockdown, we singularly injected or co-injected *sox9a2* (marked in cyan) and *gsdf* (marked with cherry) into olvas vasa-DMY-knockdown embryos of the F3 generation. Although singular injections failed to complete suppression of meiosis and proliferative mitosis but co-injection re-established the male phenotype in the XY gonad leading to complete formation of the testis, producing fertile sperm. This confirms that *gsdf* and *sox9a2* are genes downstream of *dmy* which, can regulate the sexual identity of medaka even in a DMY-independent manner. We conclude that in medaka *dmy* directly or indirectly upregulates the male sex-determining pathway by activating *gsdf* and *sox9a2* expression.

†: This laboratory was closed on 31 March, 2011.

Publication List

〔Original papers〕

- Charkraborty, T., Shibata, Y., Zhou, L.Y., Katsu, Y., Iguchi, T., and Nagahama, Y. (2011). Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Mol. Cell. Endocrinol. *333*, 47-54.
- Charkraborty, T., Katsu, Y., Zhou, L.Y., Miyagawa, S., Nagahama, Y., and Iguchi, T. (2011). Estrogen receptors in medaka (*Oryzias latipes)* and estrogenic environmental contaminants: An in vitro-in vivo correlation. J. Steroid Biochem. Mol. Biol. *123*, 115-121.
- Fernandino, J.I., Popesku, J.T., Paul-Prasanth, B., Xiong, H., Hattori, R.S., Oura, M., Strussmann, C.A., Somoza, G.M., Matsuda, M., Nagahama, Y., and Trudeau, V.L. (2011). Analysis of sexually dimorphic expression of genes at early gonadogenesis of *Pejerrey Odontesthes bonariensis* uing a heterologous microarray. Sex. Dev. *5*, 89-101.
- Mita, M., Yamamoto, K., and Nagahama, Y. (2011). Interaction of relaxin-like gonad-stimulating substance with ovarian follicle cells of the starfish *Asterina pectinifera*. Zool. Sci. *28*, 764-769.
- Mita, M., Yamamoto, K., Nakamura, M., and Nagahama, Y. (2011). Hormonal action of relaxin-like gonad-stimulating substance (GSS) on starfish ovaries in growing and fully grown states. Gen. Comp. Endocrinol. *172*, 85-89
- Ngamniyom, A., Magtoon, W., Nagahama, Y., and Sasayama, Y. (2011). Expression levels of bone morphogenetic protein 2b in fins of adult Japanese medaka (*Oryzias latipes*) exposed to sex steroid hormones. J. Fish. Aquat. Sci. *6*, 119-129.
- Okubo, K., Takeuchi, A., Chaube, R., Paul-Prasanth, B., Kanda, S., Oka, Y., and Nagahama, Y. (2011). Sex differences in aromatase gene expression in the medaka brain. J. Neuroendocrinology *23*, 412-423.
- Paul-Prasanth, B., Shibata, Y., Horiguchi, R., and Nagahama, Y. (2011). Exposure to diethylstilbesterol during embryonic and larval stages of medaka fish (*Oryzias latipes*) leads to sex reversal in genetic males and reduced gonad weight in genetic females. Endocrinology *152*, 707-717.
- Raghuveer, K., Sudhakumari, C.C., Senthilkumaran, B., Kagawa, H., Dutta-Gupta, A., and Nagahama, Y. (2011). Gender differences in tryptophan hydroxylase-2 mRNA, serotonin, and 5-hydroxytryptophan levels in the brain of catfish, *Clarias gariepinus*, during sex differentiation. Gen. Comp. Endocrinol. *171*, 94-104.

〔Review Article〕

- Shibata, N., Nakamoto, M., Shibata, Y., and Nagahama, Y. (2011). Endocrine regulation of oogenesis in the medaka, *Oryzias latipes*. In Medaka: A Model for Organogenesis, Human Disease and Evolution, H. Takeda et al. eds. (Springer), pp. 267-283.

Laboratory of Molecular Genetics for Reproduction

Associate Professor
TANAKA, Minoru

NIBB Research Fellows:	*NAKAMURA, Shuhei**
	YAMAMOTO, Yasuhiro
Postdoctoral Fellow:	*NAKAMURA, Shuhei*
Graduate Student:	*NISHIMURA, Toshiya*
Visiting Scientist:	*HAAG, Eric*
Technical Assistants:	*KINOSHITA, Chie*
	WATAKABE, Ikuko
	KOBAYASHI, Kayo
Secretary:	*YONEMITSU, Masako*

Laboratory scope

Our laboratory aims to reveal the molecular mechanisms of the formation of the gonads and sex differentiation. We use medaka fish (*Oryzias latipes*) for these purposes and have been generating transgenic medaka (Figure 1) enabling us to identify different cell lineages by fluorescence and to analyze the process of gonad formation and sex differentiation in vivo. Additionally, in order to identify the genes essential for gonadogenesis, we carried out a mutational screening of medaka with defective gonads and are performing a positional cloning. With these two unique analytical methods (visualizing cells and mutants), we are attempting to unveil the fundamental mechanisms of sex differentiation and plasticity common to many organisms.

I. Celluar biphasic process critical for manifestation of the sex

In gonochoristic vertebrates such as medaka and humans, a gene on the sex chromosome is responsible for the determination of sex. Once the process of sex determination is triggered by the gene, the animal begins to develop into either female or male and does not change the direction during its life cycle. The sex differentation is unidirectional. On the other hand, it has been described that sex is a consequence of balancing between female and male process (biphasic process) because sex reversal is often reported even in gonochoristic vertebrates.

As the results of our previous studies, we have revealed that germ cells are critical for the biphasic process. In the absence of germ cells, we found that medaka exhibit complete male secondary characteristics at both endocrine and gene levels (Kurokawa et al., 2007 PNAS) while mutants with an excess number of germ cells cause complete feminization (Morinaga et al., 2007 PNAS). The sex reversal to female is the secondary effect of the over-proliferation of germ cells due to the impairment of an ancient type of the TGFβ signal, the anti-Müllerian hormone (AMH) system (manuscript submitted). Importantly these sex reversals are independent of presence or absence of the sex determination gene on the Y chromosome. This means that the balancing between germ cells and somatic cells is essential for proper manifestation of sex directed by the sex determination gene. We have therefore proposed that the germ cells critically contribute to the biphasic process of sex in medaka.

We have also identified the niche structure (called germinal cradle) that harbors germline stem cells in the medaka ovary for the first time in vertebrates (Nakamura et al., 2010 Science). Since the cells that constitute the cradle express the AMH ligand and receptor, the cradles are the place not only for regulation of proper and continuous production of eggs but also for the biphasic process of manifestation of sex (manuscript submitted). The cradles are also characterized with the expression of *sox9b*, which is specifically expressed in male developing gonads in mammals and is essential for mammalian testis formation (figure 2).

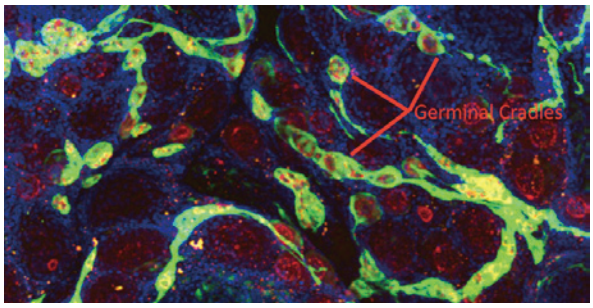


Figure 1. Red bars indicate location of ovarian niche (germinal cradles) expressing the *sox9b* gene. The germ cells are labeled with red.

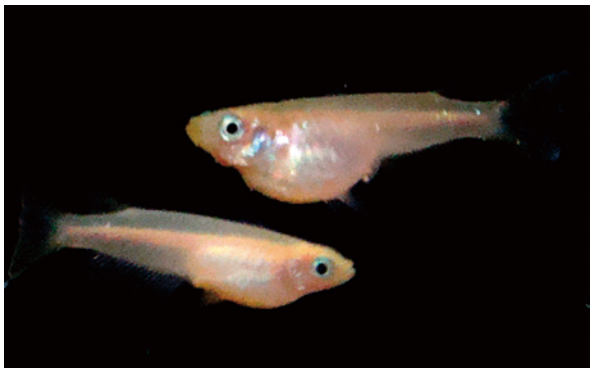


Figure 2. Hetrozygous *sox9b*-mutant medaka have a normal appearance but some exhibits female to male sex reversal.

II. *Sox9b* is essential for germ cell maintenance but not critically contribute to testis determination.

As mentioned above in mammals, *Sox9* is essential for initiation of testis formation and is under transcriptional regulation of mammalian testis determining gene, *Sry*, on the Y chromosome. Consistent with the function of mammalian *sox9* gene, there are several reports on the upregulation of *sox9* expression in testis of other vertebrates. These reports may collectively suggest the conserved role of *sox9* in testis determination across the vertebrate species. However, in medaka, *sox9* is expressed not only in Sertoli cells of the testis but also in germinal cradles with germline stem cells. This puts into doubt the conventional story of a conserved role of *Sox9*. Therefore we have isolated two *sox9b* mutant medaka with different alleles.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

The phylogenetic and syntenic analyses show that *sox9b* is one of the co-orphologues of mammalian *Sox9* in medaka. We have found that only *sox9b* is expressed in the developing somatic (supporting) cells. This indicates that *sox9b* is the functional homologue of mammalian *Sox9* in medaka gonads.

The detailed examination of developing gonads shows that the number of germ cells are reduced in the *sox9b* mutant. This is consistent with a decreasing level of mitotic activity and an increasing level of apoptosis of germ cells in the mutant. The reduced levels parallel the mutant allele number, suggesting the dose-dependent effect of *sox9b* function in the phenotype. Very interestingly, even in the homozygous mutant, the gonad is formed, indicating that identity of the supporting cells to develop into the gonadal somatic cells is retained with the lack of *sox9b* function. In addition to the decreasing germ cell phenotype, the heterozygous mutant with XX chromosomes (genetically female) exhibits masculinization. This is a completely opposite phenotype of sex reversal (male to female) from that observed in mammals

We have found that the extracellular matrix (ECM) that separates interstitial regions from germ cells and supporting cells is largely disorganized in the mutant gonads. The germ cells often protrude blebs from the discontinuous ECM, suggesting that cellular association is impaired in the mutant. Chimeric analyses between the wild type and the mutant clearly demonstrates that mutant cells, which have the ability to develop into the gonads, are inclined to be expelled from the chimeric gonads (figure 3). The degree of contribution is dependent on the number of functional *sox9b* alleles. From these results, sox9b functions to maintain the germ cells through the regulation of ECM (Nakamura et al., 2012 PLoS ONE) .

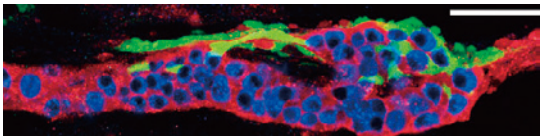


Figure 3. Chimeric gonad between wild type (red) and *sox9b*-mutant (green) cells. The mutant cells have the ability to develop into gonadal somatic cells but contribute less to the gonad than the wild type cells. Blue cells are germ cells.

Next we addressed the possible involvement of *sox9b* in the sex determination process. As mentioned above, the loss of sox9b function results in development into male gonads. The expression analysis demonstrates that the gonads in the XX mutant express male-specific genes, showing that in the XX mutants testicular development proceeds normally as in wild-type XY medaka. The sex reversal phenotype is rescued by one copy of a functional *sox9b* gene, Unlike in mammals, transgenic medaka with three copies of functional *sox9b* do not show any sign of masculinization. From these data, we have concluded that *sox9b* is not a critical contributor of male sex determination.

Then the next question is how the sex reversal to male occurs in the *sox9b* XX mutant. We have suspected that the loss of germ cells might cause the sex reversal. To confirm this hypothesis, we have tried to recover the number of germ cells in the *sox9b* mutant. Since the heterozygous *hotei* mutant possesses increasing mitotic activity, a compound mutant with *hotei* mutant allele and *sox9b* mutant allele were generated and analyzed. The heterozygous compound mutant had the normal number of germ cells and did not display sex reversal. This demonstrates that the sex reversal is primarily due to the loss of germ cells (Nakamura et al., 2012 PLoS ONE).

III. Functional divergence of *sox9* explains the different configuration of ovaries between medaka and mammals.

In medaka, *sox9b* contributes to the maintenance of germ cells including germline stem cells. Our results show that the testis-determining function of mammalian sox9 appends to the conserved role of sox9 in germ cell maintenance as neofunctionalization. With the acquisition of male determining function, sox9 expression was lost in the mammalian developing ovary. This is very well consistent with the loss of, or low number of, germline stem cells in the mammalian ovary. (figure 4) .

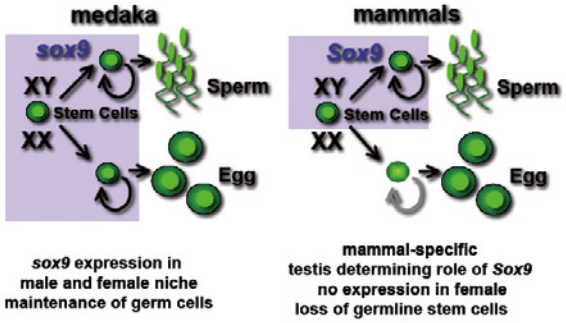


Figure 4. As sox9 acquired male determining functionality its expression was lost in the mammalian ovary.

Publication List

〔Original paper〕

- Hano, T., Oshima, Y., Kinoshita, M., Tanaka, M., Mishima, N., Wakamatsu, Y., Ozato, K., Shimasaki, Y., and Honjo, T. (2011). Evaluation of the effects of ethinylestradiol on sexual differentiation in the olvas-GFP/STII-YI medaka (transgenic *Oryzias latipes*) strain as estimated by proliferative activity of germ cells. Aquatic Toxicol. *104*, 177-184.

〔Review papers〕

- Nakamura, S., Kobayashi, K., Nishimura, T., and Tanaka, M. (2011). Ovarian germline stem cells in the teleost fish, medaka (*Oryzias latipes*). Int. J. Biol. Sci. *7*, 403-409.
- Naruse, K., Tanaka, M., and Takeda, H. (eds). (2011). "Medaka" a model for organogenesis, human disease, and evolution. total 387 pages. Springer-Verlag, Tokyo ISBN 978-4-431-92690-7.

LABORATORY OF PLANT ORGAN DEVELOPMENT

Director-General
OKADA, Kiyotaka

Assistant Professor:	*TATEMATSU, Kiyoshi*
Postdoctoral Fellows:	*IGARASHI, Hisako*
	TOYOKURA, Koichi
	URAWA, Hiroko
	YABE, Kimihiko
Visiting Scientists:	*IKEUCHI, Momoko*
	IWASAKI, Akira
	NAKATA, Miyuki
	TAMESHIGE, Toshiaki
	*TOYOKURA, Koichi**
Technical Assistants:	*HARA, Reiko*
	MATSUMOTO, Miwako
	NAKAMORI, Chihiro
	SUGIMOTO, Nagisa
	TSUZUKI, Yumiko
Secretary:	*SAKAGAMI, Mari*

Plant organs, leaves, flowers, and roots show impressive, symmetrical shapes, based on an ordered arrangement of differentiated cells. The organs are formed from a group of undifferentiated cells located at the tip of the stem or the root. In the case of leaves, the process of organogenesis starts with the formation of a leaf primordium in the peripheral zone of the shoot apical meristem (SAM) at a fixed position, following an order called phyllotaxis. Cells in the primordium then proliferate and differentiate according to three spatially fixed axes: the apical-basal axis, the lateral (central-marginal) axis, and the adaxial-abaxial (foreside-backside) axis. In the course of proliferation and differentiation, plant cells are believed to exchange information with neighboring or separated cells in order to regulate organ architecture. We are trying to understand the mechanisms of information exchange between plant cells during the development of lateral organs, such as leaves, sepals, petals, stamens and carpels by using genetic, biochemical, microsurgical and one-cell gene induction approaches.

I. Genetic approach

Recent studies of *Arabidopsis* mutants show several genes are involved in the axes-dependent control of lateral organ development. The adaxial- and abaxial-specific tissue differentiation in the leaf primordium is determined by the precise expression of the adaxial marker genes, *HD-Zip III* including *PHABULOSA* (*PHB*), and the abaxial marker genes, *FILAMENTOUS FLOWER* (*FIL*) and *YABBY*. Using the reporter gene-system, we visualized the function domain of microRNA165/166 (miR165/166), which targeted the *HD-Zip III* messenger RNA, and showed that miR165/166 act in the cells locating in the abaxial side and determine the adaxial-specific expression of PHB. One of the *MIR165/166* genes, *MIR165A*, is expressed in the abaxial epidermal cells. We revealed by the visualizing reporter genes that *MIR165A* is enough to repress the PHB expression in the cells located in the abaxial side. These results suggested that miR165 is likely to move cell to cell. We also analyzed the function domain of miR165 when primary transcript of *MIR165A* was expressed by the *FIL*-promoter. this revealed that miR165 can act in the entirety of the leaf primordia, suggesting that there is no physical barrier interfering with miRNA movement between the adaxial- and the abaxial-side.

To examine the mechanisms of establishment of the adaxial-abaxial axis, we isolated novel mutants which show altered patterns of *FIL* promoter::*GFP* expression, and named them *enlarged fil-expression domain* (*enf*). One of them, *enf1*, forms leaves with enlarged and reduced *FIL*-expression domains, indicating that *ENF1* is involved in the fixation or maintenance of the position of the adaxial-abaxial boundary (Toyokura et al., 2011). We revealed that the *ENF1* gene encodes SUCCINIC SEMALDEHYDE DEHYDROGENASE, which catalyzes the conversion of succinic semialdehyde (SSA) to succinate, and is strongly expressed in leaf primordia although its expression was not found in the SAM. Exogenous application of SSA at the side of the SAM induced the adaxial-characters on the abaxial side of newly formed leaves (Figure 1). These results indicate that SSA and/or its derivatives affect the axis-dependent cell fate in leaf primordia. We also isolated some suppressor mutants of *enf1*, which show normal leaf shapes. We determined the genes, which have the mutation for the suppression of *enf1* phenotype, using next-generation sequence methods, and revealed that one of them has the mutation in a gene which encodes a transaminase enzyme.

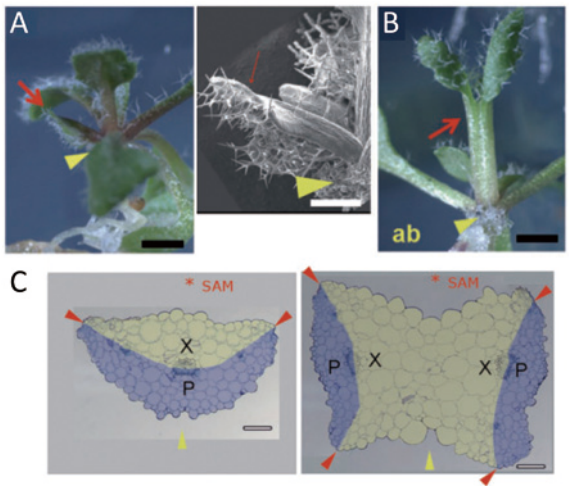


Figure 1. Effect of exogenous application of SSA on the adaxial-abaxial polarity in leaf primorda. We applied SSA-containing lanolin paste on the side of the Arabidopsis SAM, and checked the newly formed leaves after 1-2 weeks. Yellow arrowheads indicate the application site, and red arrows represent the newly formed leaves. (A) The lateral views of extreme effect (class I) of SSA taken by stereomicroscopy (left) and scanning electron microscopy (right). Newly formed leaves have the adaxial-characteristics on the abaxial-side. (B) The abaxial view of strong effect (class II) of SSA. Newly derived leaf developed with two laminas with upper sides facing each other. (C) The sections of the leaf petiols of the control leaf (left) and class II leaf (right). In class II leaves, the adaxial identity was observed not only on the side facing the SAM but also on the abaxial side.

In contrast, another mutant, *enf2*, has leaves with an enlarged *FIL*-expression domain, and the *ENF2* gene

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

encoded a plastid-localized unknown protein. Chloroplast development was repressed in a severe allele of the *enf2* mutant. Exogenous application of inhibitors for the gene expression of chloroplast genome-encoding genes to *Arabidopsis* seedlings mimics the defects of the *FIL*-expression pattern by *enf2* mutation. These results suggest that the expression of chloroplast genome-encoding genes is required for the determination of adaxial-abaxial polarity.

A line of unique oblong cells is found at the marginal edge of leaves. We noticed that a homeobox-related gene, *PRESSED FLOWER* (*PRS*) and its homolog, *WOX1*, are required for forming the margin-specific cells. The analyses of *prs wox1* double mutants indicate that the margin-specific cells act as a physical barrier separating the epidermal cells of the adaxial-side surface from those of the abaxial-side surface. Genetic analyses also showed that *PRS* and *WOX1* function in blade outgrowth downstream of the adaxial-abaxial polarity. The expression of *PRS* and *WOX1* was observed in two middle mesophyll layers and the marginal region of leaf primordia. We also revealed that the expression of *PRS* and *WOX1* are upregulated by the function of *FIL* and *YAB* genes, and repressed by those of the abaxial-specific genes, *KANADI*. We propose that the blade outgrowth and the adaxial-abaxial patterning during leaf developments are controlled by the middle domain-specific function of *PRS* and *WOX1* genes.

To reveal how floral organs fix their forms through development processes, we analyzed mutants named *folded petals* (*fop*). In the early stage of flower development, *fop* petals are similar to those of wild type, but the petals cannot grow through the narrow space between the sepal and the anther in the flower buds. In *fop* mutants, petals grow straight when the sepals are removed in the early stage of flower development. We found that the petal epidermal cells of *fop* petals bear epicuticular nanoridges like as those of wild type. FOP proteins are related to wax/cutin synthesis or transport. Thus, we proposed that wax/cutin components secreted by FOP proteins on the surface of the petal epidermis might act as a lubricant before the epicuticular nanoridge formation.

II. Biochemical approach

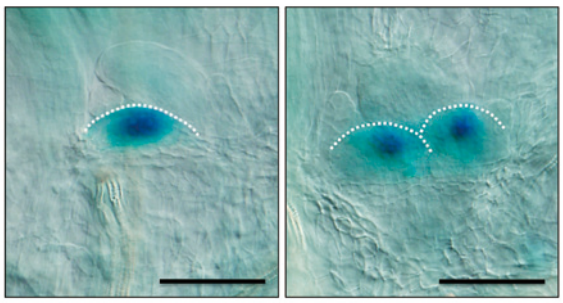


Figure 2. A purified fraction increasing the number of the SAMs in Arabidopsis seedlings. Purified fractions obtained from the apoplastic region of the curds of cauliflower were applied to Arabidopsis *CLV3* promoter:GUS transgenic lines. A purified fraction increased the number of the SAMs (Right) compared to the control (Left). GUS expression driven by the *CLV3* promoter indicates the position of the SAM. White dashed lines indicate the shape of the SAM. Bars indicate 200 μm.

We are taking a biochemical approach to study of the intercellular signaling system by analyzing small peptides as candidates for intercellular signaling ligands, which are present in the apoplastic region of the SAM. Small peptides were collected from apoplast fractions of the curds of cauliflower (*Brassica oleracea* L. var. *botrytis*). Through a biological assay using purified fractions, we obtained a fraction, which increased the number of SAMs after exogenous application of the fraction to Arabidopsis seedlings (Figure 2). Then we analyzed the peptide sequences included in the fraction by LC-MS/MS methods, and a putative lipid transfer protein was identified. We are preparing a recombinant protein of the candidate protein and examining effects of the recombinant protein on the development and growth of Arabidopsis seedlings.

III. Microsurgical approach

We are also carrying out microsurgical approaches using novel laser-ablating microscopy to investigate the cell-to-cell signaling system working during leaf development. When we ablated a small number of cells at the peripheral of the SAM of young *Arabidopsis* seedlings, some of the newly generated leaves changed to a filamentous structure lacking the adaxial-abaxial identity, suggesting that a flow of signal(s) from the SAM to the leaf primordia has a role of fixing the abaxial-adaxial polarity. We also examined the leaf serration mechanism by ablating some cells at the margin in serrated leave of *E. californica*, which form the leaflet acropetally. When leaflet incipient site is ablated, a new leaflet initiated at intact tissue near leaf tip, escaping from the ablated site, suggesting that a regular space of the leaflet initiation site is actively kept from the leaf tip.

IV. One-cell gene induction approach

As a new tool for examining the intercellular communication system, we are developing a one-cell gene-induction system *in planta* using the InfarRed Laser Evoked Gene Operator (IR-LEGO) system, and showed gene expression in only a single cell of the root. When *WUSSEL* (*WUS*) gene, which functions in maintenance of the SAM, was ectopically expressed in the root, callus or a shoot-like structure was generated at the root tip. We observed induction of ectopic cell division when *WUS* was ectopically expressed in lateral-root-cap cells.

Publication List

〔Original papers〕

- Toyokura, K., Watanabe, K., Oikawa, A., Kusano, M., Tameshige, T., Tatematsu, K., Matsumoto, N., Tsugeki, R., Saito, K., and Okada, K. (2011) Succinic semialdehyde dehydrogenase is involved in the robust patterning of Arabidopsis leaves along the adaxial-abaxial axis. Plant Cell Physiol. *52*, 1340-1353.
- Ueda, M., Matsui, K., Ishiguro, S., Kato, T., Tabata, S., Kobayashi, M., Seki, M., Shinozaki, K., and Okada, K. (2011) Arabidopsis *RPT2a* encoding the 26S proteasome subunit is required for various aspects of root meristem maintenance, and regulates gametogenesis redundantly with its homolog, *RPT2b*. Plant Cell Physiol. *52*, 1628-1640.

DIVISION OF MOLECULAR NEUROBIOLOGY

Professor
NODA, Masaharu

Associate Professor
SHINTANI, Takafumi

Assistant Professors:	*SAKUTA, Hiraki* *HIYAMA, Takeshi*
Technical Staff:	*TAKEUCHI, Yasushi*
NIBB Research Fellows:	*YOSHIDA, Masahide* *KUBOYAMA, Kazuya*
Postdoctoral Fellows:	*FUJIKAWA, Akihiro* *SUZUKI, Ryoko* *NISHIHARA, Eri* *MATSUMOTO, Masahito*
Graduate Students:	*TANI, Sachiko* *SAKURABA, Jyuichi* *MIYAZAKI, Yuriko*
Technical Assistants:	*HATTORI, Nobuko* *TOMITA, Nao* *MIURA, Seiko* *NAKANISHI, Norie* *DOKYO, Yumi* *MIYAZAKI, Emi* *WADA, Kotoe*
Secretary:	*KODAMA, Akiko*

We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using the visual systems of chicks and 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 a fundamental feature 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 axon 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.

APC2 is distributed along microtubules in growth cones as well as axon shafts of retinal axons. The knockdown of *Apc2* in chick retinas reduced the stability of microtubules in retinal axons and yielded abnormal behaviors including a reduced response to ephrin-A2 and misprojection in the tectum without making clear target zones. Recently, we generated *Apc2*-deficient mice by a gene-targeting technique. We have found that the mutant mouse displays severe laminary defects in some brain regions including the cerebral cortex and cerebellum. We are now analyzing the phenotype of the *Apc2*-deficient mouse in more detail to clarify the function of APC2 in the development of the CNS.

II. Development of direction-selective retinal ganglion cell subtypes

Visual information is transmitted to the brain by roughly a dozen distinct types of retinal ganglion cells (RGCs) defined by characteristic morphology, physiology, and central projections. However, because few molecular markers corresponding to individual RGC types are available, our understanding of how these parallel pathways develop is still in its infancy.

The direction of image motion is coded by direction-selective (DS) ganglion cells in the retina. Particularly, the ON DS ganglion cells are critical for mediating the optokinetic reflex. We generated a knock-in mouse in which *SPIG1*-expressing cells are labeled with GFP. We successfully visualized both upward- motion-preferring and downward-motion-preferring ON DS ganglion cells (SPIG1$^+$ and SPIG1$^-$ ganglion cells, respectively) by a combination of genetic labeling and conventional retrograde labeling in the medial terminal nucleus.

A key circuit module of DS ganglion cells is a spatially asymmetric inhibitory input from starburst amacrine cells in the retina. However, it was not known how and when this circuit asymmetry is established during development. Therefore, we photostimulated mouse starburst cells targeted with channelrhodopsin-2 (CR-2) while recording from single SPIG1$^+$ DS cells. We then followed the spatial distribution of synaptic strengths between starburst and DS cells during early postnatal development before these neurons can respond to physiological light stimuli, along with confirmation of connectivity by monosynaptically restricted trans-synaptic rabies viral tracing. As a result, we found that random or symmetric synaptic connections from starburst amacrine cells are established as early as postnatal day 6, and that asymmetric inhibitory synaptic inputs are subsequently developed over a 2-day period (Figure 1).

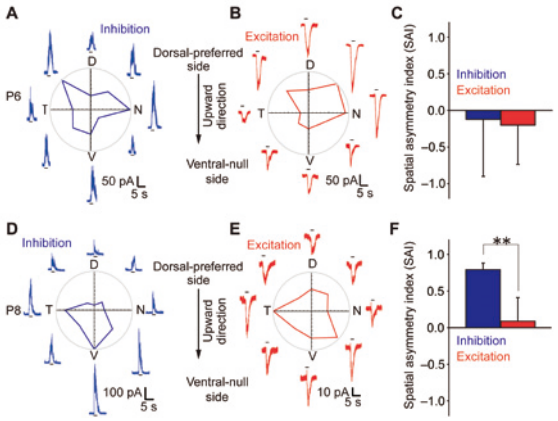


Figure 1. CR-2-assisted circuit mapping at P6 and P8. Recordings from SPIG1$^+$ cells at P6 (A–C) and P8 (D–F). (A–E) Inhibitory (A, D) and excitatory (B, E) postsynaptic currents elicited in a SPIG1$^+$ cell by the

photostimulation of the surrounding starburst amacrine cells. Polar plots are also shown. (C, F) Spatial asymmetry index (SAI) for inhibition and excitation. Error bars, s.d.

Analysis of gene-expression profiles in the two types of ON DC ganglion cells is now under way. This will shed light on molecular mechanisms underlying the differentiation and distinct circuit formation of the two DS ganglion cell types.

III. Physiological roles of protein tyrosine phosphatase receptor type Z

Protein-tyrosine phosphatase receptor type Z (Ptprz, also known as PTPζ/RPTPβ) is a member of the R5 receptor-like protein tyrosine phosphatase (RPTP) subfamily. Ptprz is predominantly expressed in the brain and its physiological importance has been demonstrated through studies with *Ptprz*-deficient mice. Ptprz modulates hippocampal synaptic plasticity: adult *Ptprz*-deficient mice display impairments in spatial and contextual learning. Ptprz is expressed also in the stomach, where it is used as a receptor for VacA, a cytotoxin secreted by *Helicobacter pylori*: *Ptprz*-deficient mice are resistant to gastric ulcer induction by VacA. Although our understanding of the physiological functions of Ptprz is thus advancing, our knowledge about its biochemical properties such as substrate specificity are still limited.

We previously identified G protein-coupled receptor kinase interactor 1 (Git1), membrane-associated guanylate kinase, WW and PDZ domain-containing 1 (Magi1), and GTPase-activating protein for Rho GTPase (p190RhoGAP) as substrates for Ptprz by developing a new genetic method named "yeast substrate-trapping system". We had already identified a dephosphorylation site at Tyr-1105 of p190RhoGAP; however, the structural determinants employed for substrate recognition of Ptprz have not been fully defined.

This year, we revealed that Ptprz selectively dephosphorylates Git1 at Tyr-554, and Magi1 at Tyr-373 and Tyr-858 by *in vitro* and cell-based assays. Of note, alignment of the primary sequences surrounding the target phosphotyrosine residue in these three substrates showed considerable similarity, suggesting a consensus motif for recognition by Ptprz (Figure 2A). We then estimated the contribution of surrounding individual amino acid side chains to the catalytic efficiency by using fluorescent peptides based on the Git1 Tyr-554 sequence *in vitro* (Figure 2B), and thereby deduced the typical substrate motif for the catalytic domain of Ptprz (Figure 2C). Furthermore, we found by database screening that the substrate motif is present in several proteins, including paxillin at Tyr-118, its major phosphorylation site (Figure 2A). Expectedly, we verified that Ptprz efficiently dephosphorylates paxillin at this site in cells.

Although initially viewed as broad specificity "housekeeping" enzymes, PTPs are actually highly selective enzymes. Our knowledge of substrates for Ptprz suggests that Ptprz dephosphorylates multiple proteins associated with actin remodeling, which plays important roles in synaptic plasticity in the adult brain and cell adhesion/migration of epithelial cells.

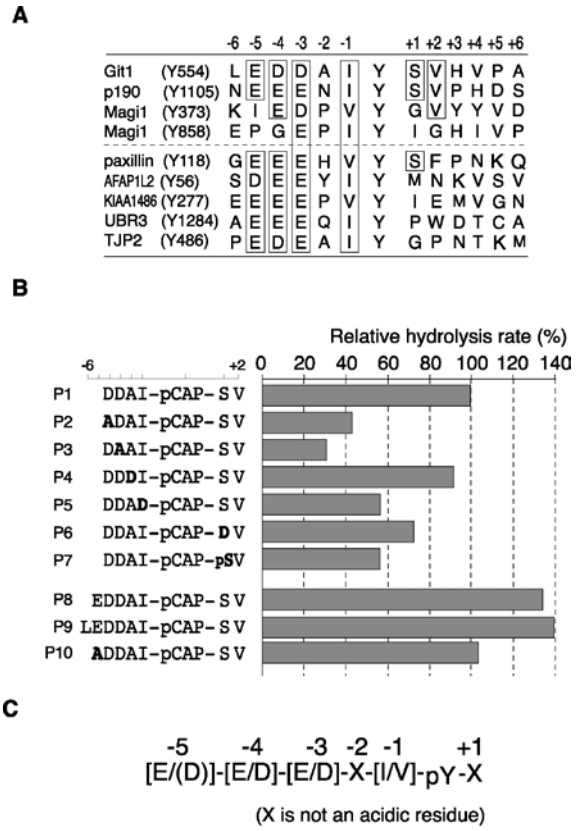


Figure 2. Identification of the substrate motif for Ptprz. (A) Primary sequences surrounding the target phosphotyrosine residues located in Git1 (Tyr-554), p190RhoGAP (Tyr-1105), Magi1 (Tyr-373 and Tyr-858), and paxillin (Tyr-118) aligned, together with four substrate candidates, AFAP1L2 (Tyr-56), KIAA1486 (Tyr-277), UBR3 (Tyr-1284), and TJP2/ZO-2 (Tyr-486). Boxed residues are conserved in at least three substrate sequences. (B) Kinetic analysis for the hydrolysis of Git1-derived peptide substrates by PtprzICR *in vitro*. The amino acid sequences of the substrate peptides (P1–10) used in this study are shown on the left of the figure. P1, P8, and P9 peptides correspond to Git1550–556, Git1549–556, and Git1548–556, respectively, except that the Tyr residue at 554 is replaced with a phosphotyrosine mimic, pCAP. P1–P7 and P10 peptides are Git1 peptide derivatives, in which the substituted amino acid residues are shown in bold. (C) Proposed consensus substrate-site motif for the PTP catalytic domain of Ptprz.

IV. Brain systems for body-fluid homeostasis

Mammals have a set of homeostatic mechanisms that work together to maintain body-fluid osmolality at near 300 mOsm/kg through the intake or excretion of water and salt. Although this homeostatic osmoregulation is vital, the mechanisms for the detection of these fluctuations have not been fully elucidated. To date, transient receptor potential vanilloid 1 (TRPV1), a cation channel, has been implicated in body-fluid homeostasis *in vivo* based on studies with the *TRPV1*-knockout mouse. However, the response of TRPV1 to hypertonic stimuli has not been demonstrated with heterologous expression systems so far, despite intense efforts by several groups. Thus, the molecular entity of the hypertonic sensor *in vivo* still remains controversial.

Very recently, we found that the full-length form of TRPV1 is sensitive to an osmotic increase exclusively at around body temperature by using human embryonic kidney 293 cells

stably expressing rat TRPV1 (HEK293-TRPV1 cells). We applied a hypertonic solution (350 mOsm) to HEK293-TRPV1 cells at various temperatures from 24 to 40°C (Figure 3A and B, HEK293-TRPV1). When the extracellular environment was changed from an isotonic (300 mOsm) to hypertonic (350 mOsm) solution at 24°C, $[Ca^{2+}]_i$ increased only slightly in HEK293-TRPV1 cells, but not in native HEK293 cells (Figure 3A and B). Surprisingly, the magnitude of the osmosensitive response markedly increased with temperature, peaking at around 36°C, which is close to normal mammalian body temperature (~37°C) (Figure 3A and B). In contrast, control HEK293 cells did not respond to the hypertonic stimulus at any temperature. Importantly, the response at 36°C showed a robust increase over a hypertonic range, but a small decrease over a hypotonic range (Figure 3C). A TRPV1 antagonist, capsazepine, and a nonspecific TRP channel inhibitor, ruthenium red, completely blocked the increase in $[Ca^{2+}]_i$. These results endorse the view that the full-length form of TRPV1 is able to function as a sensor of hypertonic stimuli.

We also demonstrated that the osmosensitivity of TRPV1 at 36°C is further enhanced by other activating stimuli, such as protons (pH) or capsaicin, indicating that osmosensitivity of TRPV 1 is synergistically enhanced by these distinct activating stimuli. (Figure 3D and E). Our findings thus indicate that TRPV1 integrates multiple different types of activating stimuli, and that TRPV1 is sensitive to hypertonic stimuli under physiologically relevant conditions.

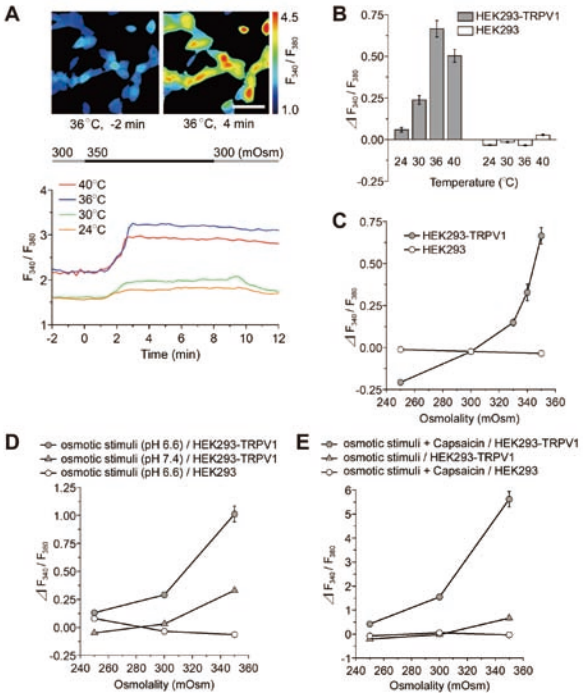


Figure 3. Osmosensitivity of TRPV 1 is synergistically enhanced by distinct activating stimuli such as temperature and protons. (A) Temperature-dependent sensitivity to osmotic stimuli. Representative pseudo-color images of $[Ca^{2+}]_i$ in HEK293-TRPV1 cells at 2 min before (upper left) and 4 min after (upper right) perfusion with a hypertonic solution (350 mOsm) at 36°C. Representative single cell traces of the fluorescence ratio at a temperature of 24, 30, 36, or 40°C (lower graph). The line on the top indicates the timing of the change from 300 mOsm (gray) to 350 mOsm (black). (B) Summary of the change in the fluorescence ratio during perfusion with the hypertonic solution at various temperatures in HEK293-TRPV1 (filled bars) and HEK293 (open bars) cells. Data are differences between fluorescence ratios before and after the change of the solution. The maximal sensitivity of the HEK293-TRPV1 cells to the hypertonic stimulation was observed at 36°C. (C) Summary of the change in the fluorescence ratio during the perfusion of various solutions differing in osmolality at 36°C. Osmolality-dependent changes in the $[Ca^{2+}]_i$ were observed in HEK293-TRPV1 cells (filled circles), but not in HEK293 cells (open circles). (D) Summary of the change in the fluorescence ratio during the perfusion of solutions with the respective osmolality and pH at 36°C. The osmotic response of the $[Ca^{2+}]_i$ in HEK293-TRPV1 cells (filled circles) was enhanced by acidification over the entire osmolality range (compare with filled triangles). This was evident especially in the hypertonic range. (E) Summary of the change in the fluorescence ratio in HEK293-TRPV1 cells upon application of 1.5 nM capsaicin solutions with different osmotic strength. Response of TRPV1 to hyperosmolality was markedly potentiated in the presence of capsaicin (compare filled circles with filled triangles). No response was observed in HEK293 cells (open circles). Error bars, s.e.m.

Publication List

〔Original papers〕

- Fujikawa, A., Fukada, M., Makioka, Y., Suzuki, R., Chow, J.P., Matsumoto, M., and Noda, M. (2011). Consensus substrate sequence for protein-tyrosine phosphatase receptor type Z. J. Biol. Chem. *286*, 37137-37146.
- Nayak, G., Goodyear, R.J., Legan, P.K., Noda, M., and Richardson, G.P. (2011). Evidence for multiple, developmentally regulated isoforms of PTPRQ on hair cells of the inner ear. Dev. Neurobiol. *71*, 129-141.
- Nishihara, E., Hiyama, T.Y., and Noda, M. (2011). Osmosensitivity of transient receptor potential vanilloid 1 is synergistically enhanced by distinct activating stimuli such as temperature and protons. PLoS ONE *6*, e22246.
- Sakamoto, K., Bu, G., Chen, S., Takei, Y., Hibi, K., Kodera, Y., McCormick, L.M., Nakao, A., Noda, M., Muramatsu, T., and Kadomatsu, K. (2011). The premature ligand-receptor interaction during biosynthesis limits the production of growth factor midkine and its receptor LDL receptor-related protein 1 (LRP1). J. Biol. Chem. *286*, 8405-8413.
- Yonehara, K., Balint, K., Noda, M., Nagel, G., Bamberg, E., and Roska, B. (2011). Spatially asymmetric reorganization of inhibition establishes a motion-sensitive circuit. Nature *469*, 407-410.

DIVISION OF BRAIN BIOLOGY

Professor
YAMAMORI, Tetsuo

Associate Professor
WATAKABE, Akiya

Assistant Professors:	*KOMINE, Yuriko*
	SADAKANE, Osamu
Assistant Professor(Specially appointed):	
	KOMATSU, Yusuke#
NIBB Research Fellow:	*OHTSUKA, Masanari*
Technical Staff:	*OHSAWA, Sonoko*
Postdoctoral Fellows:	*TAKAJI, Masafumi#*
	*OHTSUKA, Masanari**
	HATA, Katsusuke#
	GOTO, Ayako#
	SASAKI, Tetsuya
Visiting Scientist:	*HIRAKAWA, Reiko*
Graduate Students:	*NAKAGAMI, Yuki**
	SHUKLA, Rammohan
Technical Assistants:	*NAKAMURA,Tohru*
	TAKEDA, Yuta#
	NAKAGAMI, Yuki
	MORITA, Junko
	IMAI, Akiko
	KOTANI, Keiko
	KON, Yayoi
	MIKAWA, Ryuta
	IMOTO, Eiko
	HIRAYAMA, Yuka#
	TAKAFUJI, Mituse#
	KAJITANI, Tomoki#
	TAKAHASHI, Yoichi#

#: SRPBS (Strategic Research Program for Brain Sciences), NIPS

We are studying genes that are expressed in specific areas of the neocortex in order to understand the principles governing the formation of the primate brain.

I. Genes expressed in specific areas and layers of the neocortex

The neocortex emerged in mammals and evolved most remarkably in primates. To understand the underlying mechanisms of the primate brain we study gene expression patterns within different areas of the neocortex.

We have reported the findings that are schematically illustrated in Figure 1.

Using differential display methods, we found three area-specific expression genes in the primate neocortex. Firstly, *occ1* is specifically expressed in the occipital cortex in the primate brain. Secondly, the other gene that showed marked difference within the neocortex is *gdf7*, a member of the BMP/TGF-β family, which is specifically expressed in the motor cortex of the African green monkey (Watakabe *et al*., J. Neurochem., *76*, 1455-1464, 2001). Thirdly, *RBP* (retinol-binding protein) is preferentially expressed in the association and higher areas in the neocortex (Komatsu *et al*., Cerebral Cortex, 15, 96-108, 2005).

To further screen area-specific molecules systematically in the monkey neocortex, we carried out another round of screening using the RLCS method (Suzuki et al. 1996; Shintani et al. 2004). In this analysis, mRNAs were purified from 4 distinct cortical areas, converted to cDNA by reverse transcription and digested with a pair of restriction enzymes for 2-dimensional analysis. Using the RLCS method we isolated genes that showed marked differences among four areas (area 46, primary motor area, TE and V1) and characterized the expression patterns. Examples of such genes we have previously reported are *testican-1, -2* (*OCC1* related family genes), *5HT1B* and *5HT2A* (primary visual area enriched), which are preferentially expressed in the primary visual cortex, and *SPARC* (an *OCC1* related gene) and *PNMA5* whose expressions are similar to RBP (an association area enriched gene) as shown in Figure 1.

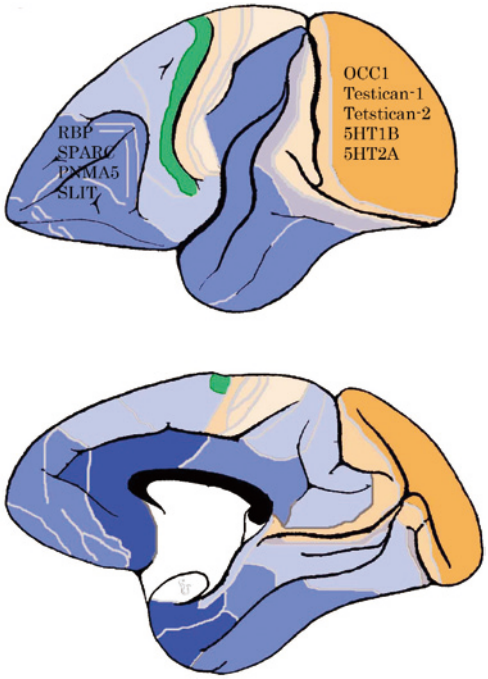


Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

This year, I reviewed our studies over the last ten years and more (Yamamori, 2011). The main discussions and conclusions are the functional significance of the two groups of the genes as shown in Figure 1. The first group of genes are those highly expressed in the primary sensory areas, particularly in the primary visual area (V1). In collaboration with Prof. Hiromichi Sato, Osaka University, we have previously shown that 5HT1B works by enhancing signal to noise (S/N) ratio and 5HT2A works as a gain controller (Figure 2). Recently, it has been reported that, fstl1 (follistatin-like 1), the mouse homologue of OCC1, is highly expressed in mouse dorsal root ganglion (DRG). The function in DRG has been revealed by the other group, showing that Fstl1 is directly bound to Na, K. ATPase, which suppresses sensory evoked presynaptic transmission by enhancing K+ influx and suppressing voltage dependent Ca++ influx (Li et al., Neuron 69, 974-987, 2011). This mechanism likely also works in primate V1 where OCC1 (macaque homologue of fstl1) is abundantly expressed. The

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

major difference between macaque V1 and mouse tissues that highly express OCC1 and fstl1, respectively, is that expression of OCC1 in V1 is activity-dependent whereas fstl1 expression in mouse tissues is not activity-dependent (Takahata et al., J. Chem Neuroanat., 35, 146-157, 2008).

These data of 5HT1B, 5HT2A and OCC1 expressions and functions strongly suggest that these genes control or modulate inputs from the retina in activity-dependent manners at the expression and functional levels (Figure 2). Such expression patterns specific to primates have come about during the course of primate evolution (Takahata et al., 2011).

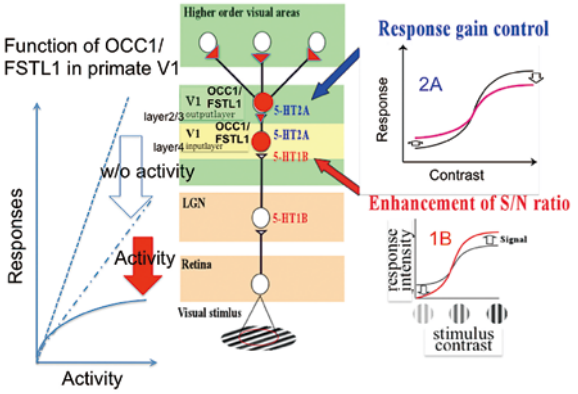


Figure 2. Roles of 5HT1B, 5HT2A and OCC1 in primate V1. Right: Possible roles of OCC1 in primate V1. As shown in rodent DRG, OCC1 is likely bound to Na, K, ATPase (NKA) and suppresses visual stimuli evoked synaptic transmission. The suppression is further enhanced by activity-dependent gene expression. Left: Responses to a stimulus with a low contrast are suppressed by the activation of 5HT1B and are enhanced by that of 5HT2A. At a high stimulus contrast, the effects are reversed. S/N ratio analysis demonstrated that the activation of 5HT1B contributes to the increase in S/N ratio. We predict that 5HT2A works as a gain controller to compensate for the gain or loss caused by 5HT1B receptors at a certain stimulus contrast. The left picture is drawn by Drs. Hiromichi Sato and Satoshi Shimegi (Osaka University) and is cited from Yamamori, T., Progress in Neurobiology 94, 201-222, 2011.

The second group of genes is selectively expressed in the association areas of the primate neocortex. Among the association area selective-expression genes, RBP, PNMA5, SPARC, and SLT2 are expressed in layers 2, 3, 5 and 6 in association areas and most abundantly prefrontal cortex and higher sensory association areas. SLIT1, on the other hand, is expressed in layers 2, 4 and 5 and most abundantly in layer 4 in the prefrontal cortex and to a lesser extent in the sensory association areas (Sasaki et al., Cerab. Cortex. 20, 2496-2510, 2010). Although the functions of these association area-selective genes are unknown, SLIT1 works to enhance dendritic branching in developing cortical neurons in rodents and thus presumably has a similar function in the primate neocortex.

II. Multisensory Information Facilitates Reaction Speed by Enlarging Activity Difference between Superior Colliculus Hemispheres in Rats

Animals can orient responses faster to multisensory stimuli than to unisensory stimuli. We have been working on studies of how animals can respond to multisensory stimulation in collaboration with Professor Yoshio Sakurai (Kyoto University) using an experimental system that evaluates audio-visual discrimination tasks in rats (Sakata et al., Exp Brain Res. 159, 409-417, 2004). Using this system, we identified the brain areas of V2L that are specifically involved in multisensory (visual and auditory) stimuli (Hirokawa et al., Neuroscience, 1402-1417, 2008). These studies were further extended to the superior colliculus (SC).

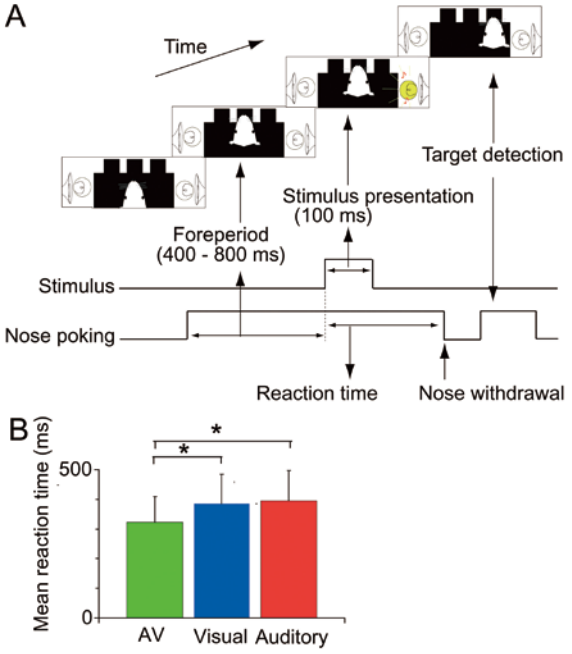


Figure 3. Two-alternative spatial discrimination tasks based on auditory and/or visual cues.
A) Timing of task events. Nose poking into the central hole initiated a trial. After a variable foreperiod, a cue stimulus was delivered from the left or right, randomly chosen from visual, auditory and audiovisual stimuli. Rats responded to the stimulus by withdrawing from the central hole and selected the direction of the cue stimulus by poking their heads into the hole ipsilateral to the stimulus. (B) Mean reaction time for each type of stimulus across sessions and rats (*p<0.001 in ANOVA and post hoc Tukey test). Error bars, standard deviation. This figure is cited from Hirokawa et al., Plos One, 2011.

The superior colliculus (SC), which receives multiple inputs from different sensory modalities, is thought to be involved in the initiation of orienting responses. However, the mechanism by which multisensory information facilitates orienting responses had not yet been understood. We demonstrate that multisensory information modulates competition among SC neurons to elicit faster responses. We conducted multiunit recordings from the SC of rats performing a two-alternative spatial discrimination task using auditory and/or visual stimuli. We found that a large population of SC neurons showed direction-selective activity before the onset of movement in response to the stimuli irrespective of stimulation modality. Trial-by-trial correlation analysis showed that the premovement activity of many SC neurons increased with faster reaction speed for the contraversive movement, whereas the premovement activity

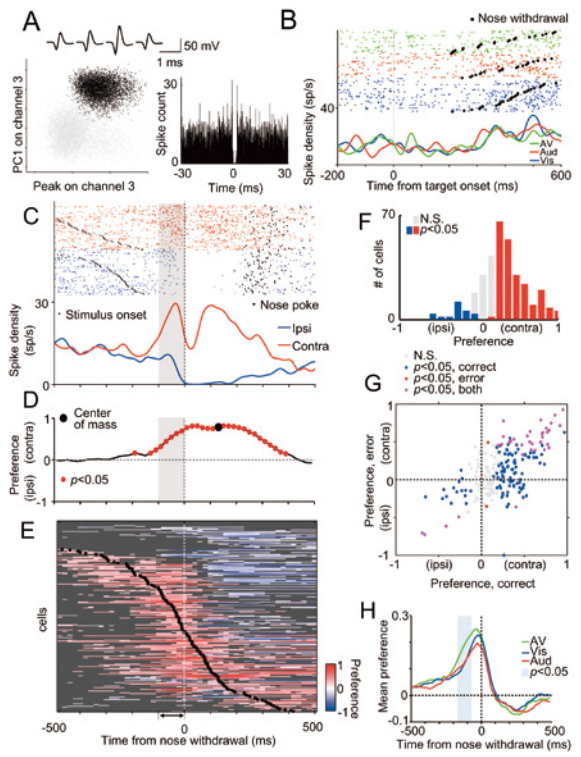


Figure 4. Direction preference preceding locomotion.
(A) Example of tetrode isolation for single unit. (Top) Average waveforms of spikes recorded on the four tetrode channels correspond to a cluster in black in the scatter plots below. The scatter plots indicate the peaks of waveforms from channel 3 plotted against principal component 1 (PC1) from channel 3 recorded from a tetrode. (Right) Corresponding autocorrelation functions with a window of ±30 ms. The bin size is 0.1 ms. (B) Rasters and spike density functions (SDFs) aligned to the onset of the target for isolated single unit (black cluster) during spatial discrimination task. The data were derived from only correct trials in responses to contralateral stimuli divided into visual, auditory and audiovisual stimulus conditions. Trials in the raster plot are sorted according to reaction time. (C) The data of the same neuron as above were aligned to movement onset and divided into ipsiversive and contraversive movement trials regardless of the modality of the stimulus. (D) Direction preference index of above unit was calculated using receiver operating characteristics (ROC) analysis for each time point ($p<0.05$, permutation test). (E) Direction preference curves for all cells (225 cells). Each row corresponds to one cell. Cells were sorted by the time of center of mass of significant positive preferences. Red and blue colors indicate preference indices with significant positive and negative values ($p<0.05$, permutation test), respectively, and gray points correspond to insignificant values. (F) Distribution of direction preference index in premovement period across population (225 cells). (G) Preference calculated for correct trials plotted against preference calculated for erroneous trials in premovement period. (H) Direction preferences calculated for each stimulus modality in each neuron and were averaged for each stimulus modality condition. The blue period shows a significant modulation of the preference under the multisensory condition (AV>unimodal, ANOVA with post hoc tukey test, $p<0.05$). This figure is cited from Hirokawa et al., 2011.

of another population of neurons decreased with faster reaction speed for the ipsiversive movement. When visual and auditory stimuli were presented simultaneously, the premovement activity of a population of neurons for the contraversive movement was enhanced, whereas the premovement activity of another population of neurons for the ipsiversive movement was depressed. Unilateral inactivation of SC using muscimol prolonged reaction times of contraversive movements, but it shortened those of ipsiversive movements. These findings suggest that the difference in activity between the SC hemispheres regulates the reaction speed of orienting responses, and multisensory information enlarges the activity difference resulting in faster responses (Hirokawa et al., 2011).

The SC has been considered as a brain region that is causally essential for integrating visual and auditory information, as demonstrated by a study of excitotoxic lesion of the SC in cats. On the other hand, our study showed that unilateral SC inactivation did not affect the facilitation of reaction speed to multisensory stimuli. It is therefore possible that different neural networks are responsive for accurate sensory detection and rapid response, respectively. In consistent with a line of this idea, we previously showed using the same technique as this study that the inactivation of the secondary visual cortex (V2L) suppresses the facilitation of reaction speed (Hirokawa et al., Neuroscience, 1402-1417, 2008).

Publication List

〔Original papers〕

- Hirokawa, J., Sadakane, O., Sakata, S., Bosch, M., Sakurai, Y., and Yamamori, T. (2011). Multisensory information facilitates reaction speed by enlarging activity difference between superior colliculus hemispheres in rats. PLoS ONE *6*, e25283.
- Kitsukawa, T., Nagata, M., Yanagihara, D., Tomioka, R., Utsumi, H., Kubota, Y., Yagi, T., Graybiel, A.M., and Yamamori, T. (2011). A novel instrumented multipeg running wheel system, Step-Wheel, for monitoring and controlling complex sequential stepping in mice. J Neurophysiol. *106*, 479-487.
- Rossini, L., Moroni. R.F., Tassi, L., Watakabe, A., Yamamori, T., Spreafico, R., and Garbelli, R. (2011). Altered layer-specific gene expression in cortical samples from patients with temporal lobe epilepsy. Epilepsia *52*, 1928-1937.

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

- Takahata, T., Shukla, R., Yamamori, T., and Kaas, J.H. Differential expression patterns of striate cortex-enriched genes among old world, new world, and prosimian primates. Cereb. Cortex. 2011 Nov. 7.

〔Review article〕

- Yamamori, T. (2011). Selective gene expression in regions of primate neocortex: implications for cortical specialization. Prog. Neurobiol. *94*, 201-222.

DIVISION OF BRAIN CIRCUITS

Professor
MATSUZAKI, Masanori

Postdoctoral Fellows:	*MASAMIZU, Yoshito*
	TANAKA, Yasuhiro
	TANAKA, Yasuyo
Visiting Scientist:	*HIRA, Riichiro*
	*OHKUBO, Fuki**
Graduate Student:	*OHKUBO, Fuki*
Technical Assistants:	*HIMENO, Miki*
	SAITO, Junko
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.

I. Development of novel techniques to photostimulate cortical neurons in vivo

We developed a method that uses Channelrhodopsin-2 (ChR2; blue-light-activated cation channel) for transcranial optogenetic stimulation. This method is based on scanning a light beam over the brain, thereby photostimulating ChR2-expressing neurons in intact mice. The laser illumination induced forelimb movement in two areas; rostral forelimb area (RFA) and caudal forelimb area (CFA). The motor forelimb areas determined by this photostimulation mapping corresponded to those determined by intracortical microstimulation (ICMS). We also revealed functional synaptic connections between RFA and CFA, by recording electrical signals in either area during the photostimulation mapping. In addition, we developed a method that uses Halorhodopsin (NpHR; yellow-light-activated chloride ion pump) for inactivating cortical neurons in vivo. Yellow light illumination on the cortical surface of NpHR transgenic mice inhibited the forelimb movement induced by ICMS in RFA. As these photostimulation methods were effective even to awake mice, these methods can be applied to the experiments described below.

II. Development of a novel operant task of head-restrained mice

To carry out two-photon calcium imaging while mice performed a self-initiated movement, we developed a head-restrained lever-pull task. Mice used the right forelimb to pull a lever for a given time and were rewarded with a water drop from a spout near the mouth, while simultaneously the lever was quickly pushed back to the wait position. Then, mice had to wait with the lever in the wait position for a while until the next trial. During the training sessions, task difficulty was increased by gradually increasing the lever-pull time. However, mice could increase or maintain the number of successful trials (>100) and the intertrial interval time decreased. After 8-9 training sessions, mice reliably performed this task. We are also improving this task for future research.

III. Spatio-temporal representation of motor information in the brain

The aim of this study is to reveal how voluntary movement is 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. To reveal how their activities are organized in the local circuit, we carried out two-photon calcium imaging in the layer 2/3 RFA and CFA of mice performing the lever-pull task after the training sessions. We have found many types of neurons in the local circuit. We are now analyzing the activity, distribution, and connections of the cortical neurons involved in sequential motor phases. The activities of the cortical neurons will be modulated by using the optogenetic stimulation methods to clarify the direction of flow of motor information. Our results will provide insights into the principles of circuit operation and the cellular basis for recovery from brain cortical damage.

Publication List

〔Original papers〕

- Ako, R., Wakimoto, M., Ebisu, H., Tanno, K., Hira, R., Kasai, H., Matsuzaki, M., and Kawasaki, H. (2011). Simultaneous visualization of multiple neuronal properties with single-cell resolution in the living rodent brain. Mol. Cell. Neurosci. *48*, 246-257.
- Kanemoto, Y., Matsuzaki, M., Morita, S., Hayama, T., Noguchi, J., Senda, N., Momotake, A., Arai, T., and Kasai, H. (2011). Spatial distributions of GABA receptors and local inhibition of Ca^{2+} transients studied with GABA uncaging in the dendrites of CA1 pyramidal neurons. PLoS ONE *6*, e22652.
- Matsuzaki, M., Ellis-Davies, G.C.R., Kanemoto, Y., and Kasai, H. (2011). Simultaneous two-photon activation of presynaptic cells and calcium imaging in postsynaptic dendritic spines. Neural Syst. Circuits *1*, 2.
- Matsuzaki, M., and Kasai, H. (2011). Two-Photon Uncaging Microscopy. Cold Spring Harbor Protocols, pdb.prot5620.
- Noguchi, J., Nagaoka, A., Watanabe, S., Ellis-Davies, G.C.R., Kitamura, K., Kano, M., Matsuzaki, M., and Kasai, H. (2011). In vivo two-photon uncaging of glutamate revealing the structure-function relationships of dendritic spines in the neocortex of adult mice. J. Physiol. *589*, 2447-2457.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

LABORATORY OF NEUROPHYSIOLOGY

Associate Professor
WATANABE, Eiji

NIBB Research Fellows: *MATSUNAGA, Wataru*
NAKAYASU, Tomohiro
Visiting Scientist: *AONO, Sachiko*

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 sensory systems of animals, we moved ahead from our previous research of the salt-sensing system to new research on the visual system.

I. Psychophysical study of Medaka fish

One of our major subjects is the psychophysical and computational studies of medaka (*Oryzias latipes*). Medaka have many advantages for behavioral work. First, genetic examination of medaka is progressing at a rapid pace, opening up new approaches to the understanding of genetic control of behavior. Second, although the central nervous system of medaka is relatively simple, its basic structure is the same as that in mammals. Thirdly, they provide invaluable comparative material for work on mammals. Examination of such a relatively simple yet vertebrate system should thus aid in the determination of the basic mechanisms of how genes affect behavior. This year, we have made progress in studies of the prey-predator interaction using medaka and zooplankton.

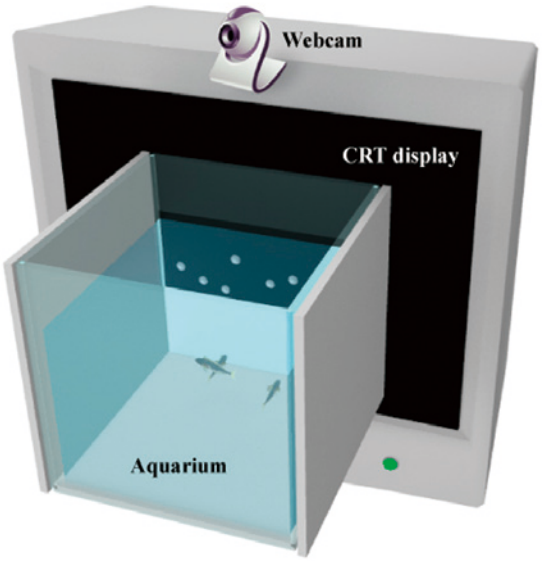


Figure 1. Virtual plankton system. The test aquarium was attached to a CRT display on which virtual plankton were shown. The behaviors of the 3 fish in the test aquarium was recorded by a webcam placed above the test aquarium.

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 (Figure 1) where the predation behavior in response to computer-generated prey was analyzed. First, we performed motion analysis of zooplankton (*Daphnia magna*) to extract mathematical functions for biologically relevant motions of prey. Next, 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 and Watanabe, Scientific Reports, *in press*).

II. Psychophysical study of Human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual system of human beings (*Homo sapiens*).

In order to interact successfully with the environment, animals must know the accurate positions of objects in space, though those positions frequently change. Neural processing, however, requires considerable time. By the time a conclusion is reached about location, the moving object has moved on to a new position in the actual world. Does our visual system compensate for this difference?

One recent focus of this debate is the flash-lag effect (Figure 2), in which a moving object is perceived to lead a flashed object when both objects are aligned in actual physical space. Last year, we proposed a simple conceptual model explaining the flash-lag effect (Delta model, Watanabe *et al*., 2010). This year, we have attempted to expand the model for application to the motor control mechanisms of the brain.

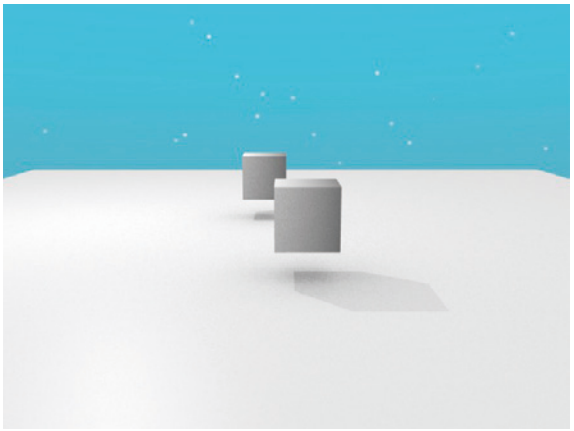
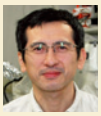


Figure 2. Flash-lag effect. A moving cube is perceived to lead in space a flashed cube when both objects are aligned in actual physical space. This effect has been utilized for understanding human motion perception. This year, we successfully produced a 3D version of the Flash-lag effect using Blender 3D software. Please refer to YouTube (DUBM-GG0gAk).

LABORATORY OF NEUROCHEMISTRY †



Professor (Concurrent)
SASAOKA, Toshikuni

Postdoctoral Fellow: *SATO, Asako*

Our major research interest is to understand the physiological role of the dopaminergic system in animal behavior, particularly locomotion and eating behaviors, using genetically altered mice.

I. Role of dopaminergic transmission in locomotion and eating behavior

The dopaminergic system is implicated in the modulation of locomotor activity, the regulation of several peptide hormones in the pituitary, the modulation of synaptic plasticity and the development of neurons. The dopaminergic system is also implicated in the control of emotion, motivation and cognition. Dysfunction of the dopaminergic system can result in several neurological and psychiatric disorders, such as Parkinson's disease and schizophrenia.

In mammals, two subgroups of dopamine receptor have been identified, referred to as D1-like receptors (D1R, D5R) and D2-like receptors (D2R, D3R and D4R) on the basis of their gene structure and their pharmacological and transductional properties. D1R and D2R are the most abundantly and widely expressed in the brain and often play a synergistic role.

To investigate the role of D1R and D2R in locomotor control and eating behavior, we utilized D1R knockout (KO) mice, D2R KO mice, and transgenic mice harboring tetracycline-regulated expression of the *D1R* gene. Daily motor activity and food/water intake in these mice were continuously monitored in home cage environment for long term (Figure 1).

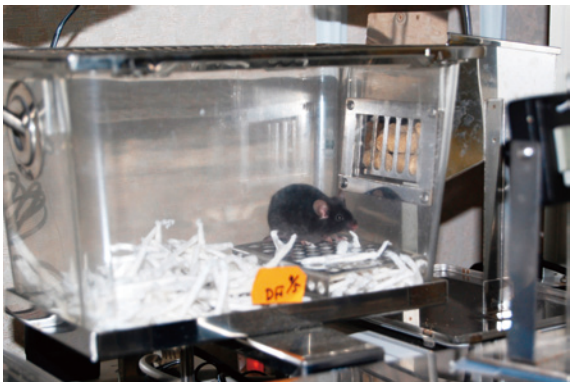


Figure 1. Experimental equipment for measurement of locomotor activity and food/water intake

II. Motor activity in D1R KO and D2R KO mice

In previous behavioral studies of D1R KO mice and D2R KO mice, changes in walking distance were analyzed in short-term sessions using beam breaks or video tracking systems. Decreased locomotor activity in D2R KO mice, and both increase and decrease in the locomotor activity in D1R KO mice have been reported in different studies. We analyzed beseline activity including all the motions (walking, rearing, grooming, climbing on the lid, and so on) in the home cages for at least 5 days. We first focused on motor activity for a 24 hr period and found that D1R KO mice were hyperactive and that D2R KO mice were hypoactive compared with wild type mice (Figure 2). To elucidate if these mice have normal circadian rhythms in activity, we analyzed motor activity in both light and dark phases. Results showed that all mice had normal circadian rhythms and that the difference in motor activity was seen during the dark phase. To further analyze the extent of activities in the dark phase, percentage of time exhibiting inactive, low, medium and high activity states were calculated. Interestingly, both D1R KO and D2R KO mice exhibited a comparable percentage of inactive state time. D1R KO mice spent more time exhibiting a high activity state, whereas D2R KO mice spent more time exhibiting a low activity state than wild type mice.

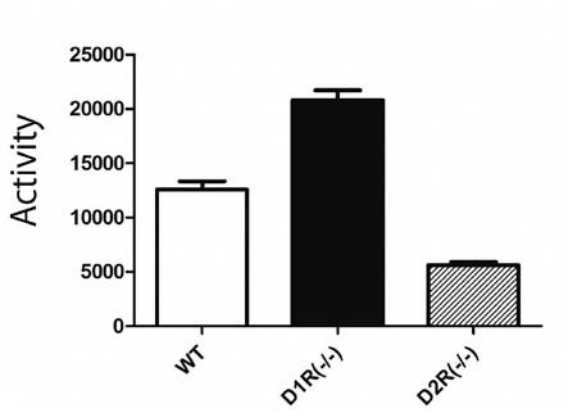


Figure 2. Motor activity of D1R KO and D2R KO mice in the home cage for 24 hr.

III. Motor activity in transgenic mice harboring controllable D1R expression

Our transgenic mouse lines showed doxycycline (Dox) controllable expression of transgenic *D1R* gene. To elucidate the effects of altered *D1R* expression, we applied Dox to the mice and monitored daily motor activity. We also examined the protein expression level of D1R in the striatum of transgenic mice. The striatum contains abundant *D1R* expression and is considered to be a major region responsible for control of motor activity. We found decrease in activity after Dox administration in transgenic mice which had no endogenous *D1R* gene, suggesting that *D1R* is required for normal activity. When Dox was applied for only 14 days, transient hyperactivity was observed as D1R expression was increased. We are analyzing the relationship between dopamine signaling via D1R and altered behavior.

†: Professor Sasaoka ended his term as a Concurrent Professor on 31 March, 2011.

DIVISION OF EVOLUTIONARY BIOLOGY

Professor
HASEBE, Mitsuyasu

Associate Professor
MURATA, Takashi

Assistant Professors:	*HIWATASHI, Yuji*
	TAMADA, Yosuke
Technical Staff:	*KABEYA, Yukiko*
NIBB Research Fellow:	*MANO, Hiroaki*
Postdoctoral Fellows:	*CHENG, Chaoyang*
	IMAI, Akihiro
	ISHIKAWA, Masaki
	ISHIKAWA, Takaaki
	KUBO, Minoru
	NAGASHIMA, Akitomo
	NOZAWA, Masafumi
	OHSHIMA, Issei
	OKAMOTO, Haruko
Graduate Students:	*AOYAMA, Tsuyoshi*
	FUKUSHIMA, Kenji
	ICHIKAWA, Shunsuke
	LI, Chen
	SUGAYA, Tomomi
Technical Assistants:	*ANDO, Sayuri*
	AOKI, Etsuko
	BABA, Nayumi
	FUKADA, Hatsumi
	GOTO, Miho
	GOTO, Misako
	HIRAMATSU, Mika
	ICHIKAWA, Michiko
	ITO, Yukiko
	KAJIKAWA, Ikumi
	KAKIGI, Rieko
	KOHARA, Mayuko
	MASUOKA, Tomoko
	NISHI, Tayo
	OOI, Shoko
	OHSHIMA, Masumi
	WAKADSUKI, Sachiko
	WASHIO, Midori
Secretary:	*KOJIMA, Yoko*

I. The lycopod *Selaginella moellendorffii* genome

Vascular plants appeared ~410 million years ago, then diverged into several lineages of which only two survive: the euphyllophytes (ferns and seed plants) and the lycophytes. We report here the genome sequence of the lycophyte Selaginella moellendorffii (Selaginella), the first nonseed vascular plant genome reported. By comparing gene content in evolutionarily diverse taxa, we found that the transition from a gametophyte- to a sporophyte-dominated life cycle required far fewer new genes than the transition from a nonseed vascular to a flowering plant, whereas secondary metabolic genes expanded extensively and in parallel in the lycophyte and angiosperm lineages. Selaginella differs in posttranscriptional gene regulation, including small RNA regulation of repetitive elements, an absence of the trans-acting small interfering RNA pathway, and extensive RNA editing of organellar genes.

Figure 1. A lycopod *Selaginella moellendorffii* whose draft genome was published.

II. Evolution from cells to tissues based on molecular mechanisms of cytokinesis

The cells of land plants and their sister group, charophycean green algae, divide by the insertion of cell plates at cytokinesis. This is in contrast to other green algae, in which the invagination of the plasma membrane separates daughter cells at cytokinesis. The cell plate appears in the middle of daughter nuclei, expands centrifugally towards the cell periphery, and finally fuses to the parental cell wall. Cell wall materials are transported to the expanding cell plate with a phragmoplast, which is mainly composed of microtubules. Centrifugal expansion of the phragmoplast is a driving force for that of the cell plate, although elucidating the molecular mechanism for the expansion was a challenge. We have found that γ-tubulin complexes on existing phragmoplast microtubules nucleate new microtubules as branches. Although elongation of the branched microtubules is likely a driving force of the phragmoplast expansion, the mechanism by which phragmoplast microtubules redistribute towards the cell periphery is unclear. Because an inhibitor of microtubule depolymerization inhibits phragmoplast expansion, analyses of microtubule depolymerization might be a key for understanding the mechanism. We developed a method for quantifying the rate of microtubule depolymerization in the phragmoplast, and found that the rate of microtubule depolymerization gradually increases from the outer surface to the inside of the phragmoplast. Based on the results, we propose a hypothesis that random branching of microtubules coupled with biased depolymerization lead directional redistribution of microtubules, which drives centrifugal expansion of the phragmoplast. Takashi Murata was this study's main researcher.

III. Evolution of molecular mechanisms in plant development

Stem cells are formed at particular times and positions during the development of multicellular organisms. Whereas flowering plants form stem cells only in the sporophyte generation, non-seed plants form stem cells in both the sporophyte and gametophyte generations. Although the molecular mechanisms underlying stem cell formation in the sporophyte generation have been extensively studied, only a few transcription factors involved in the regulation of gametophyte stem cell formation have been reported. The

moss *Physcomitrella patens* forms a hypha-like body (protonema) and a shoot-like body (gametophore) from a protonema apical cell and a gametophore apical cell, respectively. These apical cells have stem cell characteristics. We found that four AP2-type transcription factors orthologous to *Arabidopsis thaliana AINTEGUMENTA/ PLETHORA/ BABY BOOM* (*APB*) are indispensable for the formation of gametophore apical cells from protonema cells. Quadruple disruption of all *APB* genes blocked gametophore formation, even in the presence of cytokinin, which enhances gametophore apical cell formation in the wild type. Heat-shock induction of an *APB4* transgene driven by a heat-shock promoter increased the number of gametophores. Expression of all APB genes was induced by auxin but not by cytokinin. Thus, the APB genes function synergistically with cytokinin signaling to determine the identity of the two types of stem cells. The primary researchers for this study were Tsuyoshi Aoyama and Yuji Hiwatashi.

Flowers are the most complex reproductive organs in land plants, whose development is regulated by MADS-box transcription factors. To understand the origin of a genetic network of floral homeotic genes, we analyzed six MIKC classic type MADS-box genes in *P. patens*. Deletion of all six genes enhanced elongation of gametophore stem and reduced production of sporophytes. This result suggests that the MADS-box genes function in both gametophyte and sporophyte generation. Investigation of the effects of all six gene deletions is currently being undertaken by Yuji Hiwatashi.

Evolution of a branched system is a conspicuous novelty in land plant evolution, although the origin and evolution of its gene network is not known because of the lack of study in the basal land plants. We found that a deletion mutant of a polycomb repressive complex 2 gene *PpCLF* forms a branched sporophyte-like organ in *P. patens*. Analyses of auxin distribution and expression patterns of class 1 KNOX genes suggest that the active site of auxin signaling is localized to the initiation site of the branch. To elucidate how the active site is formed in the sporophyte-like organ, spatial expression patterns of the proteins related to auxin biosynthesis, inactivation, and transport are under investigation. This work was mainly done by Yuji Hiwatashi.

IV. Molecular mechanisms of reprogramming of gametophore leaf cells to pluripotent stem cells in the moss *Physcomitrella patens*

Differentiated cells can be reprogrammed to become undifferentiated pluripotent stem cells with abilities to both self-renew and give rise to most cell types in the organism. An induction of reprogramming is more easily manipulated in plants than in animals, although the genetic and molecular bases of the difference are mostly unknown. This is likely because the callus usually used in reprogramming studies in seed plants is a cell mixture composed of reprogrammed and unreprogrammed cells. We noticed that *P. patens* should overcome this problem by its rapid reprogramming ability from a single cell (see http://www.nibb.ac.jp/evodevo/ERATO/movie/MacMovie.mp4). Cells in a dissected leaf of *P. patens* are reprogrammed to become chloronema apical cells with pluripotency within 24 hours. We can continuously observe the reprogramming process of a specific cell under a microscope.

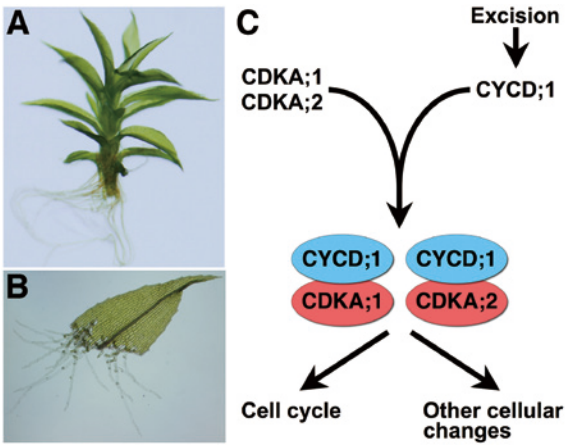


Figure 2. Reprogramming of leaf cells to chloronema apical cells. (A) A gametophore. (B) A leaf at 48 hours after excision. (C) A model showing the dual roles of cyclin-dependent kinase A (CDKA) during reprogramming.

One of the key factors of reprogramming is the change in the genome-wide chromatin modification. In the differentiated cells, a gene expression profile that fits the cell function is stably maintained through chromatin modifications. The active modifications such as trimethylation of histone H3 at lysine 4 (H3K4me3) are enriched at certain genes required for the cell function, and the repressive modifications including H3K27me3 are enriched at other unnecessary genes. In contrast, in the pluripotent stem cells, most genes are ready to be activated. Many genes with H3K27me3 also have H3K4me3 in animal pluripotent stem cells, and this bivalent state is presumed to keep genes poised for transcription. Thus, in the process of reprogramming, the epigenomic profile of differentiated cells should be changed into a pluripotent stem cell-specific epigenomic profile. However, the mechanisms of the establishment of such epigenomic profiles are almost unknown. We are currently attempting to reveal these mechanisms underlying reprogramming of *P. patens* leaf cells to chloronema apical cells with the combination of chromatin immunoprecipitation-sequencing (ChIP-Seq) using a next generation sequencer and live imaging of chromatin modifications. We have successfully established the 4D (3D + time) live-imaging method of a single *P. patens* nucleus, and also produced the H3K27me3 detector using *Drosophila melanogaster* Polycomb protein, which is known to bind to H3K27me3. We are now analyzing the ChIP-Seq data, performing the 4D live imaging for H3K27me3 during the reprogramming, and producing the live-imaging detector for H3K4me3. This study is mainly conducted by Takaaki Ishikawa and Yosuke Tamada.

V. Molecular mechanisms of mimicry

Mimicry is an intriguing phenomenon in which an organism

closely resembles another, phylogenetically distant species. An excellent example is the flower-mimicry of the orchid mantis *Hymenopus coronatus*, in which pink and white coloration and petal-like structures on its walking legs enable this insect to blend perfectly into flowers. To elucidate the evolutionary mechanism of this complex mimicry at the molecular level, we first focused on the mechanism of body coloration in the orchid mantis. HPLC and mass spectrometric analyses indicated that xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink body coloration of late-stage nymphs. On the other hand, the 1st-instar nymph of the orchid mantis with yellowish red color contains three ommochrome pigments; xanthommatin, decarboxylated xanthommatin and a labile yellow pigment which was previously uncharacterized. These results suggest that the orchid mantis alters its body color by changing the composition of ommochrome pigments during post-hatching development. This work was mainly done by Hiroaki Mano.

VI. Molecular mechanisms of host shifting

Adaptation to a novel environment often requires evolution of multiple traits. In phytophagous insects a precise combination of performance and preference traits for particular host plants is crucial for host shifting because a new host plant can be incorporated into an insect's diet if adults accept it for oviposition and if the larvae are able to complete their development on it. However, very little is known about the genetic bases of the performance and preference, which are fundamental to infer the process and evolutionary consequence of host shifting. To address the molecular mechanism of host shifting, we use two host races of a tiny moth, *Acrocercops transecta*, as a model system. A QTL analysis revealed that only a restricted region of a single autosome was responsible for the larval performance. This indicates that host shifting from *Juglans* to *Lyonia* in *A. transecta* involved changes in few genes with large effect, suggesting that a small number of genetic changes to larval performance allowed the successful host shifting. To test whether preference genes are physically linked with performance genes or not, a mapping analysis of preference genes is in progress. This study was conducted mainly by Issei Ohshima.

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

The molecular mechanisms and evolutionary significance of plant movement, including seismonastic and nyctinastic movements, are enigmatic. We are working to establish chemically mutagenized lines that lack movement to compare fitness to wild types. We are also attempting to set up a method for transformation to characterize the genes involved in movement. To achieve this goal, we use a cotyledonary node explant, which can regenerate multiple shoots in the presence of 6-benzylaminopurine (BAP), as a target of Agrobacterium-mediated gene transfer. Although the node explant is highly recalcitrant to Agrobacterium infection, we successfully obtained several lines of transformed calluses that were capable of developing new shoots. We are now trying to regenerate whole plants from these transformed shoots in addition to further improvement of transformation efficiency. This study was conducted mainly by Hiroaki Mano.

VIII. Evolution of pitcher leaves in carnivorous plants

Development and evolution of the unique morphology of pitcher-shaped leaves of the carnivorous plant family Sarraceniaceae remains problematic. Since the 1870's, the pitcher leaves have been hypothesized to have a similar developmental program to that of peltate leaves. However, this hypothesis could not explain the formation of the keel, a structure specific to pitcher leaves. To understand the development and evolution of pitcher leaves, we analyzed expression patterns of leaf developmental gene orthologs in *Sarracenia purpurea*. Unexpectedly, the results suggested that adaxial-abaxial patterning of pitcher leaves was different from those of peltate leaves and have enabled us to hypothesize the evolutionary process of pitcher leaves. This study was conducted mainly by Kenji Fukushima.

Publication List

〔Original papers〕

- Aya, K., Hiwatashi, Y., Kojima, M., Sakakibara, H., Ueguchi-Tanaka, M., Hasebe, M., and Matsuoka, M. (2011). The Gibberellin perception system evolved to regulate a pre-existing GAMYB-mediated system during land plant evolution. Nature Communications *2*, 544.
- Banks, J.A., Nishiyama, T., Hasebe, M., Bowman, J.L., Gribskov, M., dePamphilis, C., Albert, V.A., Aono, N., Aoyama, T., Ambrose, B.A., *et al*. (2011). The Selaginella genome identifies genetic changes associated with the evolution of vascular plants. Science *332*, 960-963.
- Ishikawa, M., Murata, T., Sato, Y., Nishiyama, T., Hiwatashi, Y., Imai, A., Kimura, M., Sugimoto, N., Akita, A., Oguri, Y., Friedman, W.E., Hasebe, M., and Kubo, M. (2011). *Physcomitrella* cyclin-dependent kinase A links cell cycle reactivation to other cellular changes during reprogramming of leaf cells. Plant Cell *23*, 2924-2938.
- Motose, H., Hamada, T., Yoshimoto, K., Murata, T., Hasebe, M., Watanabe, Y., Hashimoto, T., Sakai, T., and Takahashi, T. (2011). NIMA-related kinases 6, 4, and 5 interact with each other to regulate microtubule organization during epidermal cell expansion in *Arabidopsis thaliana*. Plant J. *67*, 993-1005.

DIVISION OF SYMBIOTIC SYSTEMS

Professor
KAWAGUCHI, Masayoshi

Assistant Professors:	*TAKEDA, Naoya*
	SUZAKI, Takuya
Technical Staff:	*FUKADA-TANAKA, Sachiko*
NIBB Research Fellow:	*OKAMOTO, Satoru*
Postdoctoral Fellows:	*YANO, Koji*
	FUJITA, Hironori
	YOKOYAMA, Hiroshi
	MIYAZAWA, Hikota
	HANDA, Yoshihiro
Visiting Scientists:	*YOSHIDA, Chie*
	*MIYAZAWA, Hikota**
Graduate Students:	*TAKAHARA, Masahiro*
	SASAKI, Takema
	YORO, Emiko
	NAKAJIMA, Michiko
Technical Assistants:	*SUZAKI, Momoyo*
	YOSHINORI, Yumi
Secretary:	*SANJO, Kazuko*

More than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. AM fungi absorb minerals, mainly phosphates, from the soil and provide them to the plants. The origin of AM symbiosis is thought to have been in the early Devonian period. On the other hand, the root nodule symbiosis that occurs between legumes and rhizobial bacteria, unlike AM symbiosis, involves host-specific recognition and postembryonic development of a nitrogen-fixing organ. Root nodule symbiosis is thought to have evolved about 60 million years ago. Despite marked differences between the fungal and bacterial symbioses, common genes required for both interactions have been identified using model legumes. Our laboratory focuses on the early stages of the interaction between these microorganisms and *Lotus japonicus* in order to reveal the molecular mechanism and the origin of these symbiotic systems.

I. Nodulation

1-1 Genetic analysis of molecular mechanism regulating nodule primordia formation

In the initial developmental process of nodulation, infection of rhizobia into the host plant root induces dedifferentiation and division of some of the cortical cells followed by initiation of nodule primordia. Genetic mechanisms regulating nodule primordia formation have remained poorly characterized due to a lack of mutants involved in such process. Our large-scale mutant screening approach resulted in isolation of several new non-nodulating mutants related to nodule primordia formation in *Lotus japonicus*.

In the mutant line #7-1, nodules were barely formed. Judging from the pattern of infection thread formation, however, the infection process of rhizobia appears to be normal, suggesting the mutation predominantly affects nodule development. In addition, the mutation caused pleiotropic defects especially in the shoot; the number of cotyledons was increased and maintenance of the shoot apical meristem (SAM) was compromised in the mutant. A gene that is responsible for the mutation was isolated by map-based cloning, and it turned out that the gene is a putative orthologue of the genes known to be regulators of the SAM formation in other plants. Future detailed functional analysis of the gene might propose the existence of a novel common genetic regulatory mechanism between nodule primordia and SAM formation.

Auxin is a key phytohormone governing cell division and differentiation in many developmental aspects in plants. In terms of nodulation, however, the role of auxin remains largely unknown. As the first approach to elucidate molecular relationship between nodule primordia formation and auxin, we engineered transgenic plants, in which the expression of a reporter gene (*GFP-NLS*) was controlled by synthetic auxin-response promoter, *DR5*. Using these lines, we monitored the spatiotemporal induction pattern of auxin response during nodulation. It was shown that cortical cells' division occurs concomitant with strong induction of auxin response (Figure 1A and B). After colonization of rhizobia into developing nodule primordia, this induction of the auxin response halted in the infection region of nodule (Figure 1C). Further analysis in combination with nodule symbiotic mutants should uncover the function of auxin in nodule primordia formation.

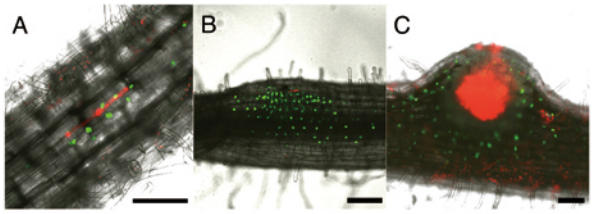


Figure 1. Spatial induction pattern of auxin response during nodule primordia formation. (A) Initial cortical cell division stage. Rhizobia start to invade into the host plant root via infection thread. (B) Beginning of the bulge of nodule primordia stage, when cortical cells actively divide. (C) Developing nodule primordia, into which rhizobia colonize. Green dots represent *DR5::GFP-NLS* expression. Red areas represent existence of rhizobia constitutively expressing *DsRED*. Scale bars: 100 μm.

1-2 Identification of *PLENTY* that mediates a novel mechanism for nodule number regulation and non-symbiotic root development

The symbiotic relationship between legume plants and rhizobia allows the host plants to grow even in nitrogen poor environments. However nodule development is an energetically expensive process. So the number of nodules must be tightly controlled by the host plants. We have been trying to elucidate the mechanism for controlling nodule numbers at the molecular level.

We previously isolated the novel hypernodulating mutant *plenty* from C^{6+} beam mutagenized seeds of *L. japonicus*. The number of nodules in *plenty* was 3-5 times more than that of wild type. Unlike previously reported hypernodulating mutants (*har1, klv and tml*), *plenty* showed

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

different characteristics. Both genetic analysis and grafting experiments using *plenty* and other hypernodulating mutants indicated that *PLENTY* functions using different signaling for controlling nodule number.

PLENTY was identified by map-based cloning. The genomic deletion was detected in the *PLENTY* gene. Furthermore, both symbiotic (hypernodulating) and non-symbiotic (short root) phenotypes of *plenty* were complemented by introducing the *PLENTY* gene (Figure 2). *PLENTY* encodes a completely unknown protein without any functional domain, although the protein is highly conserved from basal plants such as algae to higher plants. PLENTY might function as a root factor associated with an unknown regulatory mechanism of nodulation.

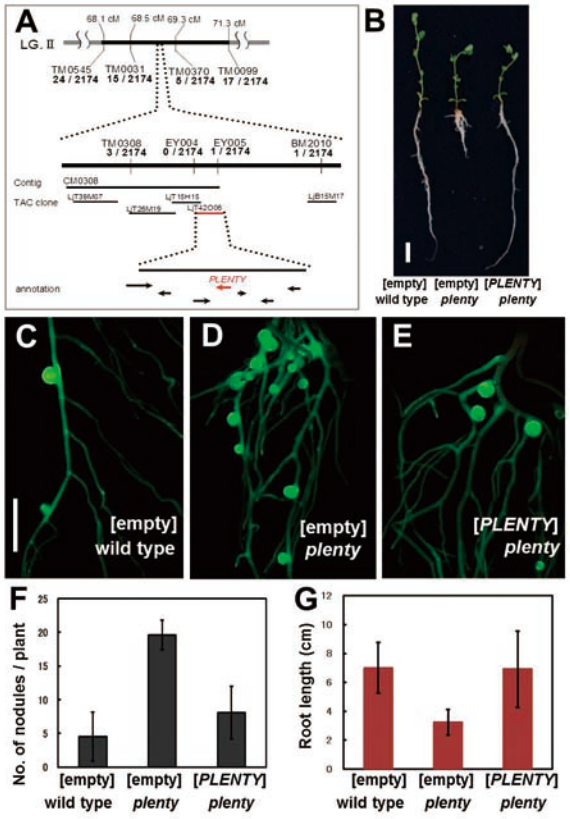


Figure 2. Identification of *PLENTY*. (A) Map-based cloning of *PLENTY*. (B-G) Complementation tests of *plenty*. *PLENTY* cDNA was introduced into *plenty* mutant via *A. rhizogenes*-mediated hairy root transformation. [empty] wild type indicates wild type with empty vector. [empty] and [*PLENTY*] *plenty* indicates *plenty* with empty vector and *PLENTY* (*Ubiquitin* $_{pro}$*:PLENTY* cDNA), respectively. Both the symbiotic (C-F) and non-symbiotic (B, G) phenotypes were rescued.

II. Arbuscular mycorrhiza symbiosis

Arbuscular mycorrhiza is mutualistic plant-fungal interaction which has several similar systems to root nodule symbiosis in host-symbiont recognition, infection process and nutrient material exchanges. Root nodule symbiosis is thought to evolve by sharing AM factors, suggesting that the AM system contains a fundamental mechanism that also regulates root nodule symbiosis. In recent studies, AM signaling factors that conduct host-symbiont recognition were isolated from both host plant and AM fungi. These results accelerated molecular analysis of the AM signaling mechanism.

We have established AM molecular marker genes *SbtM1* and *PT4*. These genes are specifically and highly induced during AM fungal infection. Green fluorescence protein (GFP) or beta-glucuronidase (GUS) fusions with the markers facilitated detection and visualization of infection processes of AM fungi. Using the tools; *SbtM*$_{pro}$*:GUS* derivatives, we have identified and analyzed *cis*-regions which specifically respond to AM fungal infection (Takeda et al., 2011). We isolated candidate genes of AM *trans*-factor from the *cis*-region with the yeast one-hybrid system and we are analyzing the genes and gene regulation system during AM fungal infection.

We also analyzed a signaling factor calcium calmodulin dependent protein kinase (CCaMK) which is a common signaling factor shared with AM and root nodule symbiosis. We found that a truncated CCaMK protein that contains only the kinase domain of the protein shows gain of function ability and activates a part of the AM signaling pathway without AM fungal infection. Introduction of the gain of function CCaMK (GOF-CCaMK) induced AM gene marker *SbtM1* expression, but not *PT4* expression. Furthermore, the AM signaling induced formation of pre-penetration apparatus (PPA). PPA is cytosolic remodeling induced by AM fungal infection, in which ER and cytosol development, and nuclear enlargement were observed before penetration of fungal hypha into the host cell. In this analysis, *SbtM1*$_{pro}$*:Venus* fusion visualized formation of the PPA and enabled detailed analysis of the cytoplasmic changes (Figure 3). The activation and signaling function of GOF-CCaMK revealed a novel aspect of AM signaling systems in *L. japonicus* (Takeda et al., accepted).

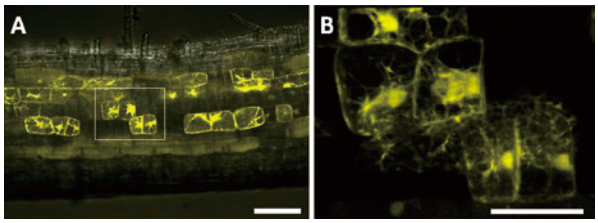


Figure 3. Pre-penetration apparatus-like structures induced by GOF-CCaMK. (A) Hairy root co-transformed with GOF-CCaMK (*CCaMK*$_{314}^{TD}$*-NLS*) and *SbtM1*$_{pro}$*:Venus* was observed with confocal microscopy and laser transmission and Venus fluorescence images were merged. (B) A stack of images obtained along the z axis of cells corresponding to the white box region in (A) showed densely developed cytosol. Bars = 100 μm (A) or 50 μm (B).

III. Reaction-diffusion pattern in the shoot apical meristem of plants

A fundamental question in developmental biology is how spatial patterns are self-organized from homogeneous structures. In 1952, Turing proposed the reaction-diffusion model in order to explain this issue. However, whether or not this mechanism plays an essential role in developmental events of living organisms remains elusive. Thus, we

investigated whether reaction-diffusion dynamics can explain the shoot apical meristem (SAM) development of plants. The SAM resides in the top of each shoot and consists of a central zone (CZ) and a surrounding peripheral zone (PZ) (Figure 4B). The SAM contains stem cells and continuously produces new organs throughout the lifespan of the plant. The formation and maintenance of the SAM are known to be essentially regulated by the feedback interaction between WUSHCEL (WUS) and CLAVATA (CLV) (Figure 4A).

We developed a mathematical model of the SAM based on reaction-diffusion dynamics of the WUS-CLV interaction, incorporating cell division and the spatial restriction of the dynamics. Consequently, we find that SAM patterning is governed by only two parameters: the stem cell proliferation mode and stem cell containment, and is classified into six groups: the fasciation pattern, multiplication pattern, fluctuation pattern, dichotomous pattern, monopodial pattern, and homeostasis pattern.

Next, we examined whether this theoretical prediction is consistent with experimental observations reported so far. Because SAM pattern formation has been intensively studied with regard to the *WUS* and *CLV* genes in *A. thaliana*, the effect of these genes on the model was investigated in detail.

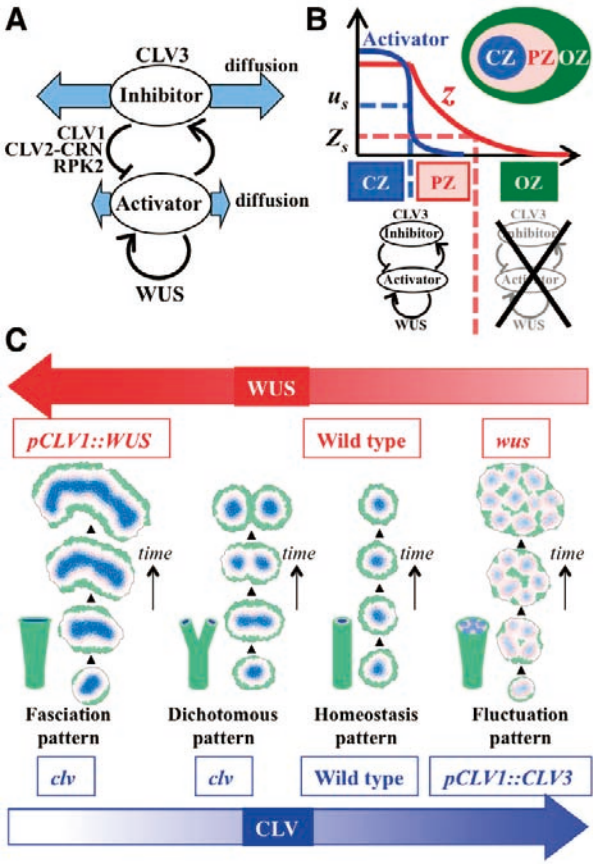


Figure 4. (A) Schematic representation of the WUS-CLV dynamics with respect to the activator-inhibitor system. (B) Schematic representation of a spatially restricted SAM. The CZ is defined as cells where the activator is highly expressed. A hypothetical molecule z is synthesized in the CZ, and diffuses to form a gradient. The PZ is differentiated by having z concentrations higher than a threshold Z_s. (C) SAM pattern changes predicted by the model are consistent with those of experimental results reported in *A. thaliana*.

As the WUS function becomes strong or the CLV function is reduced, the SAM pattern shifts in the following order: the fluctuation pattern, the homeostasis pattern, the dichotomous pattern, the fasciation pattern (Figure 4C). Our model successfully explains the various SAM patterns observed in plants, for example, homeostatic control of SAM size in the wild type, enlarged or fasciated SAM in *clv* mutants, and initiation of ectopic secondary meristems from an initial flattened SAM in *wus* mutant (Figure 4C).

As a result, we conclude that our model captures the essence of SAM pattern formation, and furthermore the reaction-diffusion dynamics is probably indispensable for SAM development in plants.

Publication List

〔Original papers〕

- Fujita, H., Toyokura, K., Okada, K., and Kawaguchi, M. (2011). Reaction-diffusion pattern in shoot apical meristem of plants. PLoS ONE *6*, e18243.
- Funayama-Noguchi, S., Noguchi, K., Yoshida, C., and Kawaguchi, M. (2011). Two *CLE* genes are induced by phosphate in roots of *Lotus japonicus*. J. Plant Res. *124*, 155-163.
- Krusell, L., Sato, N., Fukuhara, I., Koch, B., Grossmann, C., Okamoto, S., Oka-Kira, E., Otsubo, Y., Aubert, G., Nakagawa, T., Sato, S., Tabata, S., Duc, G., Parniske, M., Wang, T. L., Kawaguchi, M., and Stougaard, J. (2011). *Clavata2* genes of pea and *Lotus japonicus* affect autoregulation of nodulation. Plant Journal *65*, 861-871.
- Okamoto, S., Nakagawa, T., and Kawaguchi, M. (2011). Expression and functional analysis of a CLV3-like gene in the model legume *Lotus japonicus*. Plant Cell Physiol. *52*, 1211-1221.
- Takeda, N., Haage, K., Sato, S., Tabata, S., and Parniske, M. (2011). Activation of a *Lotus japonicus* subtilase gene during arbuscular mycorrhiza is dependent on the common symbiosis genes and two cis-active promoter regions. Molecular Plant-Microbe Interactions *24*, 662-670.

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

- Hakoyama, T., Niimi, K., Yamamoto, T., Isobe, S., Sato, S., Nakamura, Y., Tabata, S., Kumagai, H., Umehara, Y., Brossuleit, K., Petersen, T.R., Sandal, N., Stougaard, J., Udvardi, M.K., Tamaoki, M., Kawaguchi, M., Kouchi, H., and Suganuma, N. The intergral membrane protein SEN1 is required for symbiotic nitrogen fixation in *Lotus japonicus* nodules. Plant Cell Physiol. 2011 Nov. 28.

〔Review articles〕

- Fujita, H., and Kawaguchi, M. (2011). Strategy for shoot meristem proliferation in plants. Plant Signaling Behavior *6*, 1851-1854.
- Kawaguchi, M. (2011). The evolution of symbiotic systems. Cellular and Molecular Life Sciences *68*, 1283-1284.

LABORATORY OF MORPHODIVERSITY

Associate Professor
KODAMA, Ryuji

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 for our morphological studies.

I. Wing morphogenesis

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

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

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

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

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 (Figure 1) 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.

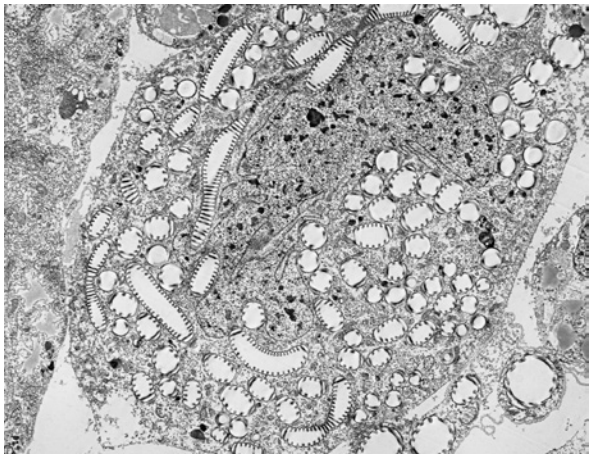


Figure 1. Transmission electron micrograph of the tracheole cell. Many cross-sections of the tracheoles can be observed within the cytoplasm of the tracheole cell. As the cell migrates, the tracheole is laid behind.

II. Other research activities

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

LABORATORY OF BIORESOURCES

Associate Professor
NARUSE, Kiyoshi

Postdoctoral Fellows:	*SASADO, Takao*
	TAKEHANA, Yusuke
	SHIBATA, Yasushi
	SHIBA, Keikun
	NAKAMOTO, Masatoshi
	OKUYAMA, Teruhiro
NIBB Research Fellow:	*KIMURA, Tetsuaki*
Research Fellows:	*KANEKO, Hiroyo*
	YOSHIMURA, Yuriko
Technical Assistants:	*KOIKE, Yukari*
	TORII, Naoko
	AJIOKA, Rie
	KOIKE, Chieko
	TESHIMA, Yuuko
	HARA, Ikuyo
	ISHIKAWA, Hiroe
	SHIBATA, Emiko
	TAKAGI, 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 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.

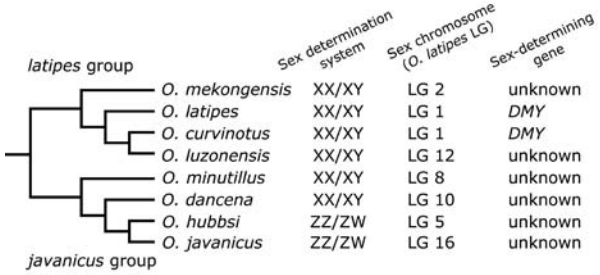


Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

Identification of these genes would provide a clue to understand the evolutionary process underlying frequent turnover of the sex determination mechanisms.

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

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

III. The function of estrogen in the medaka ovary

Estrogen has been generally considered to play a critical role in the ovarian differentiation of teleost fish by Yamamoto's model. In medaka, estrogen treatment has induced functional male-to-female sex reversal. To clarify the function of estrogen during ovarian development, we examined the role of ovarian aromatase (*arom*), which is responsible for catalyzing the conversion of testosterone to estrogen. We isolated two tilling mutant strains of *arom*. In these tiling mutants, one amino acid in aromatase ORF altered the STOP codon. In the tilling mutant of *arom*, the ovaries seemed to develop normally. However, in adult fish, yolk accumulation and formation of ovarian cavity were not observed. In some ovaries, spermatogenesis was observed. These results suggest that estrogen is not involved in early ovarian differentiation but has a critical role in maintenance of ovarian differentiation.

IV. Positional cloning of pigment cell mutants in medaka

All kinds of pigment cells are derived from neural crest cells. How each type of pigment cells differentiate and what differences are producing various pigment cell types is a very interesting question. Medaka have four types of pigment cell (melanophore, leucophore, xanthophore and iridocyte). The leucophore is unique because only some species have it. To elucidate how leucophore differentiate from neural crest cells and why it exists only in some fishes, we have successfully identified the causal gene of leucophore mutants (luecophore free (*lf*) and leucophre free 2 (*lf-2*)). We identified *slc2a15b* as the causal gene of the *lf* mutant. The *slc2a15b* expression exists but does not persist in the *lf* mutant. *slc2a15b* also exists in species without leucophore, but these use carotenoid as a pigment. Thus, *slc2a15b* may have an important role in

use of carotenoid as pigments. The *lf-2* phenotype was rescued by *pax7a*. *pax7a* is needed for differentiation of leucophore and xanthophore in medaka.

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

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA and BAC/ Fosmid clones and hatching enzymes, as well as integrated information on medaka (Figure 2). In 2011, we continued providing the TILLING screening system library to NBRP Medaka users 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.

Figure 2. NBRP Medaka website

Publication List

〔Original papers〕

- Chakraborty, T., Shibata, Y., Zhou, L.Y., Katsu, Y., Iguchi, T., and Nagahama, Y. (2011). Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Molecular and Cellular Endocrinology *333*, 47-54.
- Kai, W., Kikuchi, K., Tohari, S., Chew, A.K., Tay, A., Fujiwara, A., Hosoya, S., Suetake, H., Naruse, K., Brenner, S., *et al*. (2011). Integration of the Genetic Map and Genome Assembly of Fugu Facilitates Insights into Distinct Features of Genome Evolution in Teleosts and Mammals. Genome Biology and Evolution *3*, 424-442.
- Kato, M., Takehana, Y., Fukuda, Y., Naruse, K., Sakaizumi, M., and Hamaguchi, S. (2011). An autosomal locus controls sex reversal in interspecific XY hybrids of the medaka fishes. Heredity *107*, 523-529.
- Kobayashi, H., Iwamatsu, T., Shibata, Y., Ishihara, M., and Kobayashi, Y. (2011). Effects of co-administration of estrogen and androgen on induction of sex reversal in the medaka *Oryzias latipes*. Zoolog. Sci. *28*, 355-359.
- Koga, A., Sasaki, S., Naruse, K., Shimada, A., and Sakaizumi, M. (2011). Occurrence of a short variant of the Tol2 transposable element in natural populations of the medaka fish. Genetics Research *93*, 13-21.
- Okuyama, T., Suehiro, Y., Imada, H., Shimada, A., Naruse, K., Takeda, H., Kubo, T., and Takeuchi, H. (2011). Induction of c-fos transcription in the medaka brain (*Oryzias latipes*) in response to mating stimuli. Biochemical and Biophysical Research Communications *404*, 453-457.
- Paul-Prasanth, B., Shibata, Y., Horiguchi, R., and Nagahama, Y. (2011). Exposure to diethylstilbestrol during embryonic and larval stages of medaka fish (*Oryzias latipes*) leads to sex reversal in genetic males and reduced gonad weight in genetic females. Endocrinology *152*, 707-717.

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

- Takehana, Y., Naruse, K., Asada, Y., Matsuda, Y., Shin-I, T., Kohara, Y., Fujiyama, A., Hamaguchi, S., and Sakaizumi, M. Molecular cloning and characterization of the repetitive DNA sequences that comprise the constitutive heterochromatin of the W chromosomes of medaka fishes. Chromosome Res. 2011 Nov. 29.

〔Review articles〕

- Naruse, K. (2011). Genetics, Genomics, and Biological Resources in the Medaka, *Oryzias latipes*. In: Medaka, A Model for Organogesis, Human Diseases and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 19-37.
- Naruse, K. Tanaka, M., and Takada, H. (2011). Medaka, A Model for Organogesis, Human Diseases and Evolution Springer Tokyo.
- Shibata, N., Nakamoto, M., Shibata, Y., and Nagahama, Y. (2011) Endocrine Regulation of Oogenesisin the Medaka, *Oryzias latipes*. In: Medaka: A Model for Organogenesis, Human Disease, and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 267-283.
- Takehana, Y. (2011). Frequent turnover of sex chromosomes in the medaka fishes. In: Medaka, A Model for Organogesis, Human Diseases and Evolution. Naruse, K., Tanaka, M., and Takeda, H. eds. (Springer), pp. 229-240.

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. Eukaryotic cells employ Tor (target of rapamycin) protein kinase to recognize cellular nutrient conditions. 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).

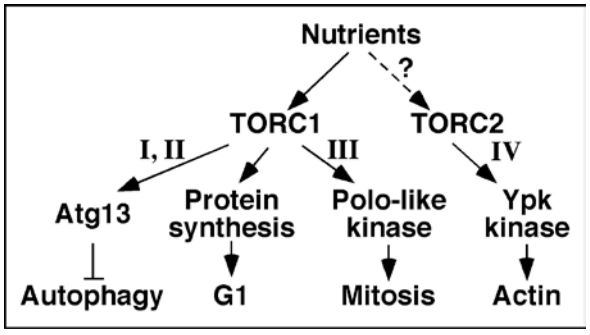


Figure 1. TOR signaling pathway of the budding yeast. Our group have found three branches of the TOR pathway.

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, such as 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 is turned out to be a substrate of TORC1, in vivo activity of TORC1 can be monitored by phosphorylation state of Atg13. Various conditions and mutants have been examined to determine what kind of nutrients TORC1 recognizes and how nutrient signal is transmitted to TORC1.

III. Localization of Polo-like kinase is controlled by TORC1 to regulate mitotic entry.

It is well known that 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. We found that this mutant, as well as yeast cells treated with rapamycin, exhibit mitotic delay with prolonged G2. We further demonstrated that this G2-arrest phenotype is due to mislocalization and resultant inactivation of Cdc5, the yeast polo-kinase. These results suggest that TORC1 mediates G2/M transition via regulating polo-kinase.

IV. Ypk2 kinase acts at the 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 AGC-type of protein kinase whose overexpression can rescue lethality of TORC2 dysfunction, is directly phosphorylated by TORC2.

Publication List

〔Original paper〕

● Yoshida, S., Imoto, J., Minato, T., Oouchi, R., Kamada, Y., Tomita, M., Soga, T., and Yoshimoto, H. (2011). A novel mechanism regulates H_2S and SO_2 production in *Saccharomyces cerevisiae*. Yeast *28*, 109-121.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor:	*OHNO, Kaoru*
Technical Assistants:	*ITO, Masako*
	NAKAMURA, Ryoko
	MATSUDA, Azusa
	FUJITA, Miyako

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).

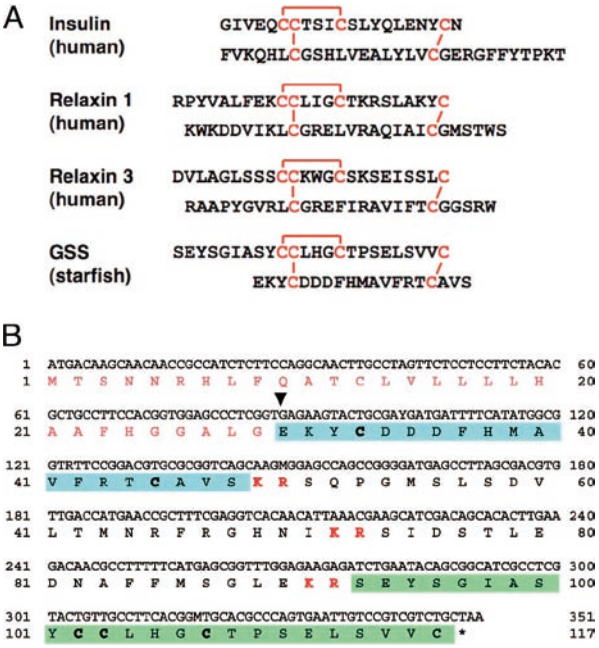


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

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

Morning glories belong to the genus *Ipomoea* that is the largest group in the family *Convolvulaceae*. Of these, *I. 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 (Figure 1). 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 cause the difference in pigmentation patterns. The recessive mutations, *duskish* of *I. nil* and *pearly-v* of *I. tricolor*, confer variegated flowers, and epigenetic mechanisms are thought to regulate flower pigmentation. We are currently characterizing detailed molecular mechanisms of these mutations.

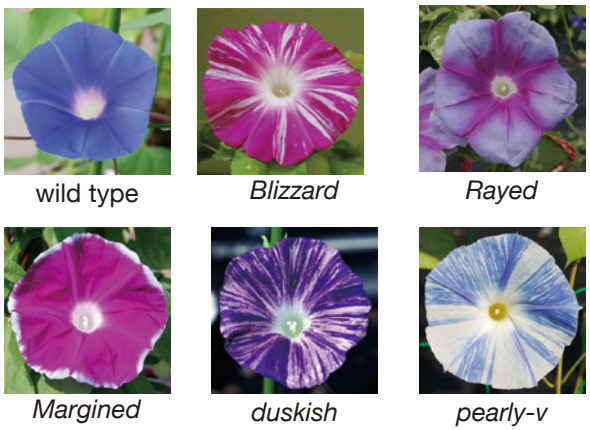


Figure 1. Flower phenotypes of the morning glories.

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*, having a genome of about 800 Mbp. We chose the Tokyo-kokei standard line for genome sequencing, and employed not only shotgun sequencing using high-throughput DNA sequencers but also BAC end sequencing. We are collaborating with several laboratories in Japan.

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 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 200 lines and 117,000 DNA clones.

Publication List

〔Original papers〕

- Higuchi, Y., Sage-Ono, K., Sasaki, R., Ohtsuki, N., Hoshino, A., Iida, S., Kamada H., and Ono, M. (2011). Constitutive expression of the *GIGANTEA* ortholog affects circadian rhythms and suppresses one-shot induction of flowering in *Pharbitis nil*, a typical short-day plant. Plant Cell Physiol. *52*, 638-650.
- Ohno, S., Hosokawa, M., Hoshino, A., Kitamura, Y., Morita, Y., Park, K.I., Nakashima, A., Deguchi, A., Tatsuzawa, F., Doi, M., Iida, S., and Yazawa, S. (2011). A bHLH transcription factor, *DvIVS*, is involved in regulation of anthocyanin synthesis in dahlia (*Dahlia variabilis*). J. Exp. Bot. *62*, 5105-5116.
- Ohno, S., Hosokawa, M., Kojima, M., Kitamura, Y., Hoshino, A., Tatsuzawa, F., Doi M., and Yazawa, S. (2011). Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. Planta *234*, 945-958.
- Saito, N., Tatsuzawa, F., Hoshino, A., Abe, Y., Ichimura, M., Yokoi, M., Toki, K., Morita, Y., Iida, S., and Honda, T. (2011). The anthocyanin pigmentation controlled by the *speckled* and *c-1* mutations of the Japanese morning glory. J. Japan. Soc. Hort. Sci. *80*, 452-460.

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

- Park, K.I., and Hoshino, A. A WD40-repeat protein controls proanthocyanidin and phytomelanin pigmentation in the seed coats of the Japanese morning glory. 2011 Dec. 28.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: *TSUGANE, Kazuo*
Visiting Scientist: *HAYASHI-TSUGANE, Mika*

An active nonautonomous DNA transposon, *nDart1-0*, belonging to the *hAT* superfamily, was identified. 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 could be easily generated under normal field conditions, and the resulting tagging lines were free of somaclonal variation. The *nDart1* transposons tend to insert into the promoter, 5' UTR region, or exon of a gene, which suggests that the *nDart1*/*aDart1-27* system is a powerful tool for rice functional genomics. Furthermore, we are developing several *indica* lines bearing the active *nDart1*/*aDart1-27* system. These lines would effectively contribute to gene functional analysis and breeding for the *indica* rice varieties.

A rice mutant displaying a heterochronically elongated internode carries a 100 kb deletion

We have isolated a recessive rice mutant, designated as *indeterminate growth (ing)*, which displays creeping and apparent heterochronic phenotypes in the vegetative period with lanky and winding culms (Figure 1). Rough mapping and subsequent molecular characterization revealed that the *ing* mutant carries a large deletion, which corresponds to a 103 kb region in the Nipponbare genome, containing nine annotated genes on chromosome 3.

Figure 1. Phenotypes of the wild type and *ing* plants. A: Three-month-old wild-type plant (left) and ing mutant (right). The bars represent 50 cm.

Of these annotated genes, the *SLR1* gene encoding a DELLA protein is the only one that is well characterized in its function, and its null mutation, which is caused by a single base deletion in the middle of the intronless *SLR1* gene, confers a slender phenotype that bears close resemblance to the *ing* mutant phenotype. The primary cause of the *ing* mutant phenotype is the deletion of the *SLR1* gene, and the *ing* mutant appears to be the first characterized mutant having the entire *SLR1* sequence deleted. Our results also suggest that the deleted region of 103 kb does not contain an indispensable gene, whose dysfunction must result in a lethal phenotype (Figure 2).

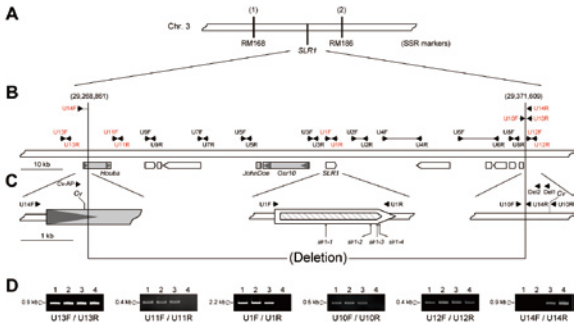


Figure 2. Characterization of the indeterminate growth (*ing*) allele. A: Rough mapping of the ing allele. B: Structure of the *ing* deletion. The large bracket indicates the deletion. C: Enlarged structures of the 5' region of *Houba*, SLR1, and the region of the 3' deletion junction. D: Detection of PCR-amplified bands with the indicated primers. Lane 1, Nipponbare; lane 2, *ING*/*ING*; lane 3, *ING*/*ing*; lane 4, *ing*/*ing*.

Examination of transpositional activity of *nDart1* at different stages of rice development

As a useful tool to elucidate gene functions, a rice transposon tagging line has been developed using an active endogenous DNA transposon, *nDart1*. It was highly desirable to evaluate the transposition timing and frequency of the *nDart1* elements during rice development to facilitate the generation of an efficient mutant isolation system. Comparison of the detected new insertions at different stages of rice development by transposon display analysis demonstrated that the last heading tiller carry a higher number of *nDart1* elements than the main culm. Moreover, it was revealed that the last heading tiller could produce progeny that carried more new insertions of *nDart1* elements, mainly as a result of the accumulation of somatic insertions in the parental plant. This report demonstrates that late tillers increase the probability of producing independent mutant lines.

Publication List

〔Original papers〕

- Hayashi-Tsugane, M., Maekawa, M., Kobayashi H., Iida, S., and Tsugane, K. (2011). A rice mutant displaying a heterochronically elongated internode carries a 100 kb deletion. J. Genet. Genomics *38*, 123-128.
- Hayashi-Tsugane, M., Maekawa, M., Qian, Q., Kobayashi H., Iida, S., and Tsugane, K. (2011). Examination of transpositional activity of *nDart1* at different stages of rice development. Genes Genet. Syst. *86*, 215-219.

〔Review article〕

- Maekawa, M., Tsugane, K., and Iida, S. (2011). Effective contribution of the *nDart* transposon-tagging system to rice functional genomics. Adv. Genet. Res. *4*, 259-272.

LABORATORY OF BIOLOGICAL DIVERSITY

YAMAGUCHI Group †

Assistant Professor: *YAMAGUCHI, Takahiro*
Postdoctoral Fellow: *NUKAZAWA, Akira*
Technical Assistant: *YAMAGUCHI, Chinami*

Angiosperm leaves generally develop as bifacial structures with distinct adaxial and abaxial identities. However, several monocot species, such as iris and leek, develop "unifacial leaves", in which leaf blades have only an abaxial identity (Figure 1). We are focusing on unifacial leaf development and evolution to understand genetic mechanisms behind diversity and evolution of organismal morphology.

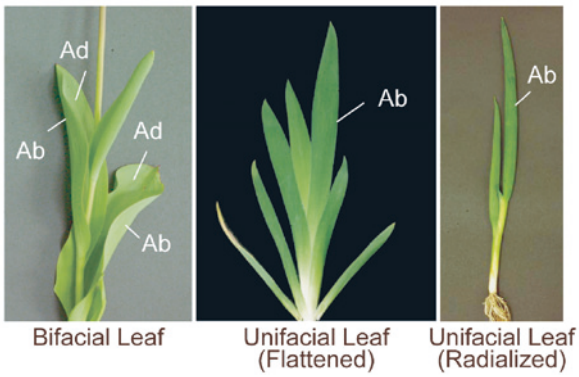


Figure 1. Bifacial and unifacial leaf structures. Ad, Adaxial side; Ab. Abaxial side.

I. Abaxialization of unifacial leaves

The development and evolution of unifacial leaves have long been matters of debate. However, nothing has been studied at the molecular genetic level. We focused on the genus *Juncus* as a model to study the evolution and development of unifacial leaves. *Juncus* contains species with a wide variety of leaf forms and is amenable to molecular genetic studies. We first characterized unifacial leaf development by investigating gene expression patterns of adaxial and abaxial determinants. As a result, we demonstrated that the unifacial leaf blade is abaxialized at the gene expression level and revealed that dominant abaxial activity leads to the unifacial leaf development.

II. Flattening of unifacial leaves

In bifacial leaves, adaxial–abaxial polarity is required for leaf blade flattening, whereas many unifacial leaves become flattened although their leaf blades are abaxialized (Figure1). This indicates independent mechanisms underlying flattened leaf blade formation in bifacial and unifacial leaves.

Using two closely related *Juncus* species, *J. prismatocarpus*, with flattened unifacial leaves, and *J. wallichianus*, with radialized unifacial leaves, we revealed that *DL* expression levels and patterns correlate with the degree of laminar outgrowth. Genetic and expression studies using interspecific hybrids of the two species revealed that the *DL* locus from *J. prismatocarpus* flattens the unifacial leaf blade and expresses higher amounts of *DL* transcripts. Thus, *DL* is a key gene in the flattening of the unifacial leaf blade. Interestingly, *DL* plays a distinct role in promoting midrib formation during bifacial leaf development. We suggest that morphological convergence of flattened leaf blades in unifacial leaves has occurred via the recruitment of *DL* function, which plays a similar cellular but distinct phenotypic role in monocot bifacial leaves (Figure 2).

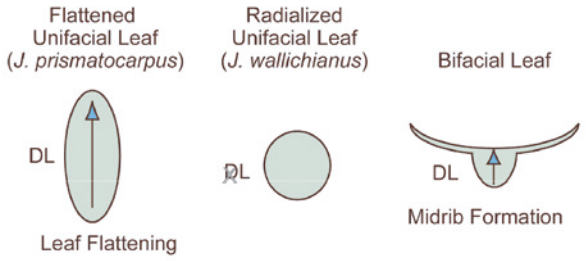


Figure 2. Mechanism of leaf blade flattening in unifacial leaves.

III. Leaf central–marginal and distal–proximal polarity specification

The mechanisms that regulate the central–marginal leaf polarity remain largely unknown. We discovered that the central–marginal polarity differentiates in the flattened leaf blade of *J. prismatocarpus*, but not in the radialized leaf blade of *J. wallichianus*. This indicates that leaf blade flattening is a key that triggers central–marginal leaf polarity differentiation. We also found the possibility that the plant hormone auxin is asymmetrically distributed in the flattened leaf primordia and induces expressions of central-marginal polarity determinants.

We also revealed that the *ROTUNDIFOLIA4* (*ROT4*) gene that encodes a plant-specific small peptide controls cell proliferation along distal-proximal axis during leaf development in *Arabidopsis thaliana*. We identified the 32 residues-long core functional region of ROT4 and showed that ROT4 acts cell autonomously without proteolytic processing (Ikeuchi et al., 2011).

Publication List

〔Original paper〕

● Ikeuchi, M., Yamaguchi, T., Kazama, T., Ito, T., Horiguchi, G., and Tsukaya, H. (2011). ROTUNDIFOLIA4 regulates cell proliferation along the body axis in Arabidopsis shoot. Plant Cell Physiol. *52*, 59–69.

†: This group was closed on 30 September, 2011.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

Assistant Professor: *JOHZUKA, Katsuki*
Technical Assistants: *ISHINE, Naomi*
MATSUZAKI, Yoko

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 created during DNA replication. Any abnormality in this process lead to segregation error or aneuploidy, resulting in cell lethality. Studies in the past decade have demonstrated that chromosome condensation is mainly achieved by condensin, a multi-subunit protein complex widely conserved from yeast to human.

Our major 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 genome during mitosis. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. We further discovered the multiple proteins interaction network recruits condensin to the RFB site.

I. Maintenance of the rDNA stability by condensin

Due to its tandem repetitive structure, the rDNA locus is unstable because of frequent homologous recombination events within the rDNA repeat. Nevertheless, most cells can maintain rDNA stability, as indicated by their relatively constant copy numbers of the repeats. A key recombination protein, Rad52 is normally localized in the nucleus but is excluded from the nucleolus. This exclusion of a recombination protein may contribute to maintaining rDNA stability. In condensin mutants, however, aberrant segregation of the rDNA locus is frequently observed and its copy numbers are dramatically decreased. We found that the Rad52 protein was excluded from the nucleolus during the mitosis in *YCS4* cells, whereas it invaded into the nucleolus in *ycs4-1* mutant cells (Figure 1). This result indicates that condensin protects from invasion of recombination enzyme during mitosis. It is suggested that rDNA condensation supports the exclusion of recombination machinery, resulting in avoidance of the accumulation of recombination intermediates within the rDNA locus, thus contributing to maintaining rDNA stability and faithful segregation.

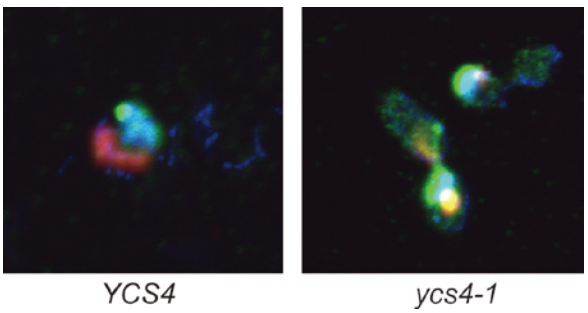


Figure 1. Nucleolar localization of Rad52 protein during mitotic phase. The wild-type (*YCS4*) and condensin mutant (*ycs4-1*) cells were arrested at metaphase by interfering with the polymerization of microtubules (nocodazole). Nucleolar organizing protein (Nop56) was detected as red and Rad52 protein was detected as green colors, respectively. In the *ycs4-1* mutant, Rad52 invades in the nucleolus (yellow).

II. Condensin-dependent chromatin folding

The RFB site, which consists of a ~150bp DNA sequence, acts as the strongest cis-element for condensin recruitment onto chromatin. If the RFB sequence 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 at an interval of 15kb distance in the cell with complete deletion of chromosomal rDNA repeat. Using such strain, condensin-dependent chromatin interaction between two RFBs was examined by chromosome conformation capture (3C) assay. We found condensin-dependent interaction between 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 the creation of a chromatin loop between those sites (Figure 2). It is thought that condensin-dependent chromatin folding is one of the basic molecular processes of chromosome condensation.

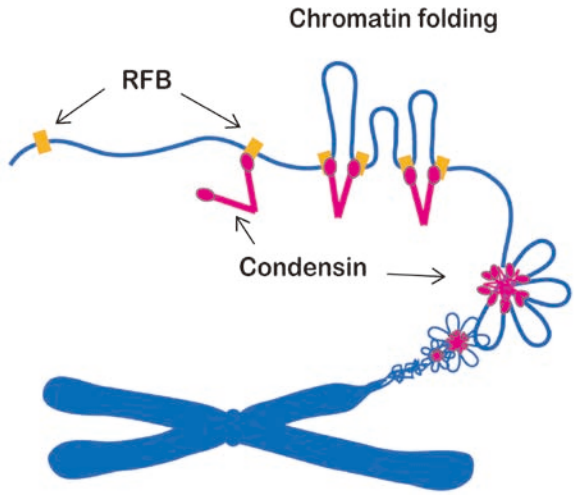


Figure 2. A Schematic model of chromosome condensation. Condensin causes chromatin interactions between adjacent binding sites (RFB, for example). This lead to a folding of chromatin fiber between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

WATANABE Group †

Assistant Professor: *WATANABE, Takaaki*
Technical Assistant: *YONEZAWA, Harumi*

Genomes have been dynamically evolving and are continually changing during development, and through diseases and environmental stress. One type of genome alteration, gene amplification, is involved in various biological phenomena, such as malignant progression of cancer, resistance to insecticides and anticancer drugs, and gene evolution. We are addressing the molecular mechanisms underlying gene amplification from a variety of perspectives.

I. Model systems for studying mechanisms for gene amplification

Long series of studies have shown that DNA double-strand breaks and inverted repeats play an important role in gene amplification. However, details of the molecular mechanisms remain to be determined. This is because previous approaches to understanding the mechanisms were based on the structural analysis of complex end products and very few model systems are available that allow chromosomal engineering and genetic analysis.

To better understand the molecular mechanisms, we have developed a new approach in which we design amplification processes and test whether the processes can produce the amplification seen in nature. Previously, we constructed a system designed to induce a rapid amplification mode, double rolling-circle replication, (DRCR, Figure 1A) via chromosomal breaks induced by site-specific endonuclease (EMBO J, 2005). This system produced intra-/extra-chromosomal products resembling those seen in mammalian cells; homogeneously staining regions (HSR) and double minutes (DMs). This result strongly suggested that amplification in mammalian cells involves DRCR.

We next examined whether recombinational processes coupled with replication can induce gene amplification via DRCR, using a distinct process, Cre-lox site-specific recombination. Here, we inferred that, if Cre recombination coupled with replication occurs, the replication fork makes an additional copy of the replicated region (Figure 1B); and that the processes from two pairs of lox sites could induce DRCR (Figure 1C). In this study, we successfully detected HSR/DM-type amplification products in yeast and Chinese hamster ovary (CHO) cells (Figure 1D and 1E). Surprisingly, over 10% of the Cre recombination-induced yeast cells undergo gene amplification. In addition, scattered-type products were also found (Figure 1F), which are frequently seen in cancer cells. From these results, we reasoned that DRCR and convergent replication are centrally involved in the amplification of drug-resistance genes and oncogene. This system can serve as a good model for amplification in mammalian cells and contribute to a better understanding of oncogene amplification and development of anticancer strategies in future.

II. Intensive rearrangement in amplified region

In amplified chromosomal regions intensive chromosome rearrangements are frequently observed, leading to an increase in the gene copy number and to a decrease in size of the amplification unit. In oncogene amplification, the complex patterns of amplification generated by the rearrangements are closely associated with poor prognosis in cancer. Interestingly, we have observed the rearrangement in all our DRCR systems.

To explore the link between the rearrangements and the DRCR process, we constructed a system that can turn on or off the occurrence of DRCR, using yeast 2μ plasmid. This system demonstrated that inversions, deletions, or duplications could be intensively induced in a DRCR-dependent manner. This result suggests that DRCR may cause the rearrangements in amplification in nature. We proposed a model in which DRCR markedly stimulates recombinational events.

III. A structural platform for gene amplification

Based on our results, we now focus on a type of genomic structure consisting of two sets of inverted repeats, designated double IR. This structure is found in the human genome and can be observed in the early stages of gene amplification. In yeast, the double IR could induce gene amplification. Now we try to construct the double IR in CHO cells and to perform its detailed functional analysis in yeast.

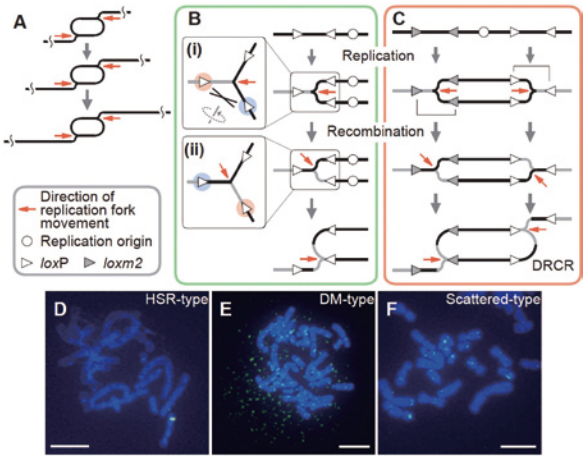


Figure 1. DRCR process, recombinational process coupled with replication, and amplification products in CHO cells.

Publication List

〔Original papers〕

● Okamoto, H., Watanabe, T., and Horiuchi, T. (2011). Double rolling circle replication (DRCR) is recombinogenic. Genes Cell *16*, 503-513.
● Watanabe, T., Tanabe, H., and Horiuchi, T. (2011). Gene amplification system based on double rolling-circle replication as a model for oncogene-type amplification. Nucleic Acids Res. *39*, e106.

†: This group was closed on 31 March, 2012.

Division of Molecular Environmental Endocrinology

Professor
IGUCHI, Taisen

Assistant Professors:	*MIYAGAWA, Shinichi*
	OGINO, Yukiko
NIBB Research Fellow:	*CHAKRABORTY, Tapas*
Postdoctoral Fellow:	*MIYAKAWA, Hitoshi*
Technical Staff:	*MIZUTANI, Takeshi*
Graduate Students:	*SATO, Masaru*
	TOYOTA, Kenji
	SUMIYA, Eri
	YATSU, Ryohei
	NAKAMURA, Takeshi
Visiting Scientists:	*ODA, Shigeto*
	HIRAKAWA, Ikumi
	TOHYAMA, Saki
	SEBILLOT, Anthony
	WON, Seok
	LANGE, Anke
Technical Assistants:	*HAYASHI, Tomoko*
	KOBAYASHI, Kaoru
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 irreversible alterations to the endocrine and reproductive systems of both sexes. The immune and nervous systems, bone, muscle, and the liver were also 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.

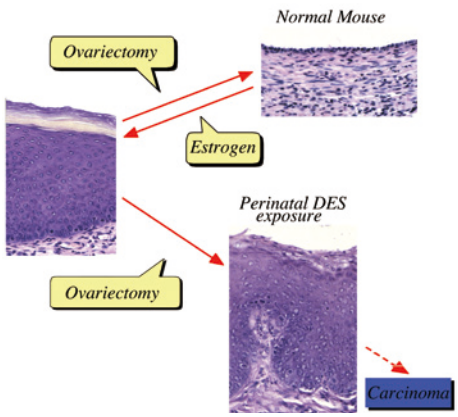


Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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. 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. Currently, we are analyzing the methylation status in the mouse vagina using MeDIP (methylated DNA immunoprecipitation) coupled with 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.

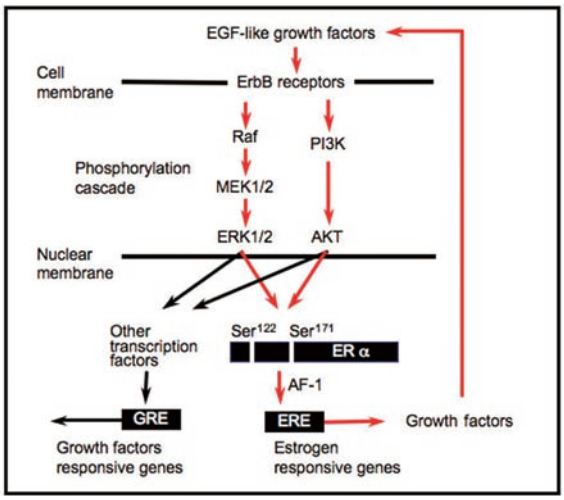


Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

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

Steroid and xenobiotic receptors (SXR) have been cloned from various animal species (fish, amphibian, 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 Isteoglossiformes. 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.

IV. Gene zoo and receptor zoo

We are establishing cDNA library banks and receptor gene banks of animal species including lancelet, lamprey, sturgeon, lungfish, gar, mangrove *Rivulus*, whale shark catshark, Japanese giant salamander, newt, *Rana rugosa*, *Silurana tropicalis*, Japanese rat snake, Okinawa habu, Florida red berry turtle, American alligator, Nile crocodile, vulture and polar bear in collaboration with the University of Pretoria, South Africa, University of Florida, Medical University of South Carolina, San Diego Zoo, USA, and the Asa Zoo in Hiroshima.

V. Sex differentiation mechanism in Daphnids

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

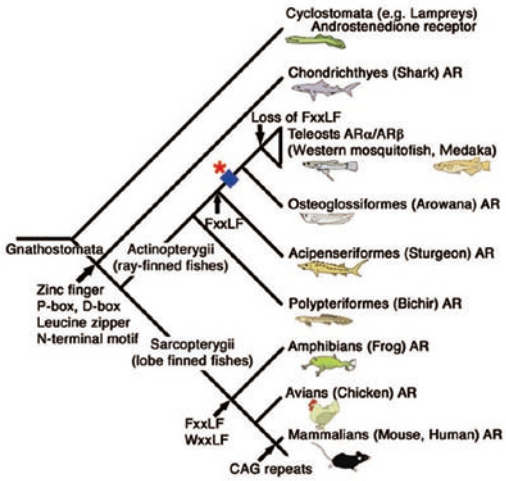


Figure 3. Evolutionary relationships of androgen receptor sequences.

information about the mode of action of the tested compounds. Therefore, we applied an ecotoxicogenomic assessment of *D. magna*. We established a *Daphnia* EST database and developed an oligonucleotide-based DNA microarray 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* 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

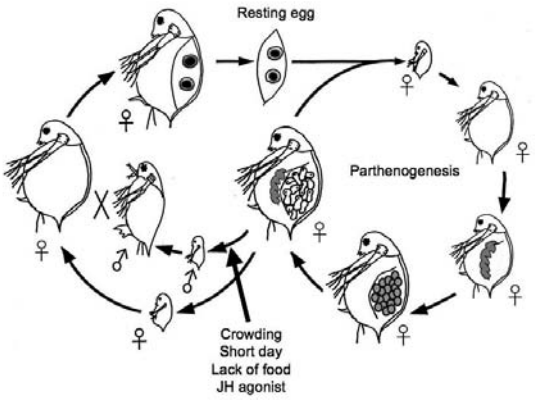


Figure 4. Life cycle of *Daphnia*.

asexual to sexual reproduction. Chemicals are able to affect the sex determination of *D. magna* 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 *D. magna*. 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* embryos which will allow us to study gain- and loss-of function analyses in more

detail in this 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*. To further explore the signaling cascade of sexual differentiation in *D. magna,* a gene expression profile of JH-responsive genes is essential. Thus, DNA microarray analysis has been performed in the gonads of *D. magna* exposed to fenoxycarb (synthesized JH agonist widely used as an insect growth regulator) and methyl farnesoate (JH identified in decapods) at the critical timing of JH-induced sex determination in *D. magna*. We are currently identifying JH-responsive genes in the ovary.

Publication List

〔Original papers〕

- Bermudez, D.S., Skotko, J.P., Ohta, Y., Boggs, A.S.P., Iguchi, T., and Guillette, L.J.Jr. (2011). Sex steroid and thyroid hormone receptor expressions in the thyroid of the American alligator (*Alligator mississippiensis*) during different life stages. J. Morphol. *272*, 698-703.
- Chakraborty, T., Katsu, Y., Zhou, L.Y., Miyagawa, S., Nagahama, Y., and Iguchi, T. (2011). Estrogen receptors in medaka (*Oryzias latipes*) and estrogenic environmental contaminants: an *in vitro-in vivo* correlation. J. Steroid Biochem. Mol. Biol. *123*, 115-121.
- Chakraborty, T., Shibata, Y., Zhou, L.Y., Katsu, Y., Iguchi, T., and Nagahama, Y. (2011). Differential expression of three estrogen receptor subtype mRNAs in gonads and liver from embryos to adults of the medaka, *Oryzias latipes*. Mol. Cell. Endocrinol. *333*, 47-54.
- Kato, Y., Kobayashi, K., Watanabe, H., and Iguchi, T. (2011). Environmental sex determination in the branchiopod crustacean *Daphnia magna*: Deep conservation of a *Doublesex* gene in the sex-determining pathway. PLoS Genetics *7*, e1001345.
- Kato, Y., Shiga, Y., Kobayashi, K., Tokishita, S., Yamagata, H., Iguchi, T., and Watanabe, H. (2011). Development of an RNA interference method in the cladoceran crustacean *Daphnia magna*. Devel. Genes Evol. *220*, 337-345.
- Lange, A., Paull, G.C., Hamilton, P.B., Iguchi, T., and Tyler, C.R. (2011). Implications of persistent exposure to treated wastewater effluent for breeding in wild roach (*Rutilus rutilus*) populations. Environ. Sci. Technol. *45*, 1673-1679.
- Matsumura, D., Haraguchi, R., Miyagawa, S., Motoyama, J., Nakagata, N., Meijlink, F., and Yamada, G. (2011). Genetic analysis of hedgehog signaling in ventral body wall development and the onset of omphalocele formation. PLoS ONE *20*, e16260.
- Miyagawa, S., Matsumaru, D., Murashima, A., Omori, A., Satoh, Y., Haraguchi, R., Motoyama, J., Iguchi, T., Nakagata, N., Hui, C.C., and Yamada, G. (2011). The role of sonic hedgehog-Gli2 pathway in the masculinization of external genitalia. Endocrinology *152*, 2894-2903.
- Moore, B.C., Milnes, M.R., Kohno, S., Katsu, Y., Iguchi, T., Woodruff, T.K., and Guillette, L.J.Jr. (2011). Altered gonadal expression of TGF-β superfamily signaling factors in environmental contaminant-exposed juvenile alligators. J. Steroid Biochem. Mol. Biol. *127*, 58-63.
- Murashima, A., Miyagawa, S., Ogino, Y., Nishida-Fukuda, H., Araki, K., Matsumoto, T., Kaneko, T., Yoshinaga, K., Yamamura, K.I., Kurita, T., Kato, S., Moon, A.M., and Yamada, G. (2011). Essential roles of androgen signaling in Wolffian duct stabilization and epididymal cell differentiation. Endocrinology *152*, 1640-1651.
- Nakajima, T., Hayashi, S., Iguchi, T., and Sato, T. (2011). The role of fibroblast growth factors on the differentiation of vaginal epithelium of neonatal mice. Differentiation *82*, 28-37.
- Nakajima, T., Iguchi, T., and Sato, T. (2011). Involvement of activin signaling in abnormalities of the mouse vagina exposed neonatally to diethylstilbestrol. Cell Tiss. Res. *344*, 527-538.
- Southam, A.D., Lange, A., Hines, A., Hill, E.M., Katsu, Y., Iguchi, T., Tyler, C.R., and Viant, M.R. (2011). Metabolomics reveals target and off-target toxicities of a model organophosphate pesticide to roach (*Rutilus rutilus*): Implications for biomonitoring. Environ. Sci. Technol. *45*, 3759-3767.
- Urushitani, H., Katsu, Y., Miyagawa, S., Kohno, S., Ohta, Y., Guillette, L.J.Jr., and Iguchi, T. (2011). Molecular cloning of anti-Müllerian hormone from the American alligator, *Alligator mississippiensis*. Mol. Cell. Endocrinol. *333*, 190-199.
- Urushitani, H., Katsu, Y., Ohta, Y., Shiraishi, H., Iguchi, T., and Horiguchi, T. (2011). Cloning and characterization of retinoid X receptor (RXR) isoforms in the rock shell, *Thais clavigera*. Aquat. Toxicol. *103*, 101-111.

〔Review articles〕

- Blumberg, B., Iguchi, T., and Odermatt, A. (2011). Editorial: Endocrine disrupting chemicals. J. Steroid Biochem. Mol. Biol. *127*, 1-3.
- Celander, M.C., Goldstone, J.V., Denslow, N.D., Iguchi, T., Kille, P., Meyerhoff, R.D., Smith, B.A., Hutchinson, T.H., and Wheeler, J.R. (2011). Species extrapolation for the 21st century. Environ. Toxicol. Chem. *30*, 52-63.
- Hayes, T.B., Beasley, V.R., de Solla, S., Iguchi, T., Ingraham, H., Kestemont, P., Kniewald, J., Kniewald, Z., Langlois, V.S., Luque, E.H., McCoy, K.A., Muñoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, S., Suzawa, M., Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costa, A.B., and Willingham, E. (2011). Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J. Steroid Biochem. Mol. Biol. 127, 64-73.
- Miyagawa, S., Sato, M., and Iguchi, T. (2011). Molecular mechanisms of induction of persistent changes by estrogenic chemicals on female reproductive tracts and external genitalia. J. Steroid Biochem. Mol. Biol. *127*, 51-57.
- Ogino, Y., Miyagawa, S., Katoh, H., Prins, G.S., Iguchi, T., and Yamada, G. (2011). Essential functions of androgen signaling emerged through the developmental analysis of vertebrate sex characteristics. Evol. Devel. *13*, 315–325.
- Watanabe, H., Kato, Y., and Iguchi, T. (2011). Application of ecotoxicogenomics for understanding mode of action of chemicals and species extrapolation. In: Handbook of Systems Toxicology. Eds. Casciano, D.A. and Sahu, S.C., John Wiley & Sons, Ltd. pp.67-72.

DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

Professor
MINAGAWA, Jun

NIBB Research Fellow:	*TAKIZAWA, Kenji*
Postdoctoral Fellows:	*TAKIZAWA, Kenji**
	OHNISHI, Norikazu
	TOKUTSU, Ryutaro
Visiting Scientist:	*CHEN, Yang*
Technical Assistant:	*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 the changing light environment. Using a model green alga, we are studying the molecular mechanisms underlying the photoacclimation of the photosynthetic machinery. We are also applying the knowledge obtained in the studies of a model green alga to various phytoplankton including diatoms in the subarctic North Pacific, prasinophytes in the subtropical Mediterranean Sea, and *Symbiodinium* in corals in tropical oceans, to explore how these environmentally important photosynthetic organisms thrive in their ecological niche.

I. Acclimation of photosynthesis

Using a unicellular green alga *Chlamydomonas reinhardtii*, we investigate the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes by means of biochemistry, molecular genetics, absorption and fluorescence spectroscopy, and bio-imaging.

1-1 State-transitions

The two photosystems—photosystem I (PSI) and II (PSII)—in the thylakoid membranes function as charge-separation devices. Each has a distinct pigment system with distinct absorption characteristics (PSI has a broad absorption peak in the far-red region as well as peaks in the blue and red regions, whereas PSII has absorption peaks in the blue and red, but not in the far-red region) and a distinct action spectrum. Thus, an imbalance of energy distribution between the two photosystems tends to occur in natural environments, where light quality and quantity fluctuate with time. Since the two photosystems are connected in series under normal conditions, green plants and algae need to constantly balance their excitation levels to ensure optimal efficiency of electron flow. State transitions occur under such conditions to redistribute the harnessed energy to minimize its unequal distribution.

Although state transitions have been widely accepted as a short-term response in plants to acclimate to the fluctuating light conditions, most of the previous investigations were conducted *in vitro*, implying that the real impact on photosynthesis remains to be characterized. This year, we visualized phospho-LHCII dissociation during state transitions using fluorescence lifetime imaging microscopy (FLIM) for the first time *in vivo*, where the fluorescence lifetime in live *C. reinhardtii* cells was monitored under a fluorescence microscope during a transition from State 1 to 2. Initially, the average lifetime of fluorescence emitted between 680-700 nm was 170 psec, which was largely due to the PSII-bound LHCII, but it shifted to 250 psec when the cells were in transition to State 2 after 5 min. Single-cell FLIM further indicated that the dissociated LHCII spreads through the cell during State 2 transitions and forms several large spotted areas. Further biochemical analyses indicated that dissociated phospho-LHCII formed a large aggregated structure, whereas unphosphorylated LHCII did not. Thus, the free phospho-LHCII aggregates appearing during State 2 transitions are in energy-dissipative form.

The molecular mechanism for q_E quenching has been a heated issue during the last two decades, and it still remains controversial. Because the unexpectedly short fluorescence lifetime of the phospho-LHCII aggregates during the state transition described above was not caused by high light illumination, they are not exhibiting q_E quenching, but rather exhibiting q_T (state transition) quenching. However, it is now tempting to speculate that LHCII aggregates are a common site of energy dissipation, i.e., that both q_E and q_T quenching are causally related by the energy-dissipative LHCII aggregates.

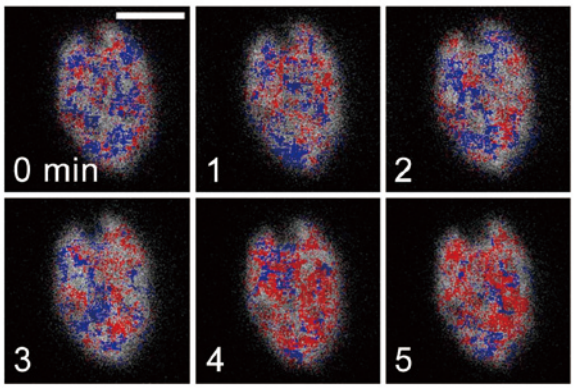


Figure 1. Visualization of the progress of a state 1-to-2 transition by means of FLIM. Blue and red dots correspond to 170 and 250 psec lifetime components, respectively.

1-2 Cyclic electron flow

In eukaryotes, photosynthesis is a process of photochemical energy transduction, which occurs via the conductance of electron flow in the thylakoid membranes of chloroplasts, resulting in the reduction of $NADP^+$ in the stroma and the concomitant generation of a proton motive force across the membranes. The NADPH generated by the electron flow and the ATP synthesized by ATP synthase utilizing the proton motive force are used to fix carbon dioxide in the Calvin-Benson cycle. Linear electron flow (LEF) and cyclic electron flow (CEF) are known as modes of electron flow in photosynthesis. In the linear pathway, electrons are transferred from PSII to $NADP^+$ by way of the cytochrome b_6f complex (Cyt b_6f) and PSI. In the cyclic

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

pathway, however, the exact pathway of electrons that originate in PSI and then return to PSI has not been clear. State transitions have long been considered as a mechanism by which the distribution of light excitation between the two photosystems is regulated. However, the performance of PSI tends to overwhelm PSII under State 2 conditions in *C. reinhardtii* because of its extensive ability to relocate LHCII proteins; this implies that state transitions might represent a mechanism by which the electron transfer chain in the thylakoid membranes is switched to the mechanism exclusively employed by PSI.

We solubilized thylakoid membranes from *C. reinhardtii* cells under State 2 conditions and loaded them onto a sucrose density gradient. A "super-supercomplex" (CEF supercomplex) with a molecular weight of approximately 1.5 million composed of the PSI-LHCI supercomplex with LHCIIs, Cyt *bf*, Fd-NADPH oxidoreductase (FNR), and the integral membrane protein PGRL1 was detected in a fraction heavier than the PSI-LHCI supercomplex. Spectroscopic analyses indicated that upon illumination, reducing equivalents downstream of PSI were transferred to Cyt *bf*, while the oxidized PSI was re-reduced by reducing equivalents from Cyt *bf*, indicating that this supercomplex is engaged in CEF. Thus, CEF takes place in a protein supercomplex where steps in LEF are rearranged to undergo an alternative pathway for the flow of electrons.

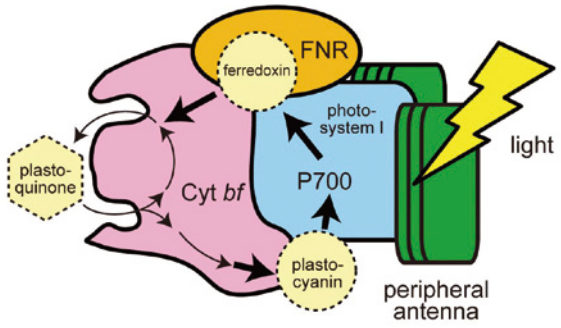


Figure 2. Cyclic electron flow by way of the CEF supercomplex.

II. Ecophysiology of marine phytoplankton

Prasinophyceae are a broad class of early-branching eukaryotic green algae. These picophytoplankton are found ubiquitously throughout the ocean and contribute considerably to global carbon-fixation. *Ostreococcus tauri*, as the first sequenced prasinophyte, is a model species for studying the functional evolution of light-harvesting systems in photosynthetic eukaryotes.

We isolated and characterized *O. tauri* pigment-protein complexes to understand the diversity and the evolutional traits of the light-harvesting systems in a primitive green alga. Two PSI fractions were obtained by sucrose density gradient centrifugation in addition to free LHC fraction and PSII core fractions. The smaller PSI fraction contains the PSI core proteins, LHCI, which are conserved in all green plants, Lhcp1, a prasinophyte-specific LHC protein, and the minor monomeric LHCII proteins CP26 and CP29. The larger PSI fraction contained the same antenna proteins as the smaller, with the addition of Lhca6 and Lhcp2, and a 30% larger absorption cross-section. When *O. tauri* was grown under high-light conditions, only the smaller PSI fraction was present. The two PSI preparations were also found to be devoid of far-red chlorophyll fluorescence (715-730 nm), a signature of PSI in oxygenic phototrophs. These unique features of *O. tauri* PSI may reflect primitive light-harvesting systems in green plants and their adaptation to marine ecosystems.

Our newest project is the study of photoacclimation of *Symbiodinium*, which live in a symbiotic relationship with corals, and other Cnidarians. We are particularly interested in those living with corals and are trying to elucidate how their photosynthetic machinery is acclimated to the variable light and temperature environments in the tropical ocean.

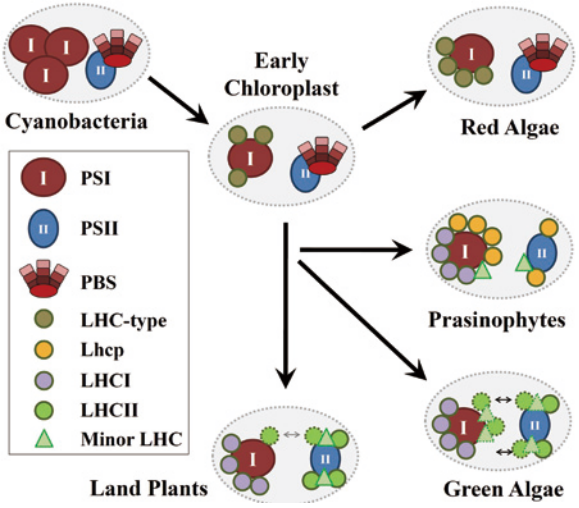


Figure 3. Evolutionary model of LHC affinity in photosynthetic eukaryotes as revealed by biochemical study of the LHC systems in *O. tauri*.

Publication List

〔Review article〕

- Minagawa, J. (2011). State transitions — The molecular remodeling of photosynthetic supercomplexes that controls energy flow in the chloroplast. Biochim. Biophys. Acta *1807*, 897-905.

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) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes from precomputed all-against-all similarity relationships 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 available, but also allows users to create their own ortholog groups on the fly using a specified set of organisms. In addition, MBGD also provides MyMBGD mode, which allows users to add their own genomes to MBGD. These features are especially useful when the user's interest is focused on comparison of genomes that are taxonomically related to some specific organisms.

The database now contains well over 1000 published genomes including 27 eukaryotic microbes and 4 multicellular organisms. To cope with further increase of genomic data in the future, we have begun to develop an efficient system for updating ortholog data, which includes a refinement procedure for constructing reference ortholog groups and an efficient procedure for incremental update of orthologous relationships (see Section II below).

MBGD is available at http://mbgd.genome.ad.jp/.

II. Development of methods for constructing orthologous groups among multiple genomes

As a core technology of our comparative genomics tools, we have 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.

We are continuing to improve the algorithm. To improve the scalability of the algorithm for comparison of thousands of genomic sequences, we are developing an efficient method to update the clustering result incrementally. By this method, one can add new genomes to a reference set of ortholog groups that is constructed from a representative set of published genomes available in the MBGD server. We are also trying to extend the algorithm for handling metagenomic data. To infer the taxonomic position of the source organism of each metagenomic sequence, we have developed a method to map each tree node of the hierarchical clustering tree generated by the DomClust algorithm onto a taxonomic tree node.

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: a "core gene pool" that comprises intrinsic genes encoding the proteins of basic cellular functions, and a "flexible gene pool" that comprises HGT-acquired genes encoding proteins which function under particular conditions. The identification of the set of intrinsically conserved genes, or the genomic core, among a taxonomic group is crucial not only for establishing the identity of each taxonomic group, but also for understanding prokaryotic diversity and evolution. 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. We developed a method for aligning conserved regions of multiple genomes, which finds the order of pre-identified orthologous groups that retains to the greatest possible extent the conserved gene orders.

We are now expanding our analysis to more diverged bacterial families to examine generality of our approach. We are also developing an enhanced algorithm that can incorporate phylogenetic relationships among input genomes.

IV. Development of a workbench for comparative genomics

We are developing 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 including phylogenetic pattern analysis, the ingroup/outgroup distinction in ortholog grouping and the core structure extraction among related genomes. The entire

RECOG system employs client-server architecture: the server program is based on the MBGD server and contains the database construction protocol used in MBGD so that users can install the server on their local machines to analyze their own genomic data, whereas the client program is a Java application that runs on a local machine by receiving data from any available RECOG server including the public MBGD server.

The central function of RECOG is to display and manipulate a large-scale ortholog table (Figure 1). The ortholog table viewer is a spreadsheet like viewer that can display the entire ortholog table, containing more than a thousand genomes. Using the zoom in/out function, it can display the entire table or a section of the main table with more detailed information. 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, such as "Neighborhood gene clustering" and "Phylogenetic pattern clustering" In addition, RECOG allows the user to input arbitrary gene properties such as sequence length, nucleotide/amino acid contents and functional classes, and compared these properties among orthologs in various genomes.

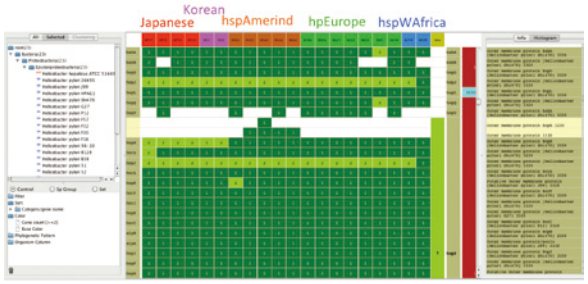


Figure 1. The RECOG system displaying ortholog groups of outer membrane proteins identified among 20 *H. pylori* strains.

V. Comparative genomics of *Helicobacter pylori*

Helicobacter pylori is a major pathogen in human gastric cancer and it is known that the East Asian strains of *H. pylori* have a stronger subtype of a major virulence factor, CagA protein, than Western strains. In collaboration with Dr. Kobayashi (Univ. Tokyo) and other researchers, we have determined the complete genomic sequences of four *H. pylori* strains isolated from Japanese patients and compared them with other published *H. pylori* genomes. Using the RECOG system and other tools (Figure 1), we tried to identify characteristic genomic features of the East Asian strains from various points of view and infer evolutionary processes and mechanisms that are related to the evolution of *H. pylori* (Figure 2). As a result, we were able to identify several genes that characterize the East Asian strains.

In addition, we found that some outer membrane proteins that are specifically duplicated in the East Asian strains are located at the boundary of the chromosomal inversion identified between the East Asian and other strains. After detailed examination of the boundary of these and other chromosomal inversions identified among *H. pylori* genomes, we were able to find a novel mechanism of genome evolution named DNA duplication associated with inversion.

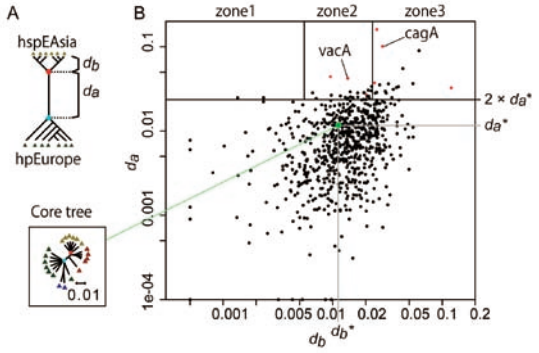


Figure 2. Identification of the genes characteristic to the East Asian strains of *H. pylori* based on phylogenetic tree topologies. Several characteristic genes were identified in addition to well-known virulence factors, cagA and vacA.

Publication List

〔Original papers〕

- Furuta, Y., Kawai, M., Uchiyama, I., and Kobayashi, I. (2011). Domain movement within a gene: a novel evolutionary mechanism for protein diversification. PLoS ONE, *6*, e18819.
- Furuta, Y., Kawai, M., Yahara, K., Takahashi, N., Handa, N., Tsuru, T., Oshima, K., Yoshida, M., Azuma, T., Hattori, M., Uchiyama, I., and Kobayashi, I. (2011). Birth and death of genes linked to chromosomal inversion, Proc. Natl. Acad. Sci. USA *108*, 1501-1506.
- Kawai, M., Furuta, Y., Yahara, K., Tsuru, T., Oshima, K., Handa, N., Takahashi, N., Yoshida, M., Azuma, T., Hattori, M., Uchiyama, I., and Kobayashi, I. (2011). Evolution in an oncogenic bacterial species with extreme genome plasticity: *Helicobacter pylori* East Asian genomes. BMC Microbiology *11*, 104.
- Yamamoto, K., Tanaka, H., Nishitani, Y., Nishiumi, S., Miki, I., Takenaka, M., Nobutani, K., Mimura, T., Ben Suleiman, Y., Mizuno, S., Kawai, M., Uchiyama, I., Yoshida, M., and Azuma, T. (2011). *Helicobacter suis* KB1 derived from pig gastric lymphoid follicles induces the formation of gastric lymphoid follicles in mice through the activation of B cells and CD4 positive cells. Mocrobes Infect. *13*, 697-708.

〔Review article〕

- Uchiyama, I. (2011). Functional inference in microbial genomics based on large-scale comparative analysis. In Omics Approaches to Protein Function Prediction, D. Kihara ed., (Springer), pp.55-92.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

Technical Staff:	*KAJIURA-KOBAYASHI, Hiroko*
NIBB Research Fellows:	*OSHIMA, Yusuke**
	*TAKAO, Daisuke**
Postdoctoral Fellows:	*ICHIKAWA, Takehiko*
	TAKAO, Daisuke
Visiting Scientist:	*OSHIMA, Yusuke*
Technical Assistants:	*SHINTANI, Atsuko*
	ISHIBASHI, Tomoko
	OKA, Naomi

Our laboratory is currently pursuing two paths of scientific inquiry 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 developmental biology.

I. Initial step for left-right asymmetry

The first L-R asymmetry in mammalian development arises on the embryonic surface. A gastrulating mouse embryo has a shallow hollow on its ventral surface, called 'the node,' with hundreds of cilia moving in a clockwise rotational manner (Figure 1; Nonaka et al., 1998). The sum of the vortical motions of the cilia, however, generates a leftward flow of the surrounding fluid rather than a vortex. The cilia can generate L-R asymmetry *de novo*, i.e. without pre-existing left-right asymmetry, by their posteriorly tilted rotation axis (Nonaka et al., 2005).

The leftward flow, called nodal flow, determines subsequent L-R development. This principle has been confirmed by our experiments, which demonstrated that embryos raised with an artificial rightward flow develop reversed L-R asymmetry (Nonaka et al., 2002).

While it is clear that nodal flow conveys asymmetric information along the L-R axis, the nature of the information remains unclear. We are now working to clarify how the direction of nodal flow is converted to the subsequent step, asymmetric gene expression.

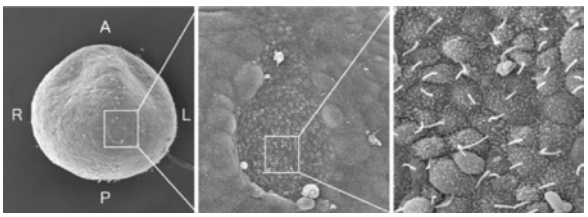


Figure 1. Left: ventral view of a 7.5-day mouse embryo. Middle: the node. Right: node cilia.

II. Imaging technologies

Long-term live imaging of large specimens, such as embryos, is very useful in developmental biology but technically challenging, mainly because of phototoxicity and the limitations of deep imaging. Light-sheet microscopy

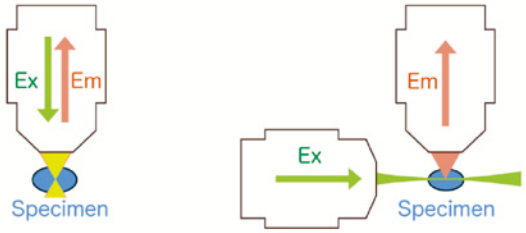


Figure 2. Principle of light-sheet microscopy. Left: light path of conventional fluorescent microscopes using single objective lens for both illumination (Ex) and detection (Em). Right: light-sheet microscopes including DSLM, where illumination light is limited to the focal plane of the detection objective.

including Digital Scanned Light-sheet Microscope (DSLM, Figure 2) is extremely suitable for this purpose, and we have applied it to analyze cell movements in intact mouse embryos at gastrulating stages (Figure 3). We have been also developing another light-sheet microscope for wider application of living samples: our embed-free and fast image acquisition system enables 4D data acquisition of freely moving *Amoeba proteus*.

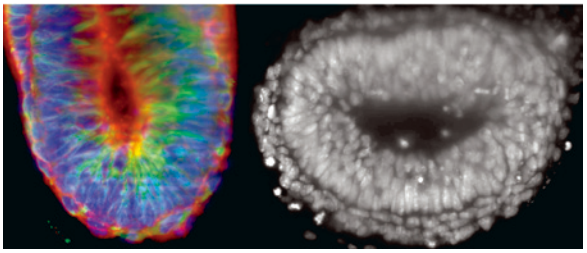


Figure 3. 6.5-day mouse embryos visualized by DSLM. Left: an optical transverse section of a fixed embryo. Right: cross section of a living one expressing GFP in the neurons.

In addition to our own research projects, we support researchers who are interested in using our DSLM and two-photon microscopes. Several collaborations including live imaging of developing brains, hair bulbs, salivary glands and renal tubules are in progress.

Publication List

〔Original paper〕

● Kishimoto., N., Alfaro-Cervello, C., Shimizu, K., Asakawa, K., Urasaki, A., Nonaka, S., Kawakami, K., Garcia-Verdugo, JM., and Sawamoto, K. (2011). Migration of neuronal precursors from the telencephalic ventricular zone into the olfactory bulb in adult zebrafish. Journal of Comparative Neurology *519*, 3549-3565.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

NIBB Core Research Facilities

Head
KOBAYASHI, Satoru

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

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

Functional Genomics Facility

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
	YAMAGUCHI, Katsushi
Postdoctoral Fellow:	*KITAZUME, Tatsuya*
Technical Assistants:	*ASAO, Hisayo*
	FUJITA, Miyako
Secretary:	*ICHIKAWA, Mariko*

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

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD5500xl (Applied Biosystems) and HiSeq2000 (Illumina), which was installed this year, the Functional Genomics Facility is committed to joint research aiming to exploring otherwise inaccessible new fields in basic biology.

During 2011 we carried out 45 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. A successful example is the mutant screening of *Arabidopsis*. We successfully identified causative mutations in EMS mutant screening by deep sequencing quickly at low cost, which is much more effective than conventional mapping-based cloning methods.

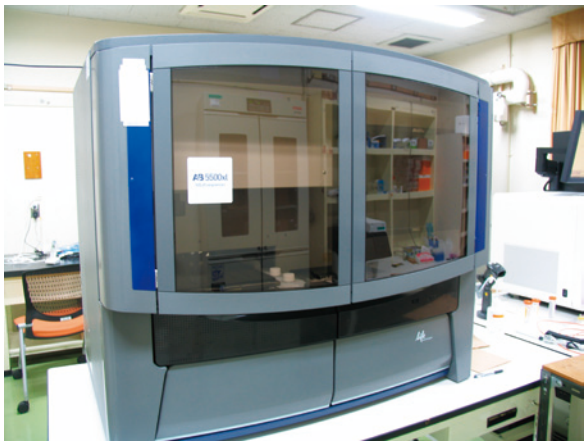


Figure 1. Next-generation sequencer SOLiD5500xl

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2011, we analyzed approximately 350 samples with mass spectrometers and 40 samples with protein sequencers.

- GC-Mass Spectrometer (JEOL DX-300)
- MALDI-TOF-MS (Bruker Daltonics REFLEX III)
- LC-Q-TOF MS (Waters Q-TOF Premier)
- Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

Other analytical instruments

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

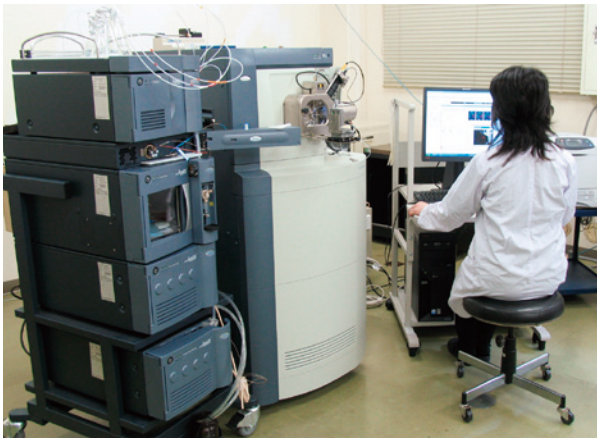


Figure 2. LC-Q-TOF-MS

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2011, we provided a two-day training course on next-generation sequence data analyses. This course is designed to introduce the basic knowledge and skills of next-generation sequence data analysis to biologists who are not familiar with bioinformatics (p. 84).

Figure 3. NIBB Genome Informatics Training Course

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

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

Technical Assistants: *HASHIYAMA, Tomomi*
SUZUKI, Miyuzu

Symbiosis Genomics

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

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

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

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

Aphid research is entering the post-genome era. We analyzed the transcriptome of aphid bacteriocytes using RNA-seq technology featuring a next-generation DNA sequencer. We found thousands of genes over-represented in the symbiotic organ in comparison with the whole body. Many genes for amino acid metabolism are found to be over-represented as expected: the plant sap-eating insect depends on the bacterial symbionts to supply essential amino acids. In addition, many kinds of novel secretion proteins that are found only in aphid species are extremely enriched in the bacteriocytes. We also found that bacteriocytes express Distal-less (Dll), a homeodomain-containing transcription factor throughout the life cycle. Future study should focus on dissecting the genetic network of these components, which should allow us to understand the genetic basis on which symbiosis generates evolutionary novelty.

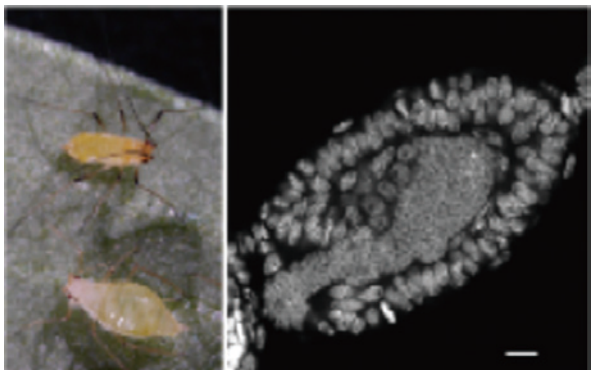


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

Publication List

〔Original paper〕

● Price, D., Duncan, R., Shigenobu, S., and Wilson, A. (2011). Genome expansion and differential expression of amino acid transporters at the aphid/*Buchnera* symbiotic interface. Mol. Biol. Evol. *28*, 3113–3126.

〔Review article〕

● Shigenobu, S., and Wilson, A.C.C. (2011). Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont. Cell. Mol. Life Sci. *68*, 1297-1309.

Spectrography and Bioimaging Facility

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Staff: *HIGASHI, Sho-ichi*
TANIGUCHI-SAIDA, Misako
Technical Assistant: *ICHIKAWA, Chiaki*
Secretary: *ISHIKAWA, Azusa*

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

Representative Instruments:

Okazaki Large Spectrograph (OLS)

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

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

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

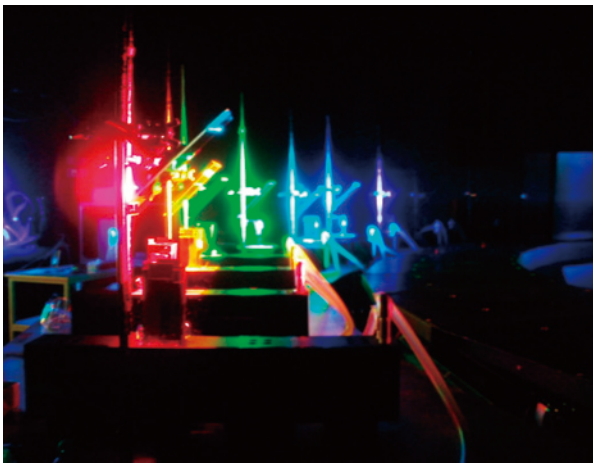


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light) were irradiated simultaneously to samples in cooling chambers.

Microscopes

This facility also has Bioimaging machines such as widefield microscopes (Olympus IX-81, BX-63 and KEYENCE BZ-8000), confocal microscopes (Olympus FV1000, Leica TCS SP2 and Nikon A1R, Carl Zeiss Duo 5) and other custom-made laser microscopes (Digital Scanned Light-sheet Microscope: DSLM and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began Collaborative Research Programs using these machines since 2010.

The DSLM was developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissue by illuminating from the side of a specimen with a light sheet (more information is described in Dr. Nonaka's section: Lab. for Spatiotemporal Regulations). Dr. Nonaka conducted and supported 7 projects of the Collaborative Research Program for the Use of the DSLM.

The IR-LEGO was developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST). This microscope can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (Kamei *et al*. Nat. Methods, 2009). Details are described in the next section. The IR-LEGO was also used for 8 Individual Collaborative Research projects, including applications for higher plants and small fish.

Publication List on Cooperation

〔Original papers〕

- Hamasaka, G., Muto, T., and Uozumi, Y. (2011). Molecular-architecture-based administration of catalysis in water: self-assembly of an amphiphilic palladium pincer complex. Angew. Chem. *50*, 4876-4878.
- Okubo, T., Kawamura, A., Takahashi, J., Yagi, H., Morishima, M., Matsuoka, R., and Takada, S. (2011). Ripply3, a Tbx1 repressor, is required for development of the pharyngeal apparatus and its derivatives in mice. Development *138*, 339-348.
- Moritoh, S., Sato, K., Okada, Y., and Koizumi, A. (2011). Endogenous arginine vasopressin–positive retinal cells in arginine vasopressin–eGFP transgenic rats identified by immunohistochemistry and reverse transcriptase– polymerase chain reaction. Mol.Vis. *17*, 3254-3261.
- Inada, H., Watanabe, M., Uchida, T., Ishibashi, H., Wake, H., Nemoto, T., Yanagawa, Y., Fukuda, A., and Nabekura, J. (2011). GABA regulate the multidirectional tangential migration of GABAergic interneurons in living neonatal mice. PLoS ONE *6*, e277048.
- Osada, H., Yoshitake, Y., Ikeda, T., Ishigaki, Y., Takata, T., Tomosugi, N., Sasaki, H., and Yonekura, H. (2011). Ultraviolet B-induced expression of amphiregulin and growth differentiation factor 15 in human lens epithelial cells. Mol. Vis. *17*, 159-169.
- Shikata, T., Iseki, M., Matsunaga, S., Higashi, S., Kamei, Y., and Watanabe, M. (2011). Blue and red light-induced germination of resting spores in the red-tide diatom Leptocylindrus danicus. Photochem. Photobiol. *87*, 590-597.

- Andrady, A.L., Hamid, H., and Torikai, A. (2011). Effects of solar UV and climate change on materials. Photochem. Photobiol. Sci. *10*, 292-300.
- Karahara, I., Takaya, E., Fujibayashi, S., Inoue, H., Weller, J. L., Reid, J.B., and Sugai, M. (2011). Development of the Casparian strip is delayed by blue light in pea stems. Planta *234*, 1019-1030.

● ***Research activity by Y. Kamei***

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Assistant: *KANIE, Yuta*

To investigate a gene function in each cell we have to express the gene in the cell *in vivo*, ideally the expression must be limited only to the single cell. Tissue or cell specific promoters were used to reveal gene functions, however promoter-driven gene expression was governed by cell fate or environment, therefore we could not control the timing of gene expression. To achieve timing-controlled gene expression we employed one of the stress responses, the heat shock response. The heat shock promoter is the transcription regulation region of heat shock proteins and all organisms have this mechanism. Positioning the target gene downstream of the promoter, we can induce the target gene expression by heating.

In infrared (IR) beams can heat water molecules, which are the main constituent of cells, hence, we can heat a single cell by irradiating IR to a target cell using a microscope. We have developed a microscope, IR laser evoked gene operator (IR-LEGO), specialized for this purpose (Figure 1). The IR-LEGO microscope can irradiate an IR laser to a single cell *in vivo* such as *C. elegans*, medaka and *Arabidopsis*, to induce the heat shock response at a desired timing.

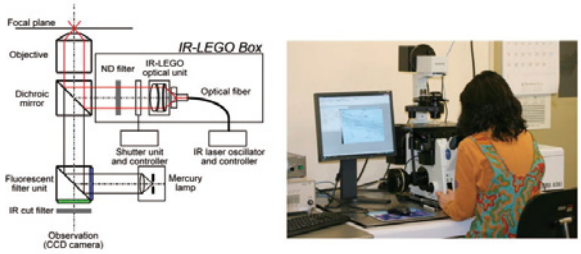


Figure 1. An infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating; however, there was no way to measure temperature in a microenvironment under microscopic observation. To achieve this we employed green fluorescent protein (GFP) as a thermometer. Since fluorescent matter has the common property of temperature dependent decrease of emission intensity, we can estimate temperature shift by emission intensity change. GFP expressing E. coli was used to measure temperature as a micro thermometer. Using this probe, we evaluated heating properties of IR-LEGO such as time course of temperature rise and 3-dimensional distribution of temperature during IR irradiation. In a model tissue which contained GFP expressing bacteria in polyacrylamide gel, temperature rose rapidly with IR irradiation and kept a constant level dependant on IR laser power (Figure 2 left). On the other hand, the heated area was limited to a small volume about as large as a typical cell (Figure 2 right).

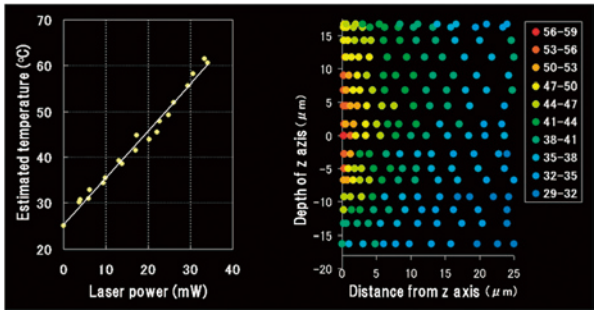


Figure 2. Heating profiles (laser power dependency of focus temperature and 3-D temperature map) of IR irradiation.

With this in mind, we tried to induce gene expression in various species. At first, we reported an IR-LEGO experiment in living *C. elegans*. Target gene expression in a target cell could be induced with only 1 s-IR irradiation. Whereas the optimal power range which can induce gene induction without cell damage was limited. Excess laser power resulted in cell death or cessation of cell division. We confirmed that an optimal irradiation, e.g. 11 mW for 1 s, induced physiological gene expression in the target cell and subsequent cell division or morphogenesis underwent normal development. Next, we tried the experiment in fishes, medaka and zebrafish, and the higher plant, *Arabidopsis*, since all organisms have a heat shock response system. We succeeded in local gene induction in the species as expected.

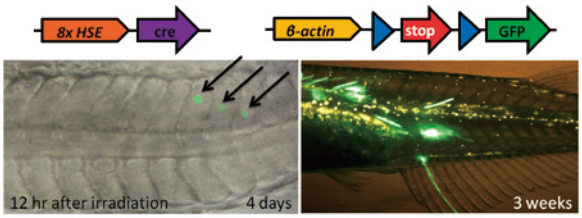


Figure 3. Cre-loxP mediated long-term GFP marking in a living medaka individual for linage tracing.

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 (Figure 3). By Applying IR-LEGO to a mutant and its rescue transgenic strain; using hsp-cre with a rescue gene which is sandwiched

by loxP sequences, we will achieve single-cell knockout experiments in living organisms, and reveal fine interaction between the cells. We are now testing this system using medaka. We have already constructed a medaka TILLING library and a screening system for reverse genetic mutant screening, furthermore we have started establishment of a cre-loxP system in medaka.

Publication List

〔Original paper〕

- Shikata, T., Iseki, M., Matsunaga, S., Higashi, S., Kamei, Y., and Watanabe, M. (2011). Blue and red light-induced germination of resting spores in the red-tide diatom *Leptocylindrus danicus*. Photochem. Photobiol. *87*, 590-597.

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

- Masuyama, H., Yamada, M. Kamei, Y., Fujiwara-Ishikawa, T., Todo, T., Nagahama, Y., and Matsuda, M. Dmrt1 mutation causes a male-to-female sex reversal after the sex determination by Dmy in the medaka. Chromosome Res. 2011 Dec. 21.

Data Integration and Analysis Facility

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

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence analysis, expression data analysis, and imaging data analysis. For this purpose, the facility maintains high-performance computers with large-capacity storage systems. On the basis of this system, the facility supports development of data analysis pipelines, database construction and setting up websites to distribute the data worldwide. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the network system in the institute and computer/network consultation for institute members.

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a shared memory parallel computer (DELL PowerEdge R905; 4 nodes/16 cores, 256GB memory), a high-performance cluster system (DELL PowerEdge M1000e+M610; 32 nodes/256 cores, 768GB memory) and a large-capacity storage system (DELL Equallogic; 35TB SAS, 26TB SATA, 750GB SSD). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be processed efficiently. Some personal computers and color/monochrome printers are also available. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for institute members. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

The facility also provides network communication services. 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/).

Figure 1. Biological Information Analysis System

Research activity by I. Uchiyama

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p. 66).

NIBB BioResource Center

Head
IGUCHI, Taisen

Vice head: *FUJIMORI, Toshihiko*

To understand the mechanisms of living organisms, studies focusing on each gene in the design of life (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 Professors:	*WATANABE, Eiji*
	TANAKA, Minoru
	NARUSE, Kiyoshi
Technical Staff:	*HAYASHI, Kohji*
	NOGUCHI, Yuji
Technical Assistants:	*INADA, Junichi*
	INADA, Yosuke
	KAJIWARA, Yuya
	MATSUMURA, Kunihiro
	ICHIKAWA, Yoko
	TAKAGI, Yukari
	SUGINAGA, Tomomi

Figure 1. The Model Animal Research Facility in the Yamate area.

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 as “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.

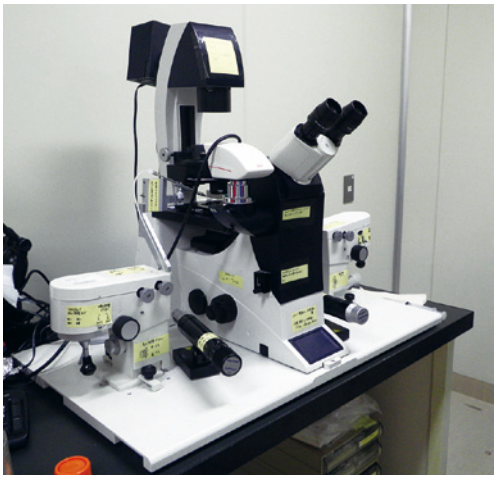


Figure 2. Equipment for manipulating mice eggs.

In 2011 (from January 1 to December 11) 3,807 mice and 717 fertilized eggs were brought into the facility in the Yamate area, and 48,862 mice (including pups bred in the facility) and 18,139 fertilized eggs were taken out.

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

A new mouse facility in the Myodaiji area opened at the beginning of 2005. The facility provides research-supporting activities to researchers in the Myodaiji area. In March 2009, we expanded the facility which includes areas for breeding, behavioral tests and transgenic studies using various kinds of recombinant viruses. In 2011 (from January 1 to December 11) 48 mice were brought into the facility in the Myodaiji area, and 1,828 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 2011 (from January 1 to the date as of December 11), 4,490 medaka and zebrafish (340 eggs, 650 embryos and 3,500 adults) were brought to the facility and 51,954 medaka and zebrafish (50.591 fertilized eggs, 473 embryos and 890 adults, including animals bred in the facility) were taken out. In the laboratory for chick embryos 180 fertilized chicken eggs were brought in and there were no fertilized eggs or chicken embryos 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 2010 we began providing the TILLING library screening system to promote the reverse genetic approach. In 2011 we shipped 219 independent medaka strains, 478 cDNA/BAC/Fosmid clones, and 120 samples of hatching enzyme to the scientific community worldwide.

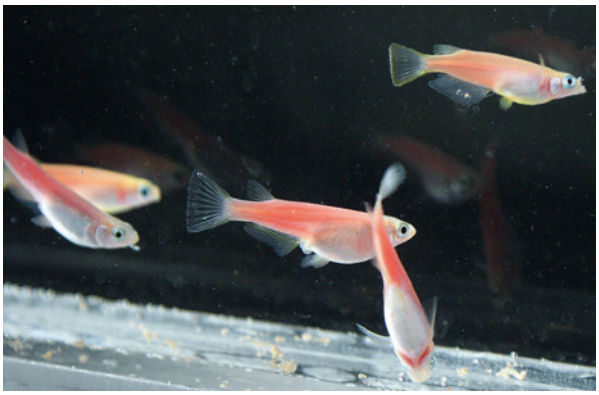


Figure 4. Gapdh-loxP[DsRed]-GFP transgenic medaka.

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 (p. 43) is studying mechanisms of the visual system using a psychophysical approach. The Laboratory of Bioresources (p. 52) 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 (p. 32) 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.

Model Plant Research Facility

● *Plant Culture Laboratory*

Assistant Professors: *HOSHINO, Atsushi*
TSUGANE, Kazuo
Technical Staff: *MOROOKA, Naoki*
KAJIURA-KOBAYASHI, Hiroko
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 48 culture boxes, 6 phytotrons, and 12 rooms with the P1P physical containment level for established and emerging model plants including a thale cress *Arabidopsis thaliana*, several carnivorous plants, a rice *Oryza sativa*, rushes *Juncus* sp., a moss *Physcomitrella patens*, and several other flowering plants. An emerging model insect, a tiny moth *Acrocercops transecta* is also reared in this laboratory. Most culture space is fully used the whole year by more than 70 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 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 plant *Mimoza pudica*, carnivorous plants, and wild-type strains of medaka fish *Oryzias* sp. Seven green houses (4, 6, 6, 6, 6, 9, and 38 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. One green house (18 m^2) with air-conditioning meets the P1P physical containment level and is 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 the spring of 2011, a bench-top bioreactor and three growth chambers (7 m^2 each) were introduced by the Japan Advanced Plant Science Network. This equipment is widely opened for outside research groups. The chambers have web cameras on each plant rack, and users can monitor their samples via the Internet. One of the cambers can control CO_2 level in addition to temperature and light conditions.

Figure 5. The growth chambers newly introduced.

● *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, constructed a BAC clone screening system, and provided 27 DNA clones and 11 mutant lines to both local and foreign biologists this year.

Research activities of the assistant professor A. Hoshino are shown on the laboratory page (p. 56).

Cell Biology Research Facility

Assistant Professor: *HAMADA, Yoshio*

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 the assistant professor Y. Hamada, the principal investigator of the Laboratory of Cell Sociology, is shown on the laboratory page (p. 16).

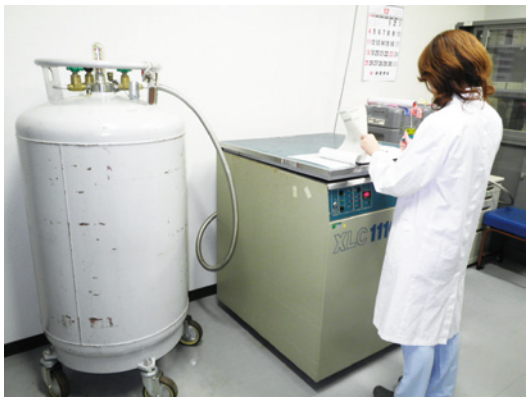


Figure 6. A user operating a cell storage tank.

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor
OGAWA, Kazuo
(Radiation Protection Supervisor)

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

Technical Assistants:
ITO, Takayo
KAMIYA, Kiyomi

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

Matsuda, Iinuma, Ito, and Kamiya maintained the Myodaiji-Area. Ogawa and Sawada worked in the Yamate-Area.

The number of registrants and the number of users from January 2011 to December 2011 are presented in Table 1.

Users counted by the monitoring system going in and out of the controlled areas numbered 3,760 persons during this period. The percentages and numbers for each area are shown in Table 2. The annual changes of registrants and the number of totals per year are shown in Figure 1.

The balance of radioisotopes received at the CRF is shown in Table 3.

The followings are CRF's special activities in 2011.

1. A special lecture was performed covering NBC (Nuclear, Biological, Chemical) hazards and the Great East Japan Earthquake disaster rescue operations by invited speakers (Okazaki fire station officers) as the 2011 training lesson for radiation workers. (See Figure 2, photo A, B.)
2. At the Myodaiji-area, the exhaust fan had deteriorated and has subsequently been exchanged. The repair work was done in accordance with Japanese law. (See Figure 2, photo C, D.)

	Myodaiji-Area	Yamate-Area
registrants	107	71
users	43	30

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2011

	Myodaiji-Area	Yamate-Area	total
users	1965	671	2636
visitors	198	129	327
total	2163	800	2963

Table 2. Users and visitors who entered each controlled area in 2011

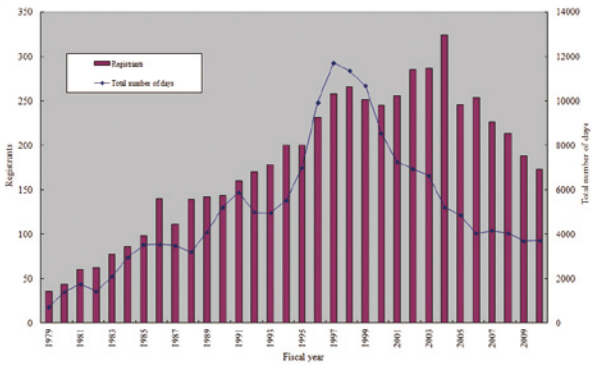


Figure 1. Annual changes of registrants and number of totals per fiscal year

	Myodaiji-Area	Yamate-Area	total
^{125}I Received	296085	7770	303855
^{125}I Used	166585	7770	174355
^{45}Ca Received	0	-	0
^{45}Ca Used	0	-	0
^{35}S Received	259000	0	259000
^{35}S Used	185000	16000	201000
^{32}P Received	499750	70000	569750
^{32}P Used	394210	48800	443010
^{14}C Received	225700	0	225700
^{14}C Used	257671	0	257671
^{3}H Received	1697779	2775000	4472779
^{3}H Used	144297	2949085	3093382
^{22}Na Received	0	-	0
^{22}Na Used	0	-	0

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2011

training course	place	numbers of participant
beginners training course for beginners*	Myodaiji	4
beginners training course for beginners*	Yamate	4
beginners training course for exparts	Myodaiji	10
beginners training course for exparts	Yamate	4
Users traning course*	Myodaiji	77
Users traning course	Yamate	54

*included English course

Table 4. Training course for radiation workers in 2011

Figure 2. The CRF's special acticities in 2011
A, B: Okazaki fire station officers lecture about NBC hazards. Photo A shows a lecture by the head of the Okazaki fire station. Photo B shows a participant wearing NBC hazard protective wear.
C, D: The exhaust fan was exchanged. Photo C shows the new fan (left) and the old fan (right). Photo D shows CRF's staff checking radiation contamination inside of the fan.

STRATEGIC PLANNING DEPARTMENT

Chair
NISHIMURA, Mikio

Associate Professor: *KODAMA, Ryuji*

The Strategic Planning Department was founded in April 2005 as a central office for assisting the director-general in preparing for NIBB's evaluation procedure and in planning a long-range strategy for the institute. Formerly the department also managed activities relating to public relations and international cooperation, however those roles of the department were separated in 2009 and are now managed by Office of Public Relations and Office of International Cooperation, respectively.

The main activities of the Department

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. The department manages these processes.

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

The department edits the annual report (this issue) which summarizes the annual activities of laboratories and facilities and is used in the evaluation procedures of the institute.

3) Assistance in fund application and long-range planning of the institute

The department assists the Director-General and Vice Director in preparing long-range plans for building the most advanced research facilities, and in application for funds from the government to realize these plans.

4) Assistance in making the Plans and Reports of the institute

The department assists in making NIBB's Medium-term Goals and Plans (for a six-year-period), and in instituting Annual Plans to realize them. The department also assists in preparing required Business and Performance Reports to answer whether we are meeting the goals set both annually and for the medium-term.

OFFICE OF PUBLIC RELATIONS

Chair
FUJIMORI, Toshihiko

Assistant Professor (Specially appointed):	*KURATA, Tomoko*
Technical Assistants:	*OTA, Kyoko*
	KAWAGUCHI, Colin

The Office of Public Relations, in order to communicate the activities of NIBB to the widest audience, performs both standard public relations duties as well as communication with scientific publications and organizations worldwide.

The main activities of the office in 2011

1) Press releases

The office 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 the pamphlet "An introduction to the National Institute for Basic Biology". Publication of leaflets introducing the individual researchers of NIBB (in Japanese). Publication of "NIBB News" (Intra-institutional newsletter, in Japanese), "NIBB English News" (Intra-institutional newsletter, in English). Design and distribution of posters for international conferences and advertisements for the graduate school's entrance examination.

4) Producing Videos

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

5) Organization of scientific outreach programs

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

OFFICE OF INTERNATIONAL COOPERATION

Chair
YOSHIDA, Shosei

Assistant Professor:	*KAMADA, Yoshiaki*
Technical Assistants:	*NAKANE, Kaori*
	TAKAHASHI, Ritsue
	SANJO, Kazuko

The Office of International Cooperation coordinates international collaboration in the field of biology, forms cooperative agreements with research institutes from around the world, exchanges researchers, and holds various academic meetings.

NIBB manages international cooperation with the European Molecular Biology Laboratories EMBL (since 2005), Germany's Max Planck Institute for Plant Breeding Research (MPIPZ) (since 2009), Singapore's Temasek Life Sciences Laboratory (TLL) (since 2010), and Princeton University of the USA (since 2010).

The main activities of the office in 2011

1) Supporting coordination of international conferences

The Office of International Cooperation coordinated the following international conferences hosted by NIBB: The 1st NIBB - Princeton Symposium "Proteomics, Metabolomics, and Beyond" (November 2011).

Other conferences scheduled for 2011 were postponed due to the earthquake on March 11th.

2) Supporting dispatching researchers and students to international conferences

The Office of International Cooperation coordinated sending NIBB researchers and graduated students to the following symposia: 3rd NIBB-TLL-MPIPZ Joint Symposium "Cell cycle and Development" (November 2011) and EMBL PhD symposium "Rhythm of Life" (November 2011).

3) Management of education related programs

The Office of International Cooperation also coordinated The 6th NIBB International Practical Course "Developmental Genetics of Medaka IV" (November, 2011)

TECHNICAL DIVISION

Head
FURUKAWA, Kazuhiko

Common Facility Group

Chief: *MIWA, Tomoki*

● ***NIBB Core Research Facilities***

Unit Chiefs: *HIGASHI, Sho-ichi*
MORI, Tomoko
Subunit Chiefs: *MAKINO, Yumiko*
YAMAGUCHI, Katsushi
Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
TANIGUCHI-SAIDA, Misako
Technical Assistants: *ICHIKAWA, Chiaki*
NISHIMURA, Noriko
YAMAMOTO, Kumi
Secretaries: *ICHIKAWA, Mariko*
ISHIKAWA, Azusa

● ***NIBB Bioresource Center***

Subunit Chiefs: *HAYASHI, Kohji*
MOROOKA, Naoki
Technical Staff: *NOGUCHI, Yuji*
Technical Assistants: *ICHIKAWA, Yoko*
TAKAGI, Yukari
SUZUKI, Keiko

● ***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

Chief: *KAJIURA-KOBAYASHI, Hiroko*

● ***Cell Biology***

Unit Chief: *KONDO, Maki*

● ***Developmental Biology***

Technical Staff: *TAKAGI, Chiyo*
UTSUMI, Hideko
OKA, Sanae
NODA, Chiyo
MIZUGUCHI-TAKASE, 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*

Reception

Secretaries: *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 2nd NIBB-EMBL PhD Mini-Symposium and 13th International EMBL PhD Students Symposium "The Rhythm of Life: Cycles in Biology"

November 16 (Wed) - 19 (Sat), 2011 | EMBL, Heidelberg

Seven PhD students from NIBB, three from Nagoya University, and one from Kyoto University (total 11 students) were funded by NIBB to participate in the 2nd NIBB-EMBL PhD Mini-Symposium 2011 (held on 16 November) and the 13th International EMBL PhD Symposium "The Rhythm of Life"(held on 17-19 November). Our students had the chance to give oral presentations at the mini-symposium and poster presentations at the PhD symposium to introduce their current research. They also attended Lab tours to visit some EMBL laboratories and facilities to exchange experimental information and discuss their research with PhD students, post-docs and PIs. (Y. Kamada)

Participating students from Japan

Nakata, Miyuki; Ohara, Yuya; Shi, Dongbo; Shibata, Michitaro; Tokue, Moe; Wanglar, Chimwar; Yoro, Emiko (NIBB); Ikegami, Keisuke; Imai, Saki; Ishida, Kai (Nagoya Univ.); Muranaka, Tomoaki (Kyoto Univ.)

Comments from students (excerpts)

・Nakata, Miyuki

Among the many great experiences I had During the EMBL PhD Symposium were chances to meet and interact with other students like myself, to tour the EMBL facilities, and to give a talk in professor Marcus Heisler's lab. Because he has been such a pioneer in my field of plant development, I sent a personal email to Professor Heisler to ask for the chance to talk with him during the symposium. He replied and suggested I give a presentation to the members of his lab. Because I have never been very comfortable giving presentations in English I was worried whether I would be understood. However, I was relieved as my presentation seemed to be received with great interest. In addition, because my ideas were approved of it has given me more confidence in my research.

・Tokue, Moe

In addition to the students from EMBL many students from around the world participated in this symposium. Seeing that people from Germany and places like Kazakhstan which is so much colder than the already very cold Germany, and Italy where fashion is so much more a part of their culture than in Germany, all work hard at their research through trial and error in the same way gave me a new feeling of connection to them all. Seeing that people from various countries with different cultures and different languages can all discuss their research together influenced me greatly.

・Wangler, Chimwar

I highly appreciate NIBB for providing us an opportunity to attend the mini PhD symposium at EMBL. It was an eye opening experience. The travelling and lodging were great. EMBL students were wonderful and co-operative. We, the NIBB students got to present our works both in oral and poster presentations. We got lots of feedback from the EMBL students and some Professors during the 13th EMBL PhD Symposium. Many researchers around the globe working on Biology had come to advise and expose their wonderful works. Their works encourage us even more to love science. Not to forget the good food over there, Heidelberg is a small, beautiful city, very much similar to Okazaki. Such a wonderful and peaceful city, no wonder many researchers come up with good inspirations and works. I would encourage many of our aspiring NIBB students not to miss this opportunity of knowledge sharing/exchange amongst different Universities and to make many new friends.

・Yoro, Emiko

This visit to EMBL was my first trip to Europe and my first English oral presentation. First I must thank the people who took the time to help me in my preparations. Of course I received a lot of advice from the members of my lab, but I also had help from the other students who visited EMBL as we repeatedly practiced together, and from the international students at NIBB who helped make my presentation easier to understand. It was a lot of work but it was also an excellent experience. Because of the help of all these people I think I gave my best presentation possible. I also feel that my motivation to give good English presentations, as well as to do good research has grown remarkably due to this experience. Going forward I hope to use this experience as an encouragement to work toward my future.

3rd NIBB-TLL-MPIPZ Joint Symposium 2011
Cell Cycle and Development

Organizers: Frederic Berger (TLL), Karuna Sampath (TLL), Toshie Kai (TLL), Toshiro Ito (TLL)

November 21 (Mon) - 22 (Tue), 2011 | Temasek Life Sciences Laboratory, Singapore

The 3rd NIBB-TLL (Temasek Life Sciences Laboratory) -MPIPZ (Max Planck Institute for Plant Breeding Research) Joint Symposium 2011 was held at TLL's facilities in Singapore. Despite it being winter, Singapore's close position to the equator keeps it quite warm, with the conference seeing 30℃ temperatures both days, and humidity of over 80%.

Through lectures on the cell cycle, development, and related fields of basic biology by researchers from Japan, Singapore, and Germany, this joint symposium set out to promote an international exchange of scientific ideas unhampered by boundaries of nation or field. The Japanese lecturers for the symposium consisted of 6 lecturers from NIBB, as well as 3 other researchers from throughout the Japanese community of basic biology scientists. Including the talks by the three keynote speakers, Prof. Mitsuyasu Hasebe (NIBB), Prof. Yoshiaki Ito (TLL), and Prof. George Coupland (MPIPZ), the topics covered included the cell cycle, cell division, fertilization, development, and differentiation, among others, with research drawn from a large pool of model species such as mice, *Arabidopsis thaliana*, *Physcomitrella patens*, zebrafish, *Xenopus laevis*, yeast, and *Volvox*. Each presentation by the researchers was both interesting, and full of personal flavor, with the question and answer sessions afterwards blossoming into fruitful cross-disciplinary discussions lasting past the initially allotted time.

The poster presentations were also quite lively, consisting of ten presentations, primarily by younger researchers, who spoke passionately about their subjects until the end of the symposium. With additional activities, such as tours of the TLL facilities and botanical gardens, as well as several lunches and dinners spent getting to know each other while sampling the delicious local cuisine, this symposium provided a wonderful opportunity for the strengthening of research ties on both a personal and organizational level, and was ultimately a very fruitful endeavor. (Y. Matsubayashi)

Speakers

Balasubramanian, Mohan (TLL), Berger, Fred (TLL), Coupland, George (MPIPZ), Nishii, Ichiro (TLL), Ingham, Phil (IMCB), Ito, Yoshiaki (NUS), Ito, Toshiro (TLL), Kai, Toshie (TLL), Kaldis, Phil (IMCB), Rorth, Pernille (IMCB), Sampath, Karuna (TLL), Simon, Ruediger (Heinrich-Heine Univ.), Turck, Franziska (MPIPZ), Yu, Cai (TLL)
Goshima, Gohta (Nagoya Univ.), Goto, Koji (RIBS Okayama), Hasebe, Mitsuyasu (NIBB), Ikui, Amy (Brooklyn College), Kamada, Yoshiaki (NIBB), Kawaguchi, Masayoshi (NIBB), Matsubayashi, Yoshikatsu (NIBB), Ueno, Naoto (NIBB), Yoshida, Shosei (NIBB)

The 1st NIBB - Princeton Symposium Proteomics, Metabolomics, and Beyond

Organizers: James Broach, Joshua Rabinowitz, Shosei Yoshida, Shuji Shigenobu, Satoru Kobayashi

November 1 (Tue) - 2 (Wed), 2011

The 1st NIBB-Princeton Symposium, "Proteomics, Metabolomics, and Beyond" was held on November 1st and 2nd, 2011 at the Okazaki Conference Center. This was the inaugural symposium of the academic exchange program between the National Institutes of Natural Sciences and Princeton University, which aims to increase scientific interaction between these institutions, with NIBB and Princeton's Department of Molecular Biology playing central roles. This symposium was originally scheduled for July 2011, but was forced to be postponed due to the hardships following the earthquake in March. This symposium gathered researchers performing unique biological investigations taking advantages of "-omics" approaches such as proteomics and metabolomics. Cutting edge research such as the interplay between extracellular nutrient and intracellular metabolism in yeast, the structural basis of metabolic enzyme localization and origin of the cytoskeleton in bacteria, comprehensive analyses of spatial distribution of lipid and protein molecules (i.e., imaging mass-spectrometry), identification of plant hormones with unique protein modifications, and real-time imaging of ATP in living cells, have made us realize that biology is changing dramatically at this very moment. NIBB's ongoing research was also introduced by sort talks and poster presentations, which stimulated intensive discussions. The participants came to an understanding of each other's ideas and interests, which we hope will ignite future communications and scientific interactions. After the symposium, participants from Princeton University and invitees from outside NIBB deepened their communications by touring around the labs and core facilities of NIBB On behalf of the organizers, we thank all the participants, the staff of the Office of International Cooperation, and the organizers' lab members, who have worked hard to conduct this symposium successfully. (Shosei Yoshida, Satoru Kobayashi, and Shuji Shigenobu)

Speakers

Broach, James (Princeton Univ.), Cristea, Ileana (Princeton Univ.), Gitai, Zemer (Princeton Univ.), Imamura, Hiromi (Kyoto Univ.) Cui, Songkui (NIBB), Kitadate, Yu (NIBB), Matsubayashi, Yoshikatsu (NIBB), Sato, Masanao (NIBB), Shigenobu, Shuji (NIBB), Taguchi, Ryo (Chubu Univ.), Tanaka, Minoru (NIBB), Yao, Ikuko (Kansai Medical Univ.)

The NIBB International Practical Course

The first NIBB International Practical Course was held in 2007 with the aim of providing young scientists around the world with opportunities to learn cutting-edge experimental skills and to communicate with experts in the field. The 6th course was held as a joint course with TLL, Singapore, in 2011 as summarized below. The course was held in a laboratory provided by NIBB and equipped with the necessary instruments and tools and a good supply of experimental materials.

The 6th NIBB International Practical Course and The 1st NIBB - TLL Joint International Practical Course "Developmental Genetics of Medaka IV"

- Period: November 14(Mon) - November 21(Mon), 2011
- Participants: 15(4 from Japan, 2 from Taiwan, one each from Hong Kong, South Korea, China, Singapore, Norway, Canada, USA, Italy, India)
- Lecturers:
  Dr. Christoph Winkler (National University of Singapore, Singapore)
  Dr. Jun KITANO (National Institute of Genetics, Japan)
  Dr. Minoru TANAKA (NIBB, Japan)
  Dr. Rie KUSAKABE (Kobe University, Japan)
  Dr. Minori SHINYA (National Institute of Genetics, Japan)
  Dr. Tomonori DEGUCHI (National Institute of Advanced Industrial Science and Technology, Japan)
  Dr. Yoshihito TANIGUCHI (Keio University, Japan)
- Course Staff from NIBB:
  Kiyoshi Naruse, Takao Sasado, Yusuke Takehana, Yasuhiro Kamei, Masatoshi Nakamoto, Tetsuaki Kimura, Yukiko Ogino
- Contents of the course:
  Transgenic Medaka (BAC homologous recombination and transposon mediated gene transfer)
  Cryopreservation of sperm and artificial insemination
  Local gene induction with infrared laser-evoked gene operator (IR-LEGO) method Forward genetics approaches with ENU mutagenesis (Demonstration)
  Reverse genetics approaches with TILLING (Targeting Induced Local Lesion IN Genome) method

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 2011 we held 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 next-generation sequences (NGS). The programs are specially designed for biologists who are not familiar with bioinformatics.

- Period: 1st course March 10 (Thu) -11 (Fri), 2011
  2nd course September 8 (Thu) -9 (Fri), 2011
- Organizer: Dr. Shuji Shigenobu (NIBB)
- Lecturers: Dr. Ikuo Uchiyama, Dr. Katsushi Yamaguchi, Dr. Shuji Shigenobu (NIBB Core Research Facilities)

- Participants: 1st course: 23 (including 3 from NIBB)
  2nd course: 15 (including 4 from NIBB)
- Topic: "Introduction to Next-generation Sequence Data Analysis"

1. Overview: NGS data analysis
2. UNIX for beginners
3. Programming for biologists
4. NGS data formats
5. NGS tools
6. Exercise

The NIBB Internship Program

The NIBB Internship program is a hands on learning experience started in 2009 as a way to promote NIBB with overseas students and to build connections through providing education to the people who will form the core of future research networks. At the same time, this program aims to internationalize the graduate students of the Graduate University for Advanced Studies (SOKENDAI), giving them the opportunity to get to know students and interns with various cultural customs.

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.

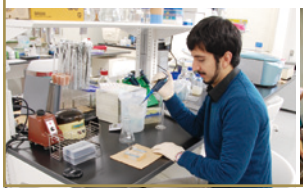
In FY 2011 there were 17 applicants, of which nine interns were selected. These interns were from universities located in 7 countries (Taiwan, Australia, India, the United States, Singapore, Egypt, and Iran) and spent periods ranging from 1 week to 1 month experiencing life as a member of a research team. Since then one of these interns has enrolled as a student of SOKENDAI.

Report from a participant
Yousef Yari Kamrani
Justus Liebig University, Giessen, Germany

My project in the lab of Professor Minagawa was about designing a new vector for gene transformation of a microalgae *Chlamydomonas reinhardtii*. The genes of interest for Professor Minagawa were those involved in photosynthetic machinery. The newly designed vector contained all the necessary elements including an antibiotic/resistance gene as a selectable marker and an appropriate inducible promoter. The gene encoding Cyan Fluorescent Protein (CFP) was selected as a reporter gene and was cloned between the Hsp70-RBCS promoter/terminator.

I liked the sense of community and the friendly atmosphere in NIBB: not only students but also the staff and professors were outstandingly supportive. Furthermore, the great lodge program and well organized student organization allowed me a very smooth transition to life in Okazaki during my internship.

I am inspired by the people I worked with and I hope to have another opportunity to come back NIBB in the future.



INDEX

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

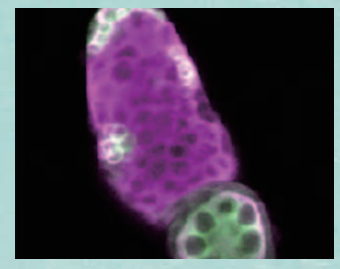
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

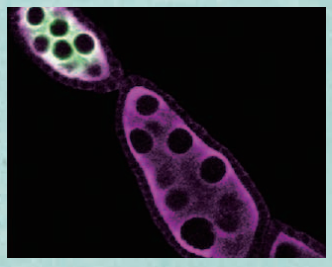
From Higashi Okazaki Station to Each Area

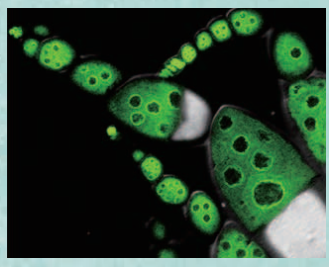
Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

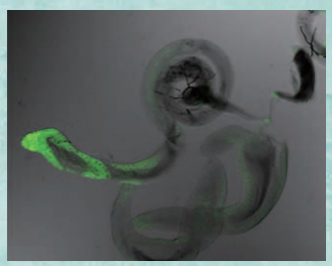
By car

Take the Tomei Expressway to the Okazaki Exit. Turn left at the City Office S.E. 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 June 2012
Edited by the Strategic Planning Department, NIBB