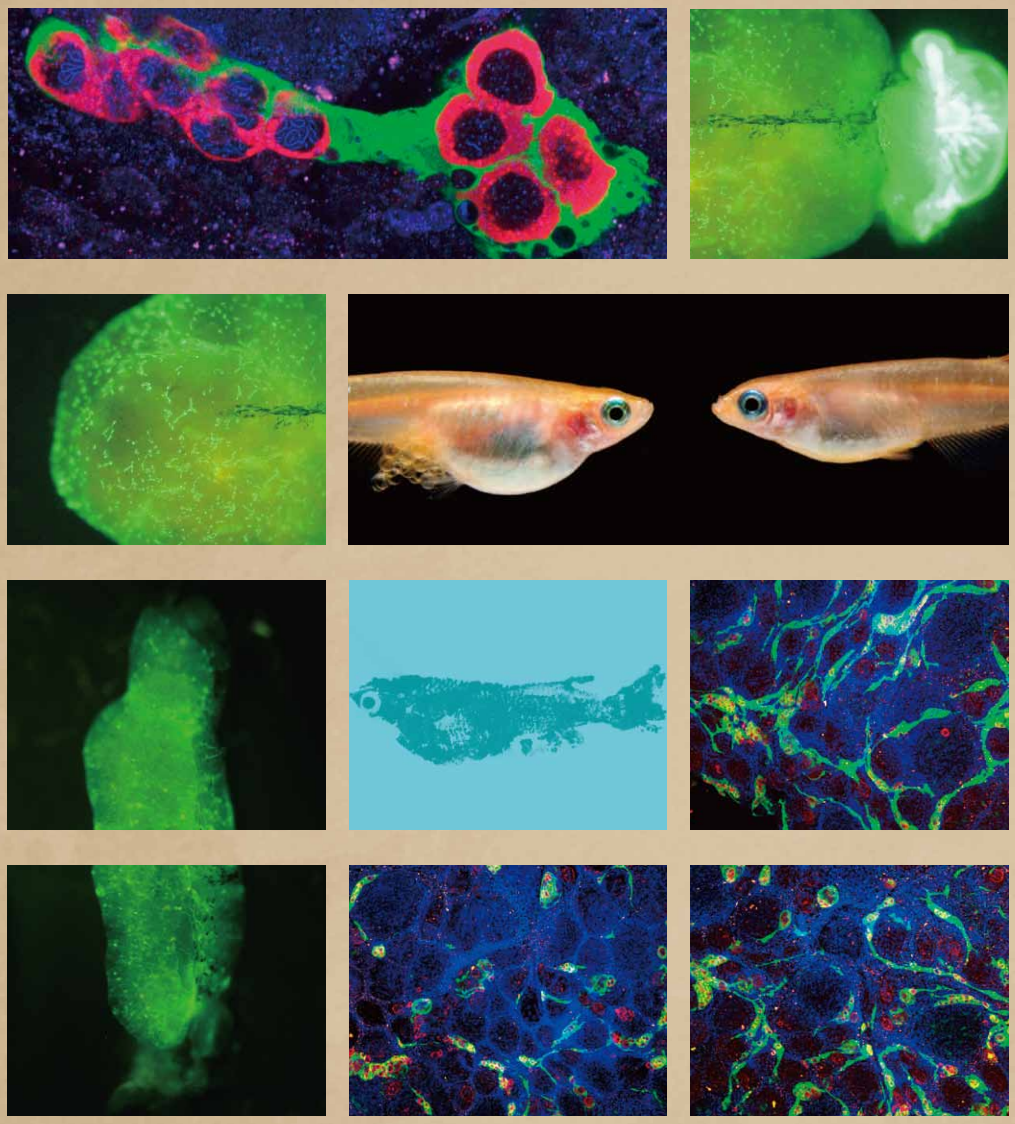


National Institute for Basic Biology

2010 ANNUAL REPORT

CONTENTS

Cover photographs and illustrations are related to studies of novel functions of germ cells on sex differentiation and the continuation of egg production. For these studies, transgenic teleost fish, medaka, and mutant medaka have been employed (Kurokawa et al., PNAS, 2007; Morinaga et al., PNAS, 2007). Transgenic medaka also lead to the first discovery of germline stem cells in the vertebrate ovary (Nakamura et al., Science, 2010). See page 31 of this report for details.

Kiyotaka Okada

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 2004, NIBB, together with four other national research institutes, established a new organization, the National Institutes of Natural Sciences (NINS), one of four Inter-University Research Institute Corporations. 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 2010 are shown in this report.

Following from 2009, we welcomed several new colleagues in 2010, including 2 professors 4 associate professors, 7 assistant professors and 5 NIBB research fellows, while one colleague 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 78. The activities of our supporting divisions are shown on the following pages 74–85.

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 happy we have the opportunity to educate graduate students.

As international collaborative activities, we hosted the 7th Okazaki Biology Conference under the title “The Evolution of Symbiotic Systems”. This conference was celebrated by the attendants because this was the first meeting designed to promote interaction between symbiosis researchers working on plant systems and researchers working on animals and other organisms (page 86). We also held two NIBB Conferences: the 56th conference on “Neocortical Organization” and the 57th on “the Dynamic Genome”, and participated in the 2nd NIBB-MPIPZ (Max Planck Institute for Plant Breeding Research) joint symposium on “Plant Science Communications 2010” in Okazaki as shown on pages 87-89. Plant researchers and students attended from TLL (Temasek Life Sciences Laboratory, Singapore), following the establishment of a Memorandum of Understanding on academic exchange between NIBB and TLL in August, 2010. In addition, we held the 5th NIBB International Practical Course on “Developmental Genetics of Zebrafish and Medaka III”, as reported on page 90.

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
May 27, 2011

ORGANIZATION OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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

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

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

Policy, Decision Making, and Administration

The Director-General oversees the operation of NIBB, assisted by the Advisory Committee for Programming and Management. The Advisory Committee, comprised of 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 International Cooperation is a central office for public relations and the management of conferences and other extramural activities.

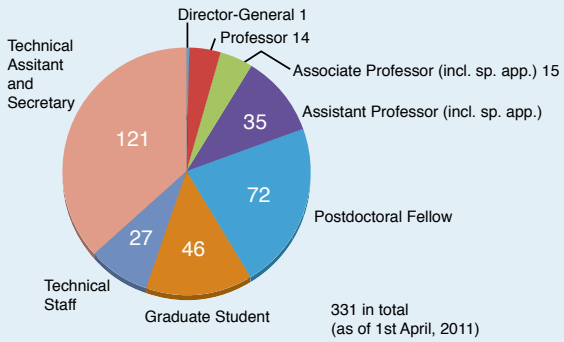
Administration of NIBB is undertaken by the Okazaki Administration Office under the direct auspices of the Administration Office of NINS.

Research and Research Support

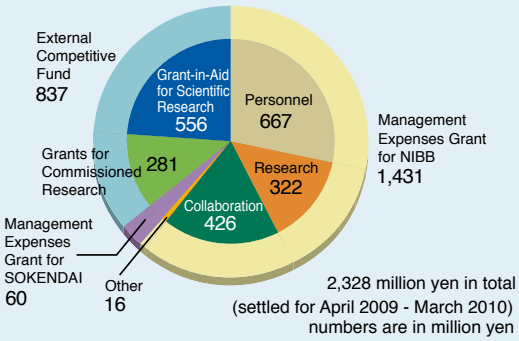
NIBB conducts its research programs through twenty-five research units and two research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research Facilities and NIBB BioResource Center were launched in 2010 to support basic biology research in NIBB and also to act as an intellectual hub to promote collaboration among the researchers of NIBB and other academic institutions. 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 the NIBB and the research facilities of the Okazaki campus. The Center for Radioisotope Facilities is one of the latter and run by the technical staff of NIBB.

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

Members in NIBB

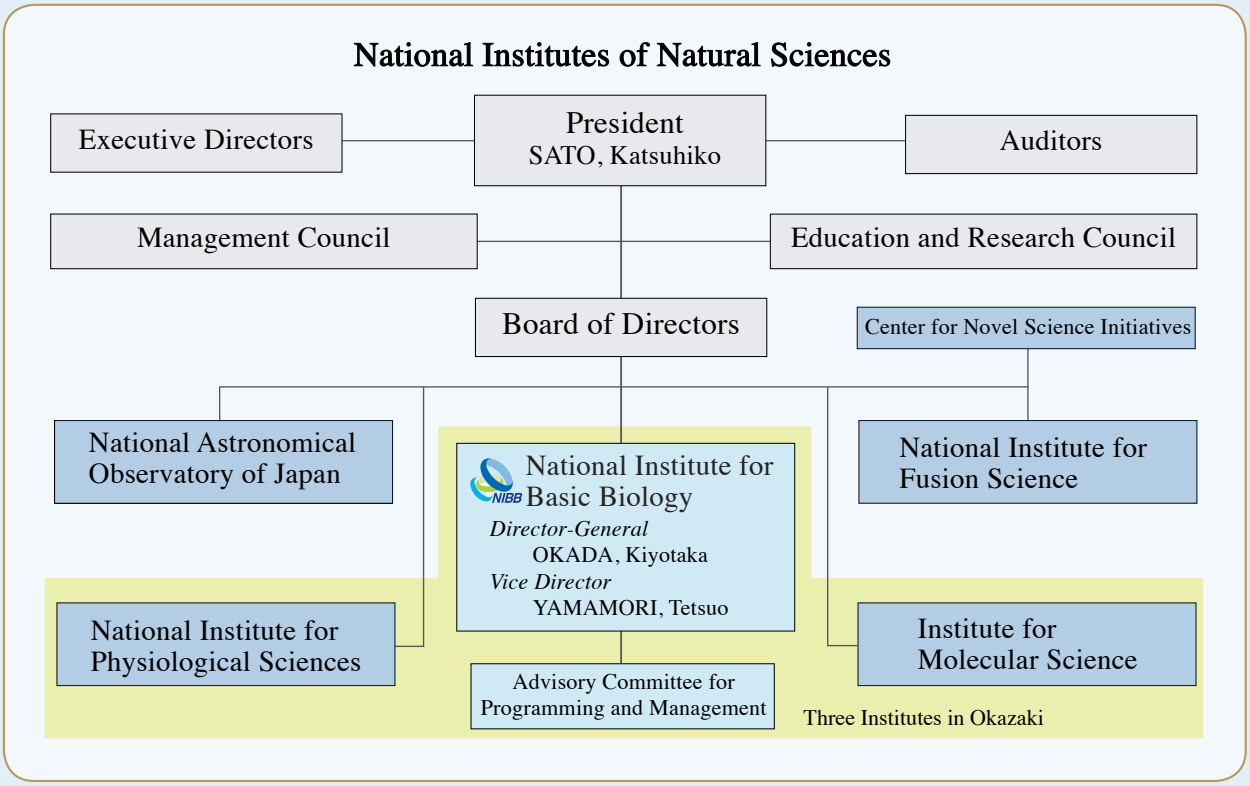


Financial Configuration of NIBB



Organization

As of April 1, 2011



Members of the Advisory Committee for Programming and Management* (as of April, 2011)

Non-NIBB members	KONDO, Takao	Professor, Nagoya University
	MIZUSHIMA, Noboru	Professor, Tokyo Medical and Dental University
	MORI, Ikue	Professor, Nagoya University
	SEHARA, Atsuko	Professor, Kyoto 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	Professor, The University of Tokyo
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	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

* Chairperson and vice-chair will be nominated at meeting scheduled for the end of June, 2011.

National Institute for Basic Biology

As of April 1, 2011

Research Units

- **Cell Biology**
  - Division of Cell Mechanism
  - Division of Intercellular Signaling Biology
  - Laboratory of Neuronal Cell Biology††††
  - Laboratory of Cell Structure
  - Laboratory of Cell Sociology
- **Developmental Biology**
  - Division for 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 Bioresource
  - 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

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

Other Research Support Sections

- **Technical Division**
- **Strategic Planning Department**
- **Office of Public Relations and International Cooperation**

Okazaki Research Facilities

- **Okazaki Institute for Integrative Bioscience**
  - Department of Development, Differentiation and Regeneration
    - Division of Developmental Genetics *
    - Division of Molecular and Developmental Biology **
  - Department of Bio-Environmental Science
    - Division of Bio-Environmental Science ***
    - Division of Neuronal Cell Biology ****
  - Department of Strategic Methodology
- **Center for Radioisotope Facilities**
- **Center for Experimental Animal**
- **Research Center for Computational Science**

*-**** These divisions also function as NIBB's divisions in the above table†-††††, respectively. Other divisions of the OIIB are not shown.

Research Facilities run jointly with National Institute for Physiological Sciences

- Electron Microscopy Room
- Disposal of Waste Matter Facility
- Instrument Design Room

GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

The National Institute for Basic Biology (NIBB) has set 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. "NIBB workshops" support travel expenses for discussions and meetings at NIBB. 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. These instruments are managed by special facilities to maintain efficient and precise operation. "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.

Collaborative research projects by year

year	2007	2008	2009	2010
Priority collaborative research projects	1	0	1	4
Collaborative research projects for model organisms/ technology development	2	3	3	2
Individual collaborative research projects	43	49	54	68
NIBB workshops	5	5	3	3
Collaborative experiments using the large spectrograph	14	11	10	8
Collaborative experiments using the DSLM	–	–	–	7
Collaborative experiments using next generation DNA sequencers	–	–	–	11
Facility Use (Training Course Facility)	–	–	–	1
total	65	68	71	94

NIBB Core Research Facilities

The NIBB Core Research Facilities were launched in 2010 to support research in NIBB and also to act as an intellectual hub to promote collaboration 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 (see page 74).

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. The Spectrography and Bioimaging Facility manages research tools, such as confocal microscopes, DSLM and the large spectrograph, and provides technical support and scientific advice to researchers. These two facilities hold specially-appointed associate professors, an expert in each field, with a mission to manage each facility as well as conducting his own academic research. The Data Integration and Analysis Facility supports 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.

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.

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. NIBB acts as a bridge between Japanese and German researchers in the field of plant sciences. In 2010, the Second NIBB-MPIPZ Joint Symposium "Plant Science Communications 2010" was held (page 89).

Collaborative programs have also been started with the Temasek Life Sciences Laboratory (TLL), of Singapore and Princeton University. Researchers from TLL participated in a NIBB-MPIPZ joint symposium in 2010 (see page 89).

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. In 2010, the 56th conference "Neocortical Organization" (page 87) and the 57th conference "The

Note: We are currently preparing a video gallery containing an English language introduction of NIBB. The gallery will be accessible from the NIBB website at: http://www.nibb.ac.jp/en/

Dynamic Genome" (page 88) were held.

International Practical Course

With the cooperation of researchers from Japan and abroad the NIBB international practical course, a practical training program, is given at a laboratory specifically prepared for its use at NIBB. The fifth course "Developmental Genetics of Zebrafish and Medaka III" was held in 2010 (see page 90). Graduate students and young researchers from various areas including Taiwan, Germany, China, and Singapore, were provided with training in state-of-the-art research techniques.

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 (see page 79).

Strains of Japanese morning glory maintained in the center

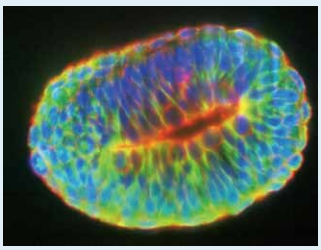
Outreach

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. Our triennial open campus event was held in 2010 at which we welcomed more than 3,200 local citizens. NIBB also cooperates in the education of undergraduate and younger students through lectures and workshops. Outreach activities are mostly managed by the Office of Public Relations and International Cooperation (see page 84).

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 model (see page 73). 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 at NIBB, members of the Advisory Committee, and company engineers to frankly discuss practical difficulties and needs regarding imaging. Bioimaging Symposium provides an opportunity for academic exchange with cutting-edge overseas researchers in the imaging field, mainly from EMBL.



DSLM image of mouse 6.5 day embryo.
Blue: DNA, Green: microtubules, Red: cortical actin.

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. In 2010, OBC7 "The Evolution of Symbiotic Systems" was held (page 86).

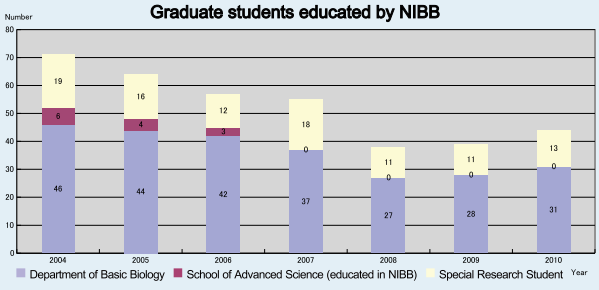
Cultivation of Future Researchers

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 Ph.D. student symposia held at EMBL, Heidelberg and provided an opportunity to give platform presentations as well posters, at least one time during their master's and doctoral program.



Personnel changes in 2010*

Newly assigned in NIBB

Name	Position	Research Unit	Date
SUZAKI, Takuya	Assistant Professor	Division of Symbiotic Systems	January 1
SHINTANI, Takafumi	Associate Professor	Division of Molecular Neurobiology	April 1
WATAKABE, Akiya	Associate Professor	Division of Brain Biology	April 1
KAMEI, Yasuhiro	Associate Professor (Specially appointed)	Spectrography and Bioimaging Facility	April 1
SHIGENOBU, Shuji	Associate Professor (Specially appointed)	Functional Genomics Facility	April 1
CHIEN, Qiuhong	NIBB Research Fellow	Division of Molecular and Developmental Biology	April 1
HIROKAWA, Junya	NIBB Research Fellow	Division of Brain Biology	April 1
OKAMOTO, Satoru	NIBB Research Fellow	Division of Symbiotic Systems	April 1
KAGEYAMA, Yuji	Assistant Professor (Specially appointed)	Division of Developmental Genetics	June 1
HASHIMOTO, Masakazu	NIBB Research Fellow	Division of Morphogenesis	July 1
MANO, Hiroaki	NIBB Research Fellow	Division of Evolutionary Biology	July 1
TAMADA, Yosuke	Assistant Professor	Division of Evolutionary Biology	August 1
HARA, Kenshiro	Assistant Professor	Division of Germ Cell Biology	August 1
MATSUZAKI, Masanori	Professor	Division of Brain Circuits	September 1
OGINO, Yukiko	Assistant Professor	Division of Molecular Environmental Endocrinology	September 16
MINAGAWA, Jun	Professor	Division of Environmental Photobiology	October 1
NAKAYAMA, Kei	Assistant Professor	Laboratory of Neuronal Cell Biology	October 1
YABE, Taijiro	Assistant Professor	Division of Molecular and Developmental Biology	October 1

Newly affiliated in other universitites and institutes

Name	New Affiliation	Position	Date
TERADA, Rie	Meijo University	Professor	April 1

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

Awardees in 2010

Name	Position	Award
SHIGENOBU, Shuji	Associate Professor (Specially appointed)	Young Scientist Initiative Award, Society of Evolutionary Studies, Japan

Note: On the unit member lists from P. 8 all members who belonged to the unit during 2010 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 2010. 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 Fellow:	*KANAI, Masatake*
Graduate Students:	*GOTO, Shino*
	NAKAI, Atsushi
	CUI, Songkui
	SHIBATA, Michitaro
Technical Assistants:	*NISHINA, Momoko*
	ARAKI, Masami
	SAITO, Miyuki
	NAKAYAMA, Tomomi
	HIKINO, Kazumi
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 peroxisome in root cells plays 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 casette transporter promotes seed gemination by inducing pectin degradation under the control of abscisic acid singnaling. 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 (*apm* mutants) based on a 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 *apm* mutants, *APM1* gene (whose defect causes the elongation of peroxisomes and mitochondria) encodes dynamin-related protein 3A (DRP3A), one member of the dynamin family. In *apm2* and *apm4*, the GFP fluorescence is

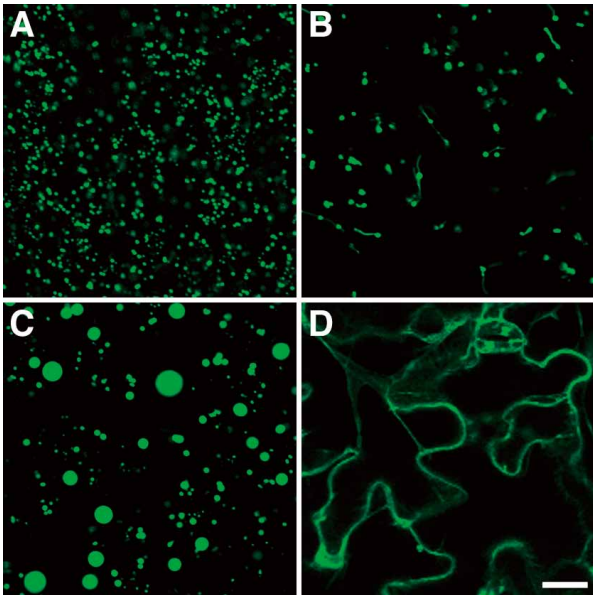


Figure 1. Phenotype of *Arabidopsis apm* mutants. GFP fluorescence in leaf cells was observed in the parent plant, GFP-PTS1 (A), *apm1* (B), *apm3* (C) and *apm9* (D) mutants. In *apm1* and *apm3* mutants, the number of peroxisomes is dramatically decreased. However, the morphology varies in both mutants. *apm1* and *apm3* have elongated and enlarged peroxisomes, respectively. In *apm9*, GFP fluorescence is observed in the cytosol because of the efficiency of protein transport to peroxisomes. Bar indicates 20 μm.

observed in the cytosol as well as in peroxisomes, showing the defect of protein transport to peroxisomes. We demonstrated that *APM2* and *APM4* encode proteins homologous to PEX13 and PEX12, respectively, and that APM2/PEX13 and APM4/PEX12 are components of the protein-translocation machinery on peroxisomal membranes. We are currently analyzing the functions of other APM proteins such as APM3 and APM9. *apm3* and *apm9* mutants exhibit enlarged peroxisomes and defect in protein transport, respectively (Figure 1). 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 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. *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* (Figure 2). *Arabidopsis nai2* mutant has no ER bodies and reduces the 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. Now we are investigating the function of NAI2 on ER body formation by heterologously expressed it in onion and tobacco cells.

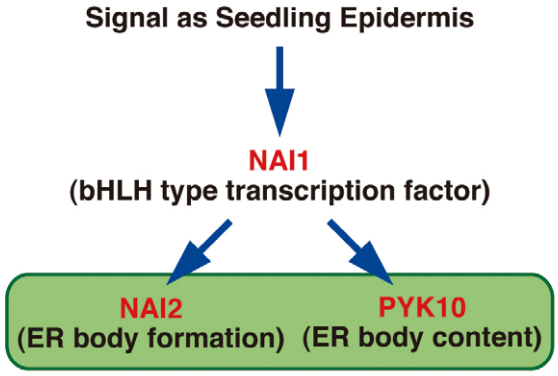


Figure 2. Model of ER body formation. NAI1 regulates the expression of *NAI2* and *PYK10* in constitutive ER bodies of seedling epidermal cells. NAI2 is responsible for the formation of ER bodies and for the accumulation of PYK10.

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 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. 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.

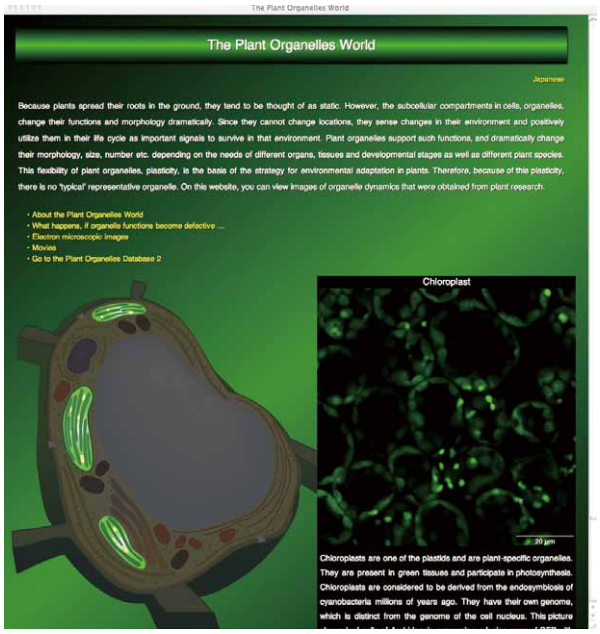


Figure 3. The graphical user interface of the homepage in 'Plant Organelles World' (http://podb.nibb.ac.jp/Orgenellome/PODBworld/en/index.html).

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- Kanai, M., Nishimura M., and Hayashi, M. (2010). A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of *ABI5*. Plant J. *62*, 936-947.
- Nakamura, S., Mano, S., Tanaka, Y., Ohnishi, M., Nakamori, C., Araki, M., Niwa, T., Nishimura, M., Kaminaka, H., Nakagawa, T., Sato, Y., and Ishiguro, S. (2010). Gateway binary vectors with the bialaphos resistance gene, *bar*, as a selection marker for plant transformation. Biosci. Biotechnol. Biochem. *74*, 1315-1319.
- Shirakawa, M., Ueda, H., Shimada, T., Koumoto, Y., Shimada, T.L., Kondo, M., Takahashi, T., Okuyama, Y., Nishimura, M., and Hara-Nishimura, I. (2010). Arabidopsis Qa-SNARE SYP2 proteins localized to different subcellular regions function redundantly in vacuolar protein sorting and plant development. Plant J. *64*, 924-935.
- Takahashi, K., Shimada, T., Kondo, M., Tamai, A., Mori, M., Nishimura, M., and Hara-Nishimura, I. (2010). Ectopic expression of an esterase, which is a candidate for the unidentified plant cutinase, causes cuticular defects in *Arabidopsis thaliana*. Plant Cell Physiol. *51*,123-131.

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

- Mano, S., Miwa, T., Nishikawa, S., Mimura, T., and Nishimura, M. The Plant Organelles Database 2 (PODB2): an updated resource containing movie data of plant organelle dynamics. Plant Cell Physiol. 2010 Nov 28.

LABORATORY OF NEURONAL CELL BIOLOGY

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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. Roles of mRNA transport and local translation in the formation of neuronal networks

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).

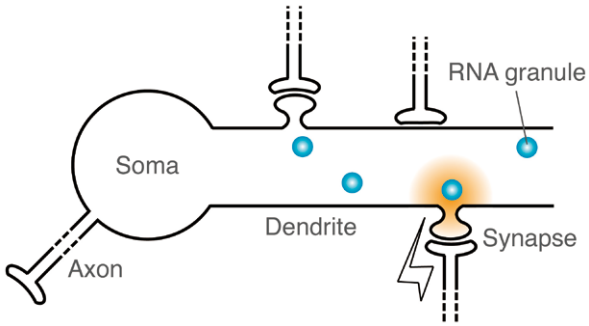

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

We have identified RNA granule protein 105 (RNG105), an RNA-binding protein, as a component of RNA granules. RNG105 dissociates from RNA granules after synaptic stimulation, which is accompanied by the induction of mRNA translation near the granules, suggesting RNG105's involvement in the delivery of its cargo mRNAs to the site for local translation and/or the control of local translation.

To understand the function of RNG105, we have generated RNG105 knockout mice. Furthermore, we have identified about 60 RNG105 cargo mRNAs in neurons. The cargo mRNAs, e.g., those encoding Na^+/K^+ ATPase (NKA) subunit isoforms, are transported to dendrites together with RNG105, but their dendritic transport is markedly reduced in neurons from RNG105 knockout mice. These results indicate the role of RNG105 in the dendritic transport of mRNAs. The RNG105 knockout neurons exhibit reduced dendritic synapse formation and reduced dendritic arborization, which results in poor development of neuronal networks (Figure 2). The perturbed formation of dendritic synapses and networks is mimicked by inhibition and knockdown of NKA subunit isoforms. Taken together, these results suggest that 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.

We are currently investigating the roles of proteins encoded by RNG105 cargo mRNAs in the formation of synapses and neuronal networks. We are also investigating *in vivo* formation and function of neuronal networks in fetuses of RNG105 knockout mice because RNG105 knockout neonates die soon after birth due to respiratory failure, which is associated with defects in fetal brainstem development. Furthermore, we are generating conditional RNG105 knockout mice to investigate the role of RNG105 in higher brain functions, e.g., memory and learning, in adult mice.

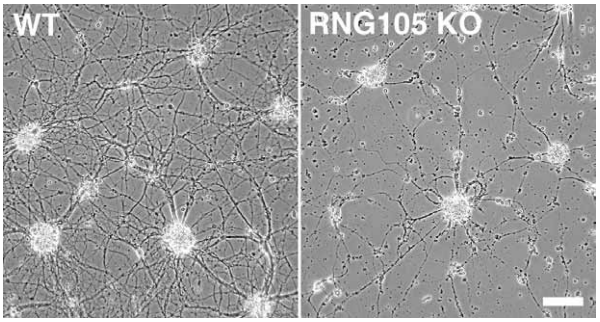

Figure 2. Phase contrast images of primary cultured neurons from wild-type (WT) and RNG105 knockout (KO) fetal brains. RNG105 knockout neurons formed poor networks compared to wild-type neurons. Scale bar, 100 μm.

II. Divergence of mRNA transporting machinery

We have also identified an RNG105 paralog, RNG140. RNG105 and RNG140 are conserved only in vertebrates and an RNG105/RNG140 homolog is found in higher invertebrates, i.e., urochordates and insects. The genes are highly expressed in the central nervous system, suggesting a link between the function of the genes and neuronal functions. RNG140 has RNA-binding domains highly conserved with RNG105 and directly binds to RNA. RNG140 as well as RNG105 induces the formation of RNA granules where mRNAs are recruited. However, RNG140-induced RNA granules do not contain RNG105, and vice versa, indicating that RNG105 and RNG140 induce distinct RNA granules (Figure 3). RNG105-induced RNA granules are similar to stress-induced stress granules in terms of

molecular components and stress inducibility, but RNG140-induced RNA granules are not similar to any other previously known RNA granules. The timing of expression of the two genes during development is also different: RNG105 is highly expressed in embryos, but RNG140 is highly expressed in adults.

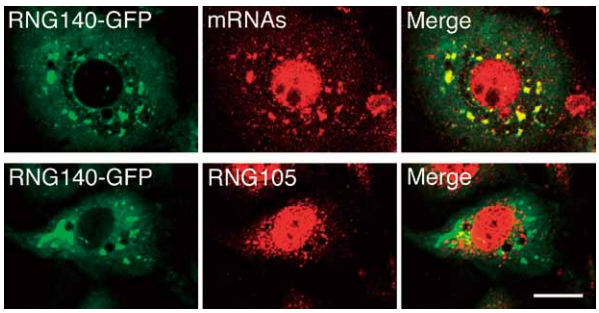

Figure 3. Staining of cultured fibroblasts expressing RNG140-green fluorescent protein (GFP). RNG140-GFP induced the formation of cytoplasmic RNA granules. The cells were stained for mRNAs with poly(dT) probes (top panels) and for RNG105 with an anti-RNG105 antibody (bottom panels). RNG140-GFP-induced granules contained mRNAs but not RNG105. Scale bar, 10 μm.

In spite of localizing to distinct RNA granules, RNG105 and RNG140 gene knockdown show similar effects on neurons: suppression of either gene reduces dendritic arborization and dendritic synapse formation. However, the knockdown effects of RNG140 are not rescued by RNG105, and vice versa, suggesting that RNG105 and RNG140 play similar roles in the development of dendrites and dendritic synapses through different pathways. Thus, RNG105 and RNG140 are localized to different kinds of RNA granules and play roles in the development of dendritic structure at distinct developmental stages.

We are currently identifying components included in the RNG140 RNA granules and investigating if the RNG140 RNA granules transport the same cargo mRNAs as RNG105 RNA granules. We will further generate knockout mice for RNG140 and RNG105- and RNG140-associated mRNAs and proteins to analyze their roles in higher order brain functions.

Publication List

〔Original papers〕

- Shiina, N., and Tokunaga, M. (2010). RNA granule protein 140 (RNG140), a paralog of RNG105 localized to distinct RNA granules in neuronal dendrites in the adult vertebrate brain. J. Biol. Chem. *285*, 24260-24269.
- Shiina, N., Yamaguchi, K., and Tokunaga, M. (2010). RNG105 deficiency impairs the dendritic localization of mRNAs for Na^+/K^+ ATPase subunit isoforms and leads to the degeneration of neuronal networks. J. Neurosci. *30*, 12816-12830.

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.

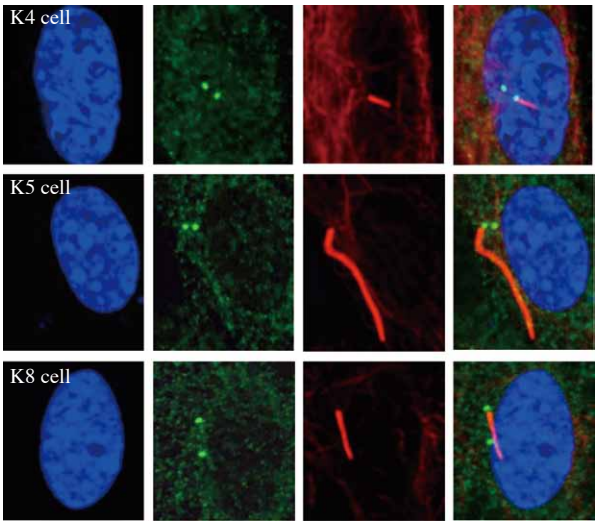

Figure 1. Antibodies stained the minus-end of the primary cilia. Green, candidate protein; red, acetylated tubulin (primary cilia); blue, DNA stained with DAPI.

LABORATORY OF CELL SOCIOLOGY

Assistant Professor
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Technical Assistants: *GONDA, Naoko*
TAKAYANAGI, Noriko

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 their 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.

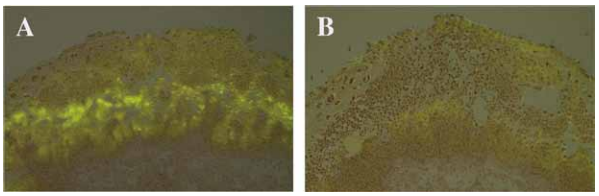

Figure 1. Programmed cell death in the developing mouse placenta. Dying trophoblast was visualized by staining with a fluorescent dye at E9.5. Wild type placenta showed extensive cell death around newly forming maternal blood beds which were surrounded by Notch2 expressing trophoblast (A), but cell death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

DIVISION OF MORPHOGENESIS

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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 cell polarity

Cell polarity is required for proper embryogenesis and organogenesis. It is well understood that there are a number of genes essential for establishing cell polarity; they include planar cell polarity (PCP) genes. In early embryogenesis, gastrulation is one of the best studied models in which cell polarization regulated by PCP genes is thought to play an essential role. Although several signaling pathways required for PCP have been identified, they seem to play permissive rather than instructive roles. To identify the trigger of cell polarization, we are currently investigating the output of cell-cell interactions that lead to cell polarization and recently found that transient intracellular Ca^{2+} is one of the earliest events that occurs prior to cell polarity formation (Shindo et al.). Furthermore, we believe that force is generated by tissue-tissue interactions and therefore mechanical stress may play an essential role in the triggering of cell polarity. To test this possibility, we are artificially applying mechanical stresses to embryonic tissues and examining cellular responses from various aspects.

Apparently, in mammals, cell polarity is also important for embryogenesis even earlier than the gastrulation stage when the primitive streak is formed. We knocked out one of two *prickle*-related genes *mpk1* that belongs to the PCP gene family and found that *mpk1*$^{-/-}$ mutants are early embryonic lethal and die between E5.5 and E6.5 mainly due to defects in cell polarization of the epiblast.

In mice, Prickle1 and Prickle2 proteins are highly expressed in the brain and they seem to have important roles for the development of higher order structures and brain functions. Recently, we also knocked out the other *prickle* gene *mpk2* encoding Prickle2. Homozygous *mpk2*$^{-/-}$ embryos developed to adult and were fertile. Interestingly, however, they exhibited lowered thresholds to the development of epilepsy. We reason that this epileptic phenotype is caused by an aberrant development of the neural network, most likely due to the disruption of neuronal cell polarity. Neurons are highly polarized cells that have dendrites and an axon. Interestingly, at least Prickle1 is localized in the post-synapse of hippocampus neurons (Figure 1). Therefore, it is possible that Prickle proteins contribute to the establishment of synaptic connection and the improper connection of neurons in *prickle* mutants might affect neuronal functions causing the epilepsy-like phenotype. To understand the molecular and cellular mechanisms for the regulation of neuronal cell polarity and the regulation of neural activity, we are currently investigating the cellular pathology of brain tissues and cellular morphology of hippocampus neurons derived from *mpk2*$^{-/-}$ embryos.

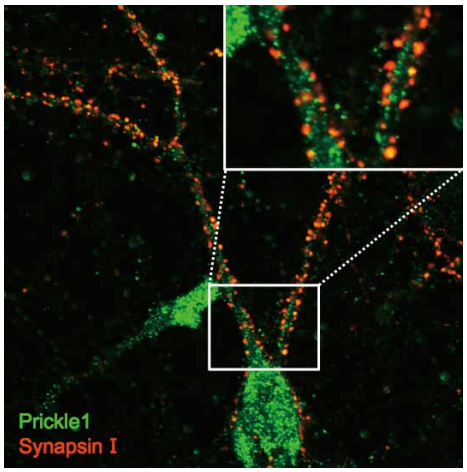

Figure 1. Localization of mouse Prickle1 in hippocampus neurons in primary culture. Prickle1-specific antibody stains punctate Prickle1 proteins adjacent to the pre-synaptic marker Synapsin I.

II. Protein ubiquitylation system involved in the Wnt/PCP pathway

Although Wnt/PCP signaling has been shown to play an essential role in the regulation of gastrulation movements, the molecular mechanisms of how Wnt signals intracellularly and how it regulates tissue movements remain elusive. We have shown that Wnt/PCP signaling activates the protein ubiquitylation/degradation system, which is essential for cell motility during *Xenopus* gastrulation. In order to clarify how the ubiquitylation system is involved in the Wnt/PCP pathway, we focused on β-TrCP (transducin-repeat containing protein), a component of E3 ubiquitin ligase

complex. β-TrCP has been shown to regulate the canonical Wnt pathway by ubiquitinating β-catenin, but it is not known whether β-TrCP is involved in the Wnt/PCP pathway. We found that expression of the dominant negative form of β-TrCP (β-TrCPΔF) impaired gastrulation movements. β-TrCPΔF significantly reduced cell-cell adhesion. In addition, β-TrCP ubiquitylates the cell adhesion molecule cadherin. It localizes to the plasma membrane and bridges cell-cell contacts through its homophilic binding. When β-TrCPΔF was expressed, cadherin was internalized and formed large aggregates in the cytoplasm (Figure 2). This result suggests that β-TrCP is required for maintaining cadherin on the plasma membrane and this function is essential for normal gastrulation. We also found that β-TrCP binds to one of the core PCP proteins, prickle. It recruits β-TrCP to the plasma membrane, and affects ubiquitylation of cadherin by β-TrCP. We are currently investigating the role of prickle in the regulation of β-TrCP activity to demonstrate the molecular mechanism of how Wnt/PCP signaling regulates gastrulation movements.

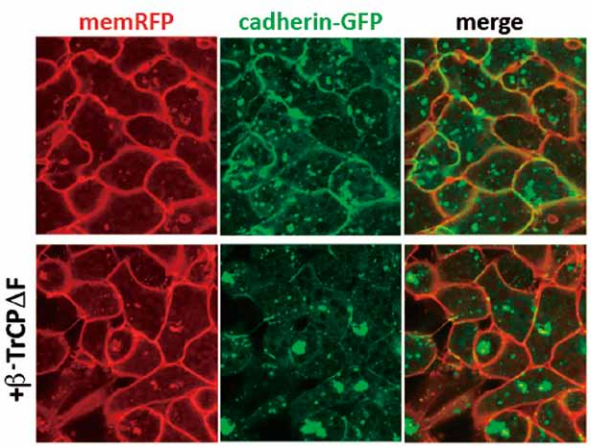

Figure 2. β-TrCP regulates cadherin localization. Membrane-tethered RFP (memRFP) and GFP-tagged cadherin were expressed in *Xenopus* embryos. Cadherin localized mainly to the plasma membrane. When the dominant negative β-TrCP (β-TrCPΔF) was expressed, cadherin was internalized and formed aggregates in the cytoplasm. This suggests that β-TrCP regulates cadherin localization and affects cell adhesion during gastrulation.

III. Cellular morphogenesis during neural tube formation

Neural tube formation is one of the prominent morphogenetic processes during embryogenesis, by which the central nervous system such as the brain and spinal cord are established. In this process, cells in the neural ectoderm undergo cell-cell intercalation, cell elongation, and apical constriction, which allow the neural tissue to form a groove along the anterior-posterior axis and converge toward the midline. Although cytoskeletal elements are responsible for such cellular behaviors, how these are regulated in this process *in vivo* is unknown.

To discern the role of the actomyosin network in neural tube formation, using zebrafish embryos, we analyzed the spatio-temporal dynamics of non-muscle myosin II, focusing on its regulatory right chain. By combining immunohistological and live-imaging analyses, we found that non-muscle myosin II was initially activated as puncta within the motile cells. These puncta were progressively incorporated to the apical region, and colocalized with the adhesive molecule. Furthermore, inhibition of non-muscle myosin II caused defects in cellular motility and morphogenesis, suggesting that non-muscle myosin II acts as the physical linkage between motile cells during zebrafish neural tube formation.

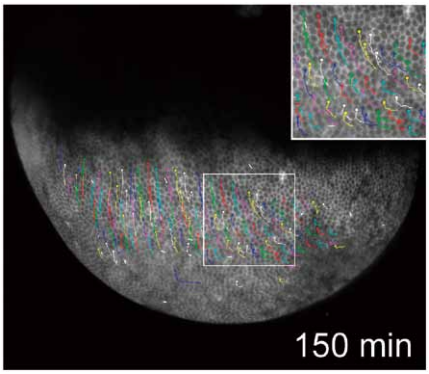

Figure 3. DSLM time-lapse images of ventral non-neural ectoderm from mid (150 min) neurula stages of a *Xenopus* embryo. The trajectories of cell movements (colored dots and lines) and cell division orientations (white short lines) were manually traced. Cells in the middle of the anterior-posterior axis moved rapidly toward the midline. Dorsal is upper side and anterior right. Insets are magnified views of the boxed areas in each image.

We also investigated the physical contribution of the non-neural ectoderm to neural tube formation using *Xenopus* embryos, since dynamic cellular behaviors occur not only in the neural ectoderm but also in the non-neural ectoderm. We tested a role of cell division using chemical inhibitors and found that cell division is not a major driving force in neural tube formation. We are currently examining other cellular mechanisms, including surface expansion, rearrangement, and radial intercalation of non-neural ectoderm cells, using digital scanned laser light sheet microscopy (DSLM) as a powerful tool. DSLM time-lapse images of neurula embryos revealed that cells in the middle of the anterior-posterior axis are highly motile, migrating toward the midline (Figure 3), implying that mechanical properties of these cells may contribute to proper neural tube formation.

IV. Regulation of notochord-specific expression of *Ci-Bra* downstream genes in *Ciona intestinalis* embryos

Brachyury, a T-box transcription factor, is expressed in ascidian embryos exclusively in primordial notochord cells and plays a pivotal role in differentiation of notochord cells. Previously, we identified ~450 genes downstream of *Ciona intestinalis Brachyury* (*Ci-Bra*), and characterized the expression profiles of 45 of these in differentiating notochord cells. We looked for cis-regulatory sequences in minimal

enhancers of 20 *Ci-Bra* downstream genes by electroporating regions within ~3 kb upstream of each gene fused with *lacZ*. Eight of 20 reporters were expressed in notochord cells. The minimal enhancer for each of these eight genes was narrowed to a region ~0.5-1.0-kb long (Figure 4). We also explored the genome-wide and coordinate regulation of 43 *Ci-Bra* downstream genes. When we determined their chromosomal localization, it became evident that they are not clustered in given region of the genome, but rather distributed evenly over 13 of 14 pairs of chromosomes, suggesting that gene clustering does not contribute to coordinate control of *Ci-Bra* downstream gene expression. Our results should provide insights into the molecular mechanisms underlying notochord formation in chordates.

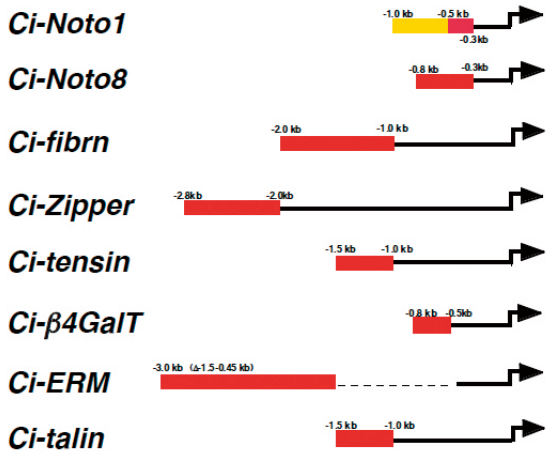

Figure 4. Minimal enhancer region in the 5' upstream sequence of eight Ci-Bra downstream genes that drive reporter expression in notochord cells. The yellow box indicates a possible regulatory region for posterior B-lineage notochord-specific expression of *Ci-Noto1*; the orange box indicates the anterior A-lineage notochord-specific regulatory region of the gene. Red boxes indicate the notochord-specific minimal enhancer region in each of the 5'upstream sequences of *Ci-Bra* downstream genes.

Publication List

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- Morita, H., Nandadasa, S., Yamamoto, T.S., Terasaka-Iioka, C., Wylie, C., and Ueno, N. (2010). Nectin-2 and N-cadherin interact through extracellular domains and induce apical accumulation of F-actin in apical constriction of *Xenopus* neural tube morphogenesis. Development *137*, 1315-1325.
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- Shindo, A., Hara, Y., Yamamoto, T.S., Ohkura, M., Nakai, J., and Ueno, N. (2010). Tissue-tissue interaction-triggered calcium elevation is required for cell polarization during Xenopus gastrulation. PLos ONE, *5*, e8897.
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- Terakubo, H., Nakajima, Y., Sasakura, Y., Horie, T., Konno, A., Takahashi, H., Inaba, K., Hotta, K., and Oka, K. (2010). Network structure of projections extending from peripheral neurons in the tunic of ascidian larva. Dev. Dyn. *239*, 2278-2287.

DIVISION OF DEVELOPMENTAL GENETICS

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Germ cells are specialized cells that can transmit genetic materials from one generation to the next in sexual reproduction. All of the other cells of the body are somatic cells. This separation of germ and somatic cells is one of the oldest problems in developmental biology. In many animal groups, a specialized portion of egg cytoplasm, or germ plasm, is inherited by the cell lineage which gives rise to germ cells. This cell lineage is called germline. The germline progenitors eventually migrate into the gonads, where they differentiate 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 formation in *Drosophila*.

I. Genome-wide search for RNAs of which translation is regulated by Nanos in the germline of *Drosophila* embryos

Several components of germ plasm have been identified in *Drosophila*. One of these components is maternal *nanos* (*nos*) RNA, which is enriched in germ plasm during oogenesis and translated *in situ* to produce Nos protein after fertilization. Nos is inherited by primordial germ cells or pole cells at the blastoderm stage and is detectable in these cells throughout embryogenesis.

Nos acts as a translational regulator for specific RNAs in the pole cells. Maternal Nos represses apoptosis and mitosis of pole cells by suppressing translation of *cyclin-B* and *head involution defective* RNA, respectively. Moreover, Nos is required for the repression of somatic cell fate in the pole cells and for the germline development within the gonads, presumably via regulating unidentified RNAs. Thus, we started a genome-wide identification of RNAs of which translation is regulated by Nos in pole cells. Nos is known to function together with the Pumilio (Pum) protein, which directly binds to distinct sequence in 3'-UTR of the target mRNAs. Recently, Gerber et al. have reported genome-wide identification of 165 Pum-binding RNAs. Based on this data, we started a systematic screen to identify target mRNAs for Nos/Pum-dependent translational regulation in pole cells. We expressed hybrid mRNAs containing GFP-coding region and 3'-UTR sequence from the Pum-binding RNAs, and then examined GFP expression in the pole cells with or without maternal Nos activity. Among twenty hybrid mRNAs, six were translationally repressed by Nos. In addition, we found that translation of two mRNAs were up-regulated by Nos. We are now examining the roles of these mRNAs in pole cell development.

II. Mechanism regulating sex determination of pole cells

"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 primordial germ cells (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. Contrary to prevailing dogma, we found in *Drosophila* that *Sex lethal* (*Sxl*) is expressed in female pole cells before gonad formation and acts autonomously in these cells to induce female development.

Sxl is transiently expressed in pole cells, during their migration to the gonads. Its expression is detected in a female-specific manner and is necessary for feminization of pole cells before they form the gonads. Furthermore, ectopic expression of *Sxl* in male (XY) pole cells is sufficient to induce female fate in these cells, and the resulting pole cells are able to produce functional eggs within female (XX) soma. Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. XX pole cells initiate female sexual identity based on their *Sxl*

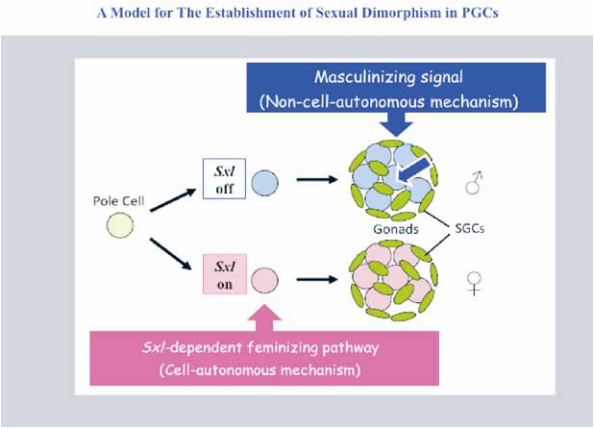

Figure 1. A model for the regulation of sexual dimorphism in pole cells.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

expression, while, lacking *Sxl* expression in XY pole cells, male sexual fate occurs primarily by a signal from gonadal soma (Figure 1). 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.

III. Mechanism regulating the formation of the niche cells in male embryonic gonads

The GSC niche in *Drosophila* testes has emerged as a useful model system for studying stem cells. In the apical tip of the adult testes, the GSCs lie in intimate contact with somatic hub cells, known collectively as the niche cells, which causes the stem cells to retain self-renewing potential. GSCs divide to produce one daughter cell that remains associated with the hub cells, while the other daughter cell detaches and initiates spermatogenesis.

Hub cells are derived from a subset of somatic gonadal cells (SGCs) that are located in the anterior region of male embryonic gonads (Figure 2). However, it remains unclear how the proper niche size and location are regulated within the developing gonads. We have demonstrated that a receptor tyrosine kinase, Sevenless (Sev), represses hub development in the anterior region of male embryonic gonads. Sev is expressed by SGCs within the posterior region of the gonads, and is activated by a ligand, Bride of sevenless (Boss), which is expressed by pole cells, to prevent ectopic hub differentiation in the posterior SGCs (Kitadate and Kobayashi, 2007).

We further found that Notch signaling induces hub differentiation (Kitadate and Kobayashi, 2010) (Figure 3). Notch is activated in almost all of the SGCs within male embryonic gonads, suggesting that the posterior SGCs, as well as the anterior SGCs, have the capacity to contribute to hub differentiation. Since hub differentiation is restricted in the anterior SGCs, the posterior SGCs should be repressed to become hub cells.

We showed that epidermal growth factor receptor (Egfr) is activated in the posterior SGCs to repress hub differentiation (Figure 4). In the absence of *Egfr* activity, ectopic niche differentiation is evident in the posterior SGCs. Moreover, hub differentiation which is normally observed in the anterior SGCs was repressed by expressing a constitutively active form of *Egfr* throughout SGCs. These observations show that Egfr is both required and sufficient to repress hub differentiation (Kitadate and Kobayashi, 2010).

Egfr is activated in the posterior SGCs by Spitz ligand emanating from pole cells, while a ligand for Notch, Serrate, is expressed in SGCs (Figure 2, 3 and 4). This implies that varying the number of pole cells alters the niche size. Indeed, a decrease in the number of pole cells causes ectopic hub differentiation, which consequently increases their chance to recruit pole cells as GSCs. When ectopic hub differentiation is repressed, the decreased number of pole cells fail to become GSCs. Thus we propose that SGCs sense PGC number through signaling from PGCs to SGCs to modulate niche size, and this serves as a mechanism securing GSCs.

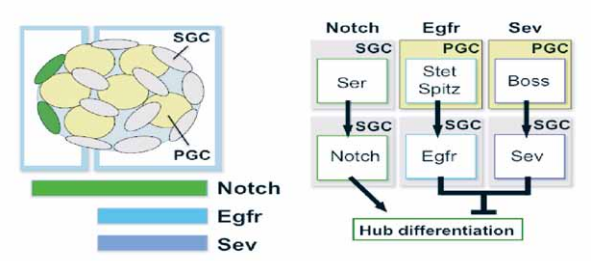

Figure 2. Hub (green) differentiation is controlled by negative regulators, Sev and Egfr and a positive regulator, Notch.

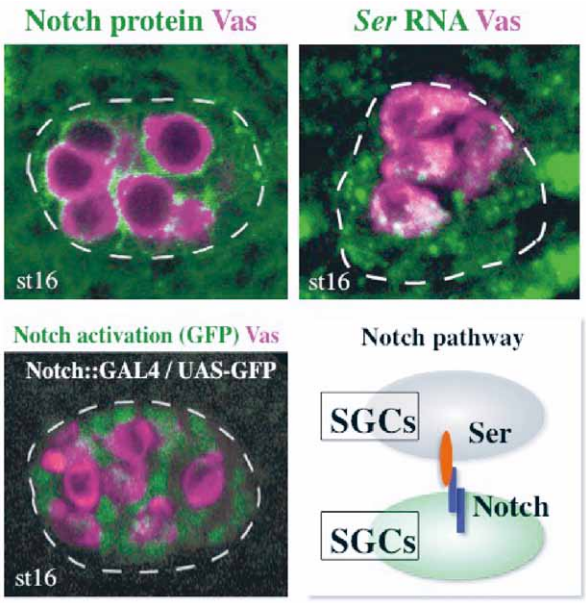

Figure 3. Expression of Notch and *Serrate* in male embryonic gonads.

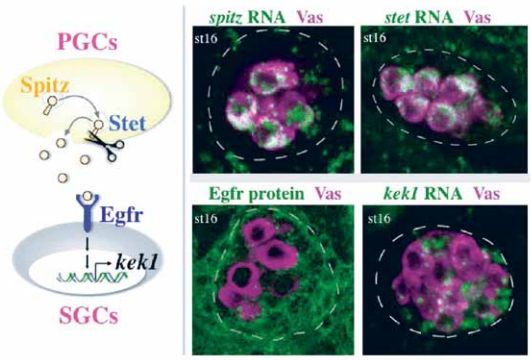

Figure 4. Expression of Egfr signaling components in male embryonic gonads.

IV. Studies on short ORF-containing transcripts in Drosophil

Transcriptome analyses in eukaryotes, including mice and humans, have identified many poly(A)-containing transcripts that only contain short ORFs (sORFs; less than 100 aa). These sORF transcripts are believed to most likely function

as non-coding RNAs (ncRNAs), but growing evidence strongly suggests that a substantial proportion of these "non-coding" transcripts are actually translated into tiny peptides.

We have reported that the *polished rice* gene polycistronically encodes extremely small peptides (11 or 32 aa residues) and regulates epidermal development in *Drosophila*. *pri* is essential for the formation of specific F-actin bundles that prefigure epithelial cellular processes, or trichomes. This year, we have been focusing on genetic interaction of *pri* and *shavenbaby* (*svb*), the master regulator of trichome formation. SVB protein is a unique transcription factor that contains both a transcriptional repression domain and an activation domain, as well as a DNA binding signature of C_2H_2-type zinc-finger. Analysis of gene expression profiles of *pri* mutant flies demonstrated that *pri* is required for activation of *svb* target genes (Kondo et al., 2010) (Figure 5). Transcription assay using an SVB-responsive reporter showed that SVB protein functions as a transcriptional repressor in the absence of *pri* products, while co-expression of *pri* results in activation of the reporter, suggesting that transcriptional activity of SVB protein depends on *pri* activity. Coincidently, in cultured cells and in living embryos, sub-nuclear localization of SVB was changed upon *pri* expression: SVB was detected in a punctate pattern in the absence of *pri* while being more diffused in the presence of *pri*. Biochemical analysis showed that *pri* induced N-terminal truncation of SVB, which results in loss of the repression domain. Truncation is proteolytic, because 1) *svb* mRNA is not modified by *pri* expression, 2) known start codons used in Drosophila do not match to the N-terminus of truncated SVB protein, 3) amino acid sequence, but not DNA sequence, of the truncation site is highly conserved in *Drosophila* species. Taken together, we conclude that *pri* converts SVB from a transcriptional repressor to an activator. These results demonstrate that sORF genes play important roles in *Drosophila* and further analysis of sORF genes should elucidate unexplored novel genome functions of eukaryotes.

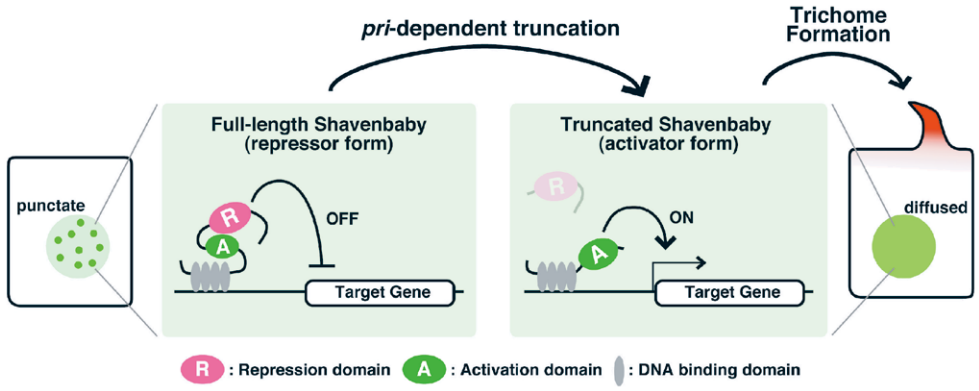

Figure 5. Biological roles of the *polished rice* gene. During trichome formation, *pri* induces truncation of SVB protein, converting transcriptional activity of the protein. Accompanied by activity conversion, subnuclear localization of SVB is also drastically changed. The truncated form of SVB protein in turn activates transcription of target genes, which results in trichome formation.

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- Kondo, T., Plaza, S., Zanet, J., Benrabah, E., Valenti, P., Hashimoto, Y., Kobayashi, S., Payre, F., and Kageyama, Y. (2010). Small peptides switch the transcriptional activity of Shavenbaby during *Drosophila* embryogenesis. Science *329*, 336-339.
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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.” 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 the paraxial mesoderm (or somites), by contrast, appear not to be simply directed by the morphogen gradient and threshold, but by a unique mechanism proceeding periodically. 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, 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. 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 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).

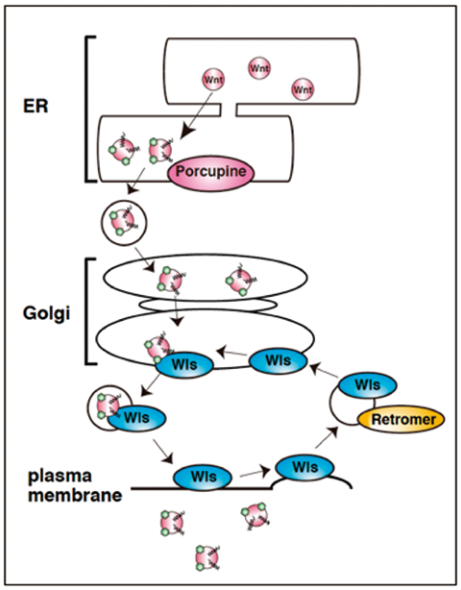

Figure 1. Wnt secretion pathway: Wnt proteins require specific machinery for their secretion. Wntless (Wls), a seven-pass membrane protein, is required for intracellular transport of Wnt proteins. After release of Wnt proteins into the extracellular space, Wls is recycled from the plasma membrane back to the Golgi apparatus by the retromer complex. Prior to the association with Wls, Wnt proteins are modified with a fatty acid, palmitoleoylate, by Porcupine in the ER.

Until now, the role of Porcn *in vivo* has been examined in only a few vertebrate studies. For instance, in humans, mutations in *PORCN* cause focal dermal hypoplasia (FDH), which is an X-linked dominant disorder, characterized by patchy hypoplastic skin and malformations of a wide variety of tissues. However, it is unclear whether the FDH phenotypes are actually caused by abnormalities in Wnt signaling. Furthermore, although some of the FDH phenotypes look similar to those caused by impaired Wnt signaling, only a few of the many defects caused by impaired Wnt-signaling components are observed in FDH patients. Thus, it is unclear whether vertebrate *Porcn* is actually

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required for Wnt signaling *in vivo*, or whether such a requirement is equivalent for all the members of the Wnt family proteins.

To investigate these questions, we used zebrafish as a model system and examined the effects of defects in Porcn function on Wnt signals in early embryonic stages because the roles of embryonic Wnt signals have been precisely characterized. In addition, we are trying to reveal the molecular mechanism underlying the extracellular transport of Wnt proteins during embryogenesis using frog embryos.

II. Roles of Ripplys in establishment of segmental patterns of somites.

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 addition to Ripply1, another structurally related-protein, Ripply2, is required for the rostro-caudal patterning.

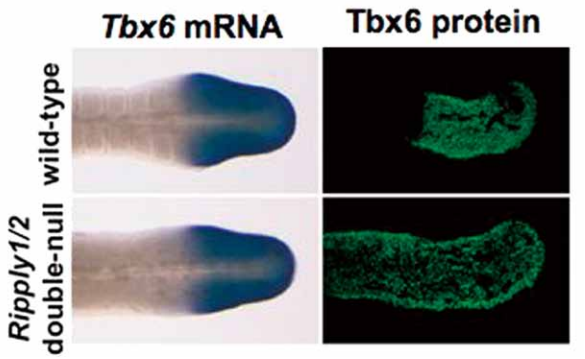

Figure 2. Ripply1 and Ripply2 determine the positions of somite boundaries through degradation of Tbx6 proteins. In Ripply1/2-deficient mouse embryos, the anterior border of the Tbx6 domain is expanded anteriorly.

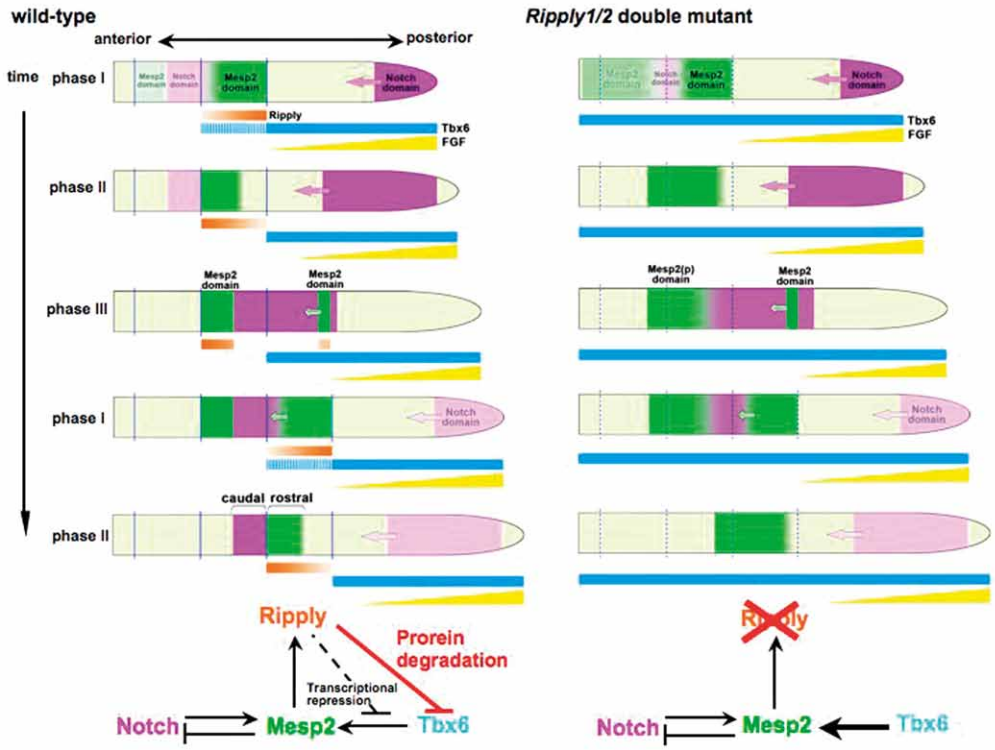

Figure 3. A model of somite segmentation. The Notch active domain travels in the posterior PSM in a posterior-to-anterior direction in phase I. Then, the anterior front of the progressing Notch domain encounters the Mesp2 expression domain, which was already generated in the previous segmentation cycle at late phase II. At this time, progression of the Notch active domain appears to be arrested at the posterior edge of the Mesp2-expressing domain, because Mesp2 inhibits Notch activity. On the other hand, a new cycle of expression of Mesp2, which is induced by traveling Notch activity, emerges in the middle-to-posterior side of the Notch active domain (phase III). In accordance with the anterior progression of the Notch domain, Mesp2 expression subsequently turns on in the Notch domain, resulting in anterior expansion of the newly formed Mesp2 domain in Tbx6 positive area (phase I in the next segmentation cycle). Ripply1/2 regulates anterior expansion of the Mesp2 domains by inhibition of Tbx6. Because of misregulation of Tbx6, boundary formation and rostro-caudal patterning were disturbed in Ripply1/2 mutant embryos.

Interestingly, mouse embryos lacking both *Ripply1* and *Ripply2* exhibit complete lack of somite boundaries, in addition to severe rostralization of somites. The positions of somite boundaries are defined by the anterior limit of the Tbx6 protein domain, which is regulated by degradation of Tbx6 proteins. A recently study showed that a transcription factor, Mesp2, is required for this degradation although it is uncertain how Mesp2 induces this degradation. Interestingly, expression of mouse *Ripply1* and *Ripply2* is dependent on Mesp2 and *Ripply1/2*-deficient embryos exhibit anterior expansion of Tbx6 protein domain (Figure 2), suggesting that *Ripply1/2* expression by *Mesp2* is important in the establishment of somite boundaries by regulating Tbx6 degradation. In addition, Ripply1/2 regulates the rostro-caudal patterning within a somite through interaction with Mesp2, Notch signaling and Tbx6. Based on these results, we proposed a model to explain the boundary formation and the rostro-caudal patterning of somites (Figure 3).

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

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. The pharyngeal arches comprise mesodermal cells, neural crest-derived mesenchyme, an outer ectodermal cover and an inner endodermal lining. 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 the cardiovascular development, while the endodermal cells located in the caudal pouches give rise to several organs, including the thymus and parathyroid gland. Thus, the pharyngeal development is a key process in the generation of these organs.

We found that *Ripply3*, another member of the Ripply family, is expressed in the caudal endoderm and ectoderm of the pharyngeal apparatus in the mouse embryo. Interesitngly, in the caudal pharyngeal endoderm, *Ripply3* is co-expressed with *Tbx1, which* is essential for pharyngeal development (Figure 4). A number of mouse genetic studies and mutational analyses in human patients have indicated that *Tbx1* is the most likely gene responsible for the phenotype of Chromosome 22q11 deletion syndrome (22q11DS). 22q11DS includes the DiGeorge syndrome (DGS), conotruncal anomaly face syndrome (CAFS) and velocardiofacial syndrome (VCFS), is characterized by the abnormal development of the pharyngeal apparatus in the form of thymic hypoplasia or aplasia, hypocalcemia arising from parathyroid hypoplasia and cardiac outflow defects. We show that Ripply3 can modulate Tbx1 activity in in vitro reporter assays. Furthermore, *Ripply3*-kock out mouse, generated by ourselves, exhibit abnormal development of pharyngeal derivatives, including ectopic formation of the thymus and the parathyroid gland, as well as cardiovascular malformation. Corresponding with these defects, *Ripply3*-deficient embryos show hypotrophy of the caudal pharyngeal apparatus. Ripply3 represses Tbx1-induced expression of *Pax9* in *in vitro* luciferase assays, and *Ripply3*-deficient embryos exhibit up-regulated *Pax9* expression. Together, our results show that Ripply3 plays a role in pharyngeal development probably by regulating Tbx1 activity.

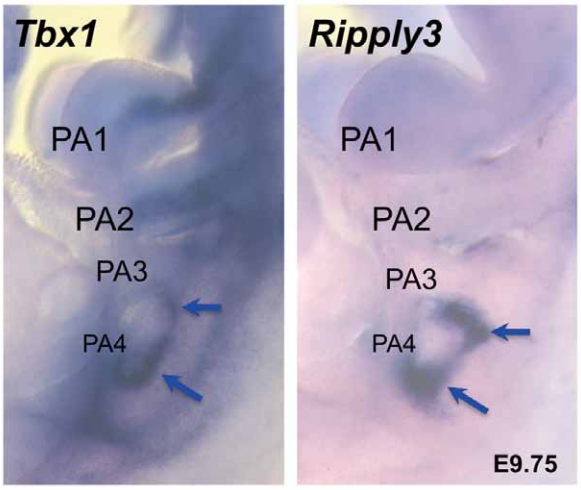

Figure 4. Expression of Ripply3 with its target, Tbx1, in the pharyngeal region of a mouse embryo.

Publication List

〔Original papers〕

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Secretary:	*KATO Azusa*

The aim of our research is to understand the events uThe 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 to 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.

I. Transcriptional regulations of key genes during pre-implantation development.

During the first 4 days after fertilization, the mouse embryo proceeds in its development in the oviduct and uterus, and reaches the blastocyst stage. In the early blastocyst stage, two cell types, namely cells of the inner cell mass (ICM) and cells of the trophectoderm (TE) can be distinguished. TE cells form extra-embryonic tissues including the future placenta. This is the beginning of cell differentiation during mouse development. In the late blastocyst stage, ICM cells form two layers, i.e., the epiblast and the primitive endoderm. Primitive endoderm cells do not contribute to cells of the embryo proper during future development, while the epiblast forms all the parts of the embryo. During these stages, several key factors are known to play important roles in cell differentiation. A few of them are already shown to change their expression levels according to the position of cells within an embryo.

We are currently focusing on Nanog, a protein with homeodomain. Nanog is known as one of the major players in establishing and maintaining pluripotency of ES/iPS cells, and development of epiblast cells in pre-implantation embryos. Expression of Nanog starts at the 4-cell stage and increases later during pre-implantation development. Its expression pattern varies between embryos during blastocyst formation until it is localized to the ICM. At the late blastocyst stage, when the epiblast and primitive endoderm cells are specified in the ICM, Nanog is localized to the epiblast. Although it was suggested that Nanog expression in early stages is stochastic, based on immuno-staining studies of fixed embryos, it remains unclear how Nanog gene expression is regulated and localized to the epiblast during pre-implantation development. To understand actual spatiotemporal changes in Nanog gene expression in each cell, continuous observation of three-dimensional images is necessary. For this purpose, expression of Nanog is visualized by using transgenic mice expressing EGFP under the control of Nanog promoter, which was provided by Dr. Shinya Yamanaka at Kyoto Univ. Embryos of this transgenic mouse line were collected and cultured in vitro. Developmental processes from 2-cell to blastocyst stage were recorded using a laser scan microscope equipped with a CO2 incubator. EGFP signal intensity in each cell was measured correlating with the position of each cell and future cell fate. Transcriptional regulation of Nanog gene expression was separated into three phases. It is initiated at the 4~8-cell stage, and the descendants of cells expressing Nanog higher than others tend to express continuously higher. And in the second phase, it is rather randomly up-regulated during early stages (8~16-cell stage) whereas it may correlate with differentiation status of cells in later stages (16~32-cell stage). Molecular mechanisms underlying these transcriptional regulations are under examination. We are also planning to establish other mouse lines to visualize other key factors by similar approaches.

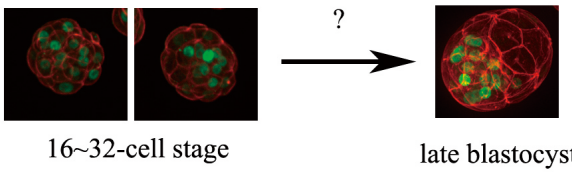

Figure 1. Localization of Nanog protein in pre-implantation embryos. Because protein localization varies between embryos, continuous observation is essential to understand actual behavior of this molecule .

II. Morphological observation of developing mouse embryos in the uterus.

One of the characteristics of mammalian development is that embryos develop in the uterus after implantation. Direct interaction between embryos and the cells of the uterus is important. In studies on developmental biology of mammalian embryos, embryos are usually removed from the uterus, and isolated embryos are analyzed. We decided to analyze early embryonic development of mice comparing the changes in the uterine epithelium. To observe the morphogenesis of embryos within the uterus, a series of sections of implanted uteruses were made, and the morphology of the embryos within the uteruses was observed after hematoxylin-eosin staining. Figure 2 shows an example of a section. At this stage, embryos in a uterus align in the same orientation, and the spaces between neighboring

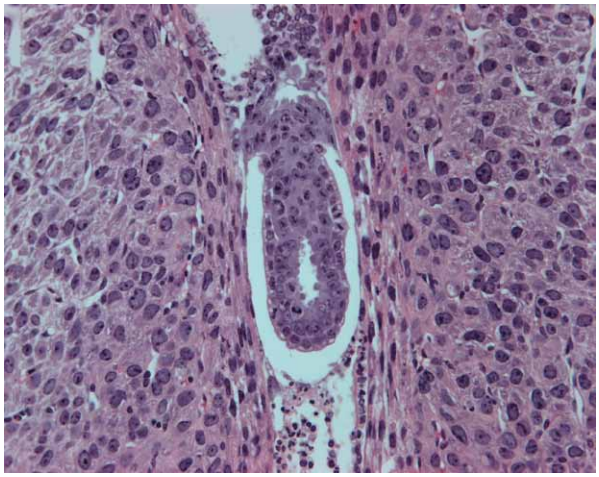

Figure 2. A section of a 5-day mouse embryo in the uterus. Embryos locate to a small gap of uterien epithelium, and align along one axis of the uterus.

embryos are similar. Precise positions of embryonic implantation and changes in the uterine epithelium correlating with embryonic development will be examined using these images.

III. Planner cell polarity in the mouse oviduct.

Planar Cell Polarity (PCP) is the asymmetric organization within epithelial cells along the plane of the epithelium in tissues. We are focusing on the epithelial cells of the mouse oviduct. The multiple cilia on the apical cell surface beat back and forth along the axis from the ovary to the uterus, and this directionally controlled beating is believed to transport eggs to the uterus. Although there are many studies in human oviducts, there are few reports on mouse oviductal ciliary movements where we can discern underlying genetic programs. To study ciliary movements in the mouse oviduct, we recorded ciliary beatings with a high speed CCD camera. We calculated the ciliary beat frequency (CBF) by automated image analysis and found that the average CBF was 10.9±3.3 Hz and 8.5±2.5 Hz (± standard deviation) during the diestrus and estrus stages, respectively. Mapping of the CBF to multiple locations in the epithelium showed that the cilia beat regularly at a local level, but have a range of frequencies within the entire plane. We also observed ova with cumulus cells were transported to the uterus side by the opened oviduct at the diestrus and estrus stages. These results suggest that the ciliated cells of the infundibulum can generate unidirectional flows and are able to deliver ova by their ciliary activities despite their discordance in beating periodicity. We also applied this method to analyze the ciliary movements of the ependymal cells.

These ciliated cells may have clear PCP from ovary to uterus. Our aim is to reveal the molecular and cellular mechanisms of regulating PCP in the mouse oviduct. We are preparing experiments to uncover molecular mechanisms underlying PCP formation in the oviducts, mainly by focusing on the involvement of PCP core group genes and their products.

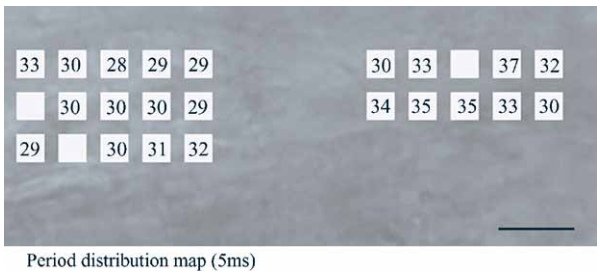

Figure 3. Cilia beat regularly at a local level with a range of frequency in the entire plane. CBF of 25 ROIs that were visualized on the oviductal epithelium (white squares). The number in each white square indicates the n translation when the autocorrelation value was at the first peak, and is equal to the period of beatings (1/200 second). White squares with no number indicate that the CBF measurement at those ROIs was invalid because the autocorrelation value at the first peak was very low. Bar = 10 μm.

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〔Original papers〕

- Tissir, F., Qu, Y., Montcouquiol, M., Zhou, L., Komatsu, K., Shi, D., Fujimori, T., Labeau, J., Tyteca, D., Courtoy, P., Poumay, Y., Uemura, T., and Goffinet, A.M. (2010). Lack of cadherins Celsr2 and Celsr3 impairs ependymal cilliogenesis, leading to fatal hydrocephalus. Nature Neurosci. *13*, 700-707.
- Zheng, L., Ishii, Y., Tokunaga, A., Hamashima, T., Shen, J., Zhao, Q.L., Ishizawa, S., Fujimori, T., Nabeshima, Y., Mori, H., Kondo, T., and Sasahara, M. (2010). Neuroprotective effects of PDGF against oxidative stress and the signaling pathway involved. J. Neurosci. Res. *88*, 1273-1284.

〔Review Articles〕

- Fujimori, T. (2010). Preimplantation development of mouse: A view from cellular behavior. Develop. Growth Differ. *52*, 252-262.
- Niwa, H., and Fujimori, T. (2010). Stem cell systems in development of mammals. Develop. Growth Differ. *52*, 251.

DIVISION OF GERM CELL BIOLOGY

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Visiting Scientist:	*SUGIMOTO, Ryo**
Secretary:	*KUBOKI, Yuko*

Mammalian spermatogenesis represents a highly potent and robust stem cell system. Decades of research, including detailed 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 2010, we revealed a couple of important features of mouse sperm stem cells. One is the discovery of a hierarchy between subpopulations of 'undifferentiated spermatogonia', in which the stem cell functions reside, and reversibility between these subpopulations. This was a proposal that warrants a re-evaluation of a long-held theory in this field. Second is the finding that stem cells are not in a state where they always experience asymmetric division and persist for the entire life span of an organism, but that stem cells replace each other frequently and support spermatogenesis as a population.

I. Hierarchy and reversibility within undifferentiated spermatogonia

Lines of morphological investigations that emerged in the 1950s established the backbone of mammalian spermatogenesis research. The morphologically most primitive spermatogonia in the adult mouse testis are A_s or A_{single} spermatogonia (single, isolated spermatogonia). Their progeny remain interconnected due to incomplete cytokinesis, forming syncytial cysts of 2^n cells (2, 4, 8, 16 etc.). It has been experimentally established that "undifferentiated spermatogonia" (A_{undif}), which contribute <1% of the entire testicular cell population and consist of A_s, A_{pr} (A_{paired}; interconnected two-cell cysts), and A_{al} ($A_{aligned}$; cysts of 4, 8, 16 or occasionally 32 cells) contain stem cells. Undifferentiated spermatogonia then transform into A_1 stage of 'differentiating spermatogonia', as they lose their vast self-renewing potential (Figure 1).

The prevailing "A_s model" (Figure 2), which was originally proposed in 1971, supposes that A_s is the only cell type that can act as stem cells, while the interconnected

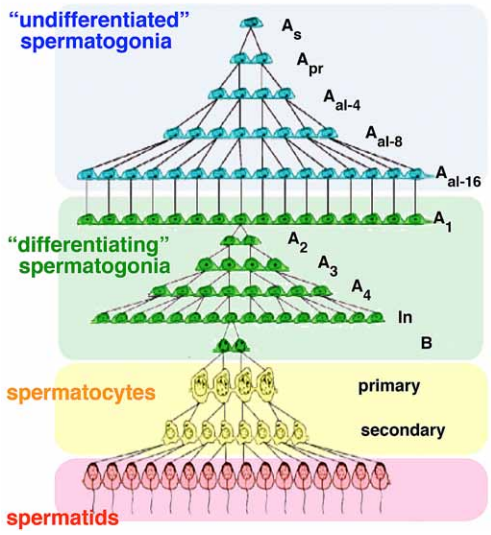

Figure 1. Spermatogenic cell types found in mature mouse testis. Spermatogonia, spermatocytes, and spermatids correspond to the mitotic, meiotic and haploid cells respectively. 'Undifferentiated' spermatogonia population harbors the stem cell functions. Reprinted from Develop. Growth Differ. *52*, 311-317 (2010), with permission.

population of A_{undiff} (A_{pr} and A_{al}) is devoid of stem cell capacity. Similarly, corollaries of this model are that all the A_s cells are functionally equivalent and uniformly act as the stem cells, and that this population plays active roles in every aspect of the stem cell functions, i.e., maintenance of steady state and regeneration after tissue insult and transplantation. However comprehensive and persuasive the "A_s model" is, this was not based on the direct analyses of the cells' behavior. Therefore, the A_s model warrants functional evaluations. It was previously shown by our group that no single stem cell population acts in every aspect of stem cell function: Cells supporting the steady state spermatogenesis are different from those that support regeneration (Nakagawa et al., Dev. Cell, 2007). However, the precise cellular identity that supports these stem cell functions remained to be elucidated.

In 2010, we revealed that a gene expression profile visualizes the heterogeneous nature of the undifferentiated spermatogonia population, in addition to the number of chained cells or the length of the syncytial cysts (Nakagawa

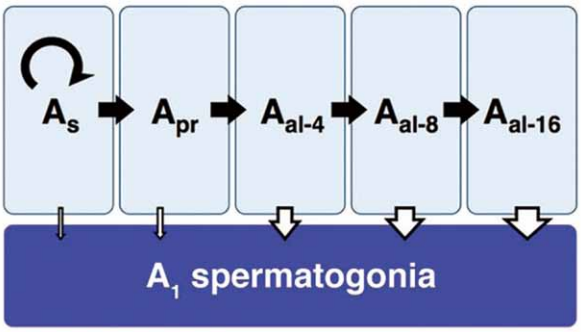

Figure 2. The 'A_s model'. Schematically shown on the basis of theories proposed by the groups of Huckins, Oakberg and de Rooij. Reprinted from Science *328*, 62-67 (2010), with permission

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

et al., Science 2010). We established that the populations of cysts that compose the same number of spermatogonia are heterogeneous in their expression of GFRα1 (glial cell line-derived neurotropic factor receptor 1; shown in magenta in Figure 3) and Ngn3 (neurogenin3; green) genes.

We then investigated the steady-state behavior of these subpopulations of undifferentiated spermatogonia by means of pulse-labeling and live imaging. It was shown that the bulk of the Ngn3+ population differentiate into longer cysts (rightward black arrows in Figure 3) and Kit+ differentiating spermatogonia (shown in blue) as represented by downward white arrows, and that the Ngn3+ cells are supplied from GFRα1+ spermatogonia (downward black arrows). As a logical consequence, the GFRα1+ population is postulated to

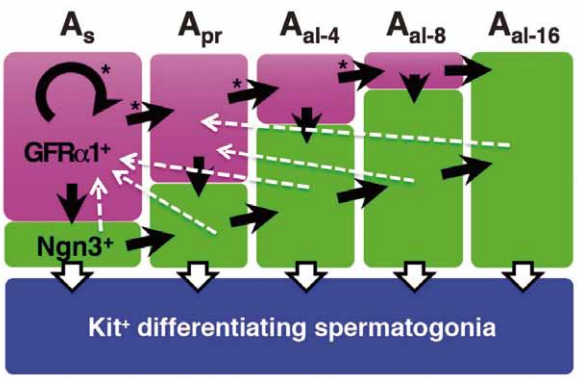

Figure 3. A modified version of 'As model'. Based on the proposal by Nakagawa et al. (2010). See text for the detail. Arrows with asterisks are not confirmed but sill hypothetical. Reprinted from Science *328*, 62-67 (2010), with permission.

function as the main body of the self-renewing compartment.

Interestingly, a very small portion of Ngn3+ cells did 'revert' back into being GFRα1+ and shorter chains (dotted arrows in Figure 3) in steady state. This accompanies fragmentation of syncytial cysts as shown in Figure 4: This phenomenon was not assumed in the mouse 'A_s model', while observed in fruit fly germlines. During regeneration,

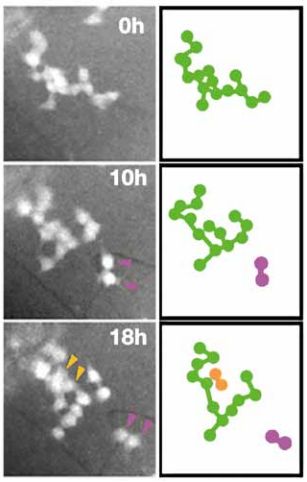

Figure 4. An example of fragmentation of A_{al-16} cyst spermatogonia observed by live imaging. Two pairs of spermatogonia (magenta and orange, morphologically defined as A_{pr}'s) were pinched off an A_{al-16} spermatogonia. Elapsed times are shown.

Ngn3+ cells return to a self-renewing stem cell state much more frequently than in steady state. These observations proposes modification of the A_s model and explain how the stem cell population change their behavior so that they remain constant in steady state, while increasing during regeneration to recover the stem cell pool quickly.

II. Rapid and stochastic turnover between the sperm stem cells

It has been believed that stem cells, including that of mammalian spermatogenesis, are preserved in steady-state cycling tissues as they repeat an asymmetric division that produces one self-renewing and one differentiating daughter cell (Figure 5a). Indeed, such a "stem cell-type" division has been observed only in a limited number of instances including fruit fly germline stem cells, but remains to be evaluated in most systems including mouse spermatogenesis.

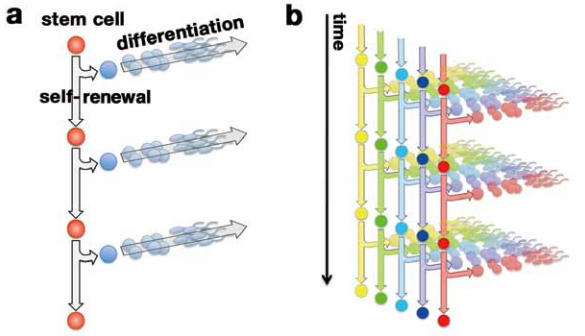

Figure 5. A classical view of stem cell behavior based on the idea of asymmetric division. (a) A single stem cell undergoes a sequence of asymmetric division that produces one self-renewing and one differentiating daughter cell. (b) Given that all the stem cells act as in (a), the stem cell repertoire and each stem cell's differentiating progeny (shown in different colors) will be stable over time in the tissue.

We analyzed the long-term behavior of steady-state mouse sperm stem cells that were pulse-labeled using an inducible cre-loxP system for over a year after pulse (Klein et al., Cell Stem Cell 2010; Nakagawa et al., Dev. Cell 2007), in collaboration with Ben Simons (Cambridge University).

If we suppose that the individual stem cells are preserved as a result of repeated asymmetric divisions, the stem cell repertoire should be constant and the number and the size of the stem cell cohorts will also be constant (Figure 5b). However, the number of the observed stem cell-derived clones decreased while the surviving clones expanded in size (Figure 6a). Mathematical analyses indicate that stem cells disappear frequently and stochastically with a surprisingly short average longevity of less than two weeks, and that the lost stem cells are replenished by the progeny of the neighboring stem cells (Figure 6b).

These findings represent a new idea for the functionality of a stem cell compartment, where cells replace each other and maintain themselves and supply differentiating progeny as a population.

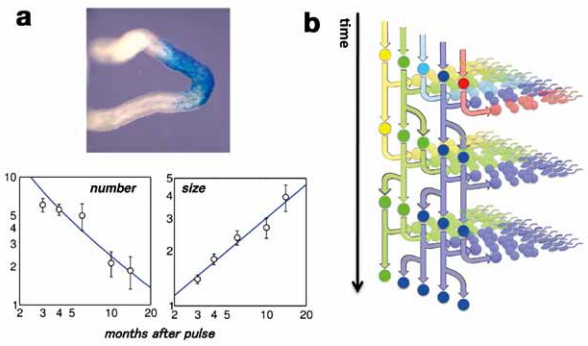

Figure 6. Actual behaviors of pulse-labeled sperm stem cells in seminiferous tubules. (a) Upper panel, a LacZ-labeled single sperm stem cell-derived clonal patch in seminiferous tubules three months after pulse. Visualized by X-gal reaction. Lower panels: Number (left, per testis) and size (right, length in mm) of the surviving pulse-labeled patches indicated periods after pulse (shown in months). (b) A schematic of stem cell behavior in steady state spermatogenesis. Stem cells show frequent replacement by their neighbors, making the stem cell repertoire unstable.

III. Perspectives

We feel that the above findings elucidate novel and fundamental features of mouse sperm stem cells. Accordingly one of the next steps is to provide a molecular basis of the reversibility underlying stem cell differentiation and reversion, as well as the mutual replacement happening between the stem cells.

We will be also investigating aspects of the environmental control of the stem cell population. One is the spatial regulation: We have previously observed that undifferentiated spermatogonia preferentially localize to the vasculature-proximal region (Yoshida et al., Science 2007), while the details of the nature of these 'niche' regions is still unknown. Second is temporal regulation: The differentiation of undifferentiated spermatogonia does not occur randomly but shows a beautiful periodicity once every 8.6 days, representing the seminiferous epithelial cycle.

We hope these studies will shed light and lead to the better understanding the mouse sperm stem cell system.

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〔Review Article〕

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DIVISION OF REPRODUCTIVE BIOLOGY †

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	SHIBATA, Emiko
	TAKAKI, Chikako
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.

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

We 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 have developed a simple, cost effective and gene-specific transgenic RNAi technology for understanding the roles of the zygotic gene products in medaka. Knockdown of DMY in XY gonads induced down-regulation of the genes associated with testicular differentiation and up-regulation of the genes associated with ovarian differentiation, resulting in a complete male-to-female sex-reversal in adult XY medaka (Figure 1). Importantly, we were able to continue a trans-generational knockdown effect of DMY until at least the F3 generation. Since the RNAi effect is long lasting and inheritable, this will provide a powerful tool for the analysis of not only embryos, but also phenotypic consequences that develop over longer periods of time.

A search for the target genes of DMY led to the identification of gonadal soma derived factor (GSDF), a member of the transforming growth factor-beta superfamily. An XY-specific up-regulation was detected in the expression levels of GSDF in the whole embryos of medaka at 6 days post fertilization, coincident with the initiation of DMY expression in XY gonads. Conversely, the expression of GSDF was found to be very weak in XX gonads during embryogenesis. Importantly, GSDF and DMY were found to be co-localized in the same cell type in XY gonads. When the XY embryos were treated with estradiol-17β (E2), in order to reverse their phenotypic sex, a decline was observed in the expression of *GSDF*. These results suggest that GSDF plays an important role in testis differentiation in medaka, probably down stream of DMY.

The molecular control of ovarian development in medaka is less understood. Recently, we cloned three estrogen receptor (ER) subtypes (ERα, β1 and β2) from medaka and examined whether these ERs were involved in ovarian differentiation. The expression of *ERβ2*, but not *ERα* nor *ERβ1*, was up-regulated in XX embryos, but not XY embryos, collected during sex determination/differentiation. It is particularly important to note that the expression of *ERβ2* was markedly increased at 6-8 dpf with a distinct peak at 7 dpf. This correlates well with the initiation of proliferative mitosis in female medaka. *In situ* hybridization revealed *ERβ2* signals in XX gonads collected at 0 dph (8 dpf). This stage-specific expression in females is consistent with the notion that ERβ2 plays an important role in ovarian differentiation in medaka. We also examined the possible involvement of R-Spondin 1 (RSPO-1), a novel regulator of the Wnt/β-catenin signaling pathway, in ovarian differentiation in medaka. *RSPO-1* is expressed in XX gonads from as early as 0 day after hatching to the adult stage, while the expression was barely detected in XY gonads. When the XY embryos were treated with E2, a marked increase was observed in the expression of *RSPO-1*. Knockdown of *RSPO-1* in XX gonads induced female-to-male sex-reversal, while overexpression of *RSPO-1* in XY gonads induced male-to-female sex-reversal. These results indicate that RSPO-1 is critical to initiate the ovary pathway in medaka.

In the Nile tilapia (*Oreochromis niloticus*), we identified that *GSDF*/*DMRT1* in XY gonads and *Cyp19a1*/*Foxl2* in XX gonads during early gonadal differentiation are critical for indifferent gonads to differentiate into either the testis or ovary. XX tilapia carrying extra copies of tilapia *DMRT1* as a transgene induced various degrees of gonadal changes including complete sex change to testis, indicating that DMRT1 plays an important role in testicular differentiation. The critical role of Foxl2 in ovarian differentiation was confirmed by male sex reversal of XX transgenic tilapia carrying a dominant-negative mutant of *Foxl2*.

II. Sexual plasticity in the adult gonochoristic fish

We treated females of two species of adult gonochoristic fish, the Nile tilapia and medaka, with aromatase inhibitors (AI) for up to five months to block the conversion of androgens to estrogens. In both species, suppression of E2 production via AI treatment caused a rapid degeneration of ovarian tissues, leading to the differentiation and development of testicular tissues. Sex-changed fish show a typical male pattern of estrogen and androgen levels, secondary sex characteristics, producing fertile sperm in the newly formed testes. The control ovaries of adult tilapia and medaka contained some isolated cysts adjacent to the ovarian cavity. These cysts of medaka generally contained a single PGC-like *vasa*-positive germ cell which was surrounded by a few somatic cells. AI treatment induced proliferation of these germ cells in the exposed medaka ovaries, indicating that the

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

PGC-like germ cells along the germinal epithelium underwent *de novo* differentiation in the absence of estrogen, giving rise to the testicular germ cells in the AI-treated gonads. These findings indicate that the cysts on the dorsal side of the adult ovaries are the origin of germ cells in the newly formed testicular tissue. Our results also indicate that gonochoristic fish maintain their sexual plasticity to adulthood and that estrogens play a critical role in maintaining the female phenotype.

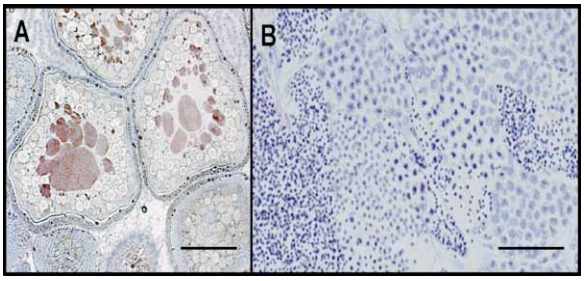

Figure 1. A, Ovary of control XX medaka. B, Gonad of AI-treated XX medaka having testicular tissue with various stages of spermatogenesis. Scale bars are 200 μm (A) and 80 μm (B).

III. Regulation of oocyte maturation and ovulation

Our studies using vertebrate (fish) and invertebrate (starfish) models have revealed that the basic mechanisms involved in oocyte maturation are the same in these two species despite the differing chemical nature of the hormonal agents involved. In both species, three major mediators have been shown to be involved (*Three step model*): a gonad-stimulating substance (GSS), 1-methyladenine (maturation-inducing hormone, MIH), and a maturation-promoting factor (MPF) in starfish, and gonadotropin (LH), 17α, 20β-dihydroxy-4-pregnen-3-one (DHP) (MIH), and MPF in fish. Importantly, these actions of MIHs have been shown to be mediated through the membrane receptors. More recently, DHP has also been shown to be involved in ovulation (follicle rupture). Interestingly, this action of DHP is mediated through nuclear progestin receptors in the granulosa cells.

We recently purified GSS from the radial nerves of starfish (*Asterina pectinifera*) and the complete amino acid sequence was determined. Phylogenetic analyses revealed that starfish GSS was a relaxin-like peptide. Chemically synthesized GSS induced not only oocyte maturation and ovulation in isolated ovarian fragments, but also unique spawning behavior followed by the release of gametes shortly after injection. Thus, this study represents the first evidence of a relaxin system in invertebrates and points towards a novel reproductive role for this peptide in starfish. This work was done in collaboration with Drs. M. Mita and M. Yoshikuni.

Publication List

〔Original papers〕

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Laboratory of Molecular Genetics for Reproduction

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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.

Figure 1. Larva of Various transgenic medaka.

I. Homeostasis in continous gamete production

To produce gametes throughout the reproductive period, there must be some mechanism of homeostasis ensuring the continuum of gamete production in the gonads. In mammalian testis, germline stem cells have been identified as a critical component for this homeostasis. On the other hand in female mammals, all the germ cells differentiate into oocytes during ovarian development in the fetus and the pool of oocytes is the sole sourse for egg production during the reproductive period in adult ovaries. This view of homeostasis has been widely accepted in mammals and is described in many textbooks.

However, many vertebrates with high fecundity produce an enormous number of eggs. In some vertebrates, the egg number reaches 10 to the order of 8 at a single spawning and they spawn eggs several times during their life cycles. It is very unlikely that the conventional theory of an oocyte pool developed during a fetal period can explain the large number of mature eggs.

Unlike mammalian ovaries, ovaries with high fecundity possess germ cells that undergo mitotic division. These immature germ cells are very likely to be the source of egg production, but the homeostasic mechanism that achieves this continuous and prolific egg production remains unknown.

II. *Sox9b*-expressing cells form ovarian cords in the germinal epithelium of ovary.

Sox9 is essential for initiation of testis formation and is under transcriptional regulation of mammalian testis determing gene, *Sry*, on the Y chromosome. *Sox9* is expressed not in the ovary but in Seroli cells of the testis that harbor germline stem cells.

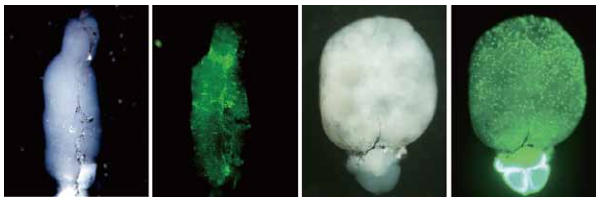

Figure 2. *sox9b* expression (green) in medaka adult testis (left two panels) and ovary (right two panels)

Previously we established transgenic medaka that recapitulate endogenous *sox9b* (medaka orthologue of mammalian *sox9*) expression by fluorescence. Interestingly, unlike mammals, medaka *sox9b* is expressed in both testis and ovary (Figure 2). The cells expressing *sox9b* reside in the ovarian structure called the germinal epithelium, which separates the stromal compartment, where oocyte growth occurs, from the ovarian cavity, into which mature oocytes are ovulated. *Sox9b*-expressing cells form a network by thin cellular processes. Since there have been no reports on this structure, the networks composed of *sox9b*-expressing cells were named “ovarian cords” (Figure 3).

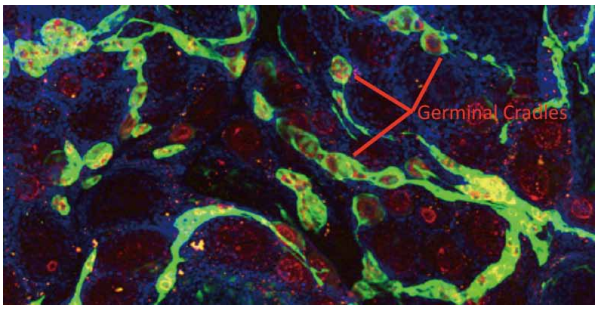

Figure 3. *Sox9b*-expressing cells (green) form a network structure called “ovarian cords” on the surface of adult medaka ovary. The germ cells (red) are endosed with the *sox9b*-expressing cells.

III. Germinal cradles in the ovarian cords contain germ cells at an early stage of oogensis.

Very intriguingly, all the germ cells at an early stage of oogenesis are colonized in the ovarian cords. Therefore we designated the colonized regions within the cords as the “germinal cradle” (Figure 4).

There are three types of germ cells found in the germinal cradles, germ cells that are isolated from the others by *sox9b*-expressing cells (Gs type), cyst-forming germ cells (Gcys type) and an early diplotene stage of oocytes (Gdip). Further

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

analysis with BrdU labeling experiments identified two populations of Gs type germ cells, rapidly and slowly dividing germ cells. Gcys germ cells divide synchronously three to five times to produce 8 to 32 clustered germ cells and then enter meiosis. Experiments with selective elimination of Gcys germ cells have suggested that Gcys cells were recovered from Gs cells. All the results have suggested that Gdip oocytes in the germinal cradles originate from slow-dividing Gs germ cells through Gcys germ cells and Gs germ cells may contain germline stem cells.

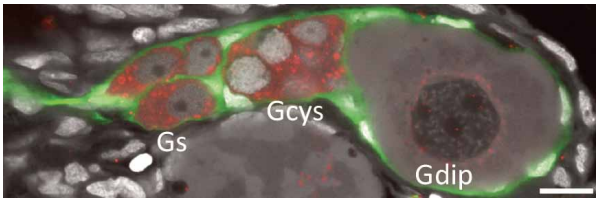

Figure 4. All the germ cells at early stages of oogenesis are clustered within ovarian cords. These colonized regions are called "germinal cradle" and contain germline stem cells.

IV. Germinal cradles in the ovarian cords harbor germline stem cells.

In order to prove that the Gs populations contain germline stem cells, clonal analysis was done. Previously we have shown that *nos2* is expressed in oogonial cells (Aoki et al, 2009 *Zool. Sci.*). Therefore we suspected that *nos2*-expressing germ cells correspond to a Gs type of germ cells and contain germline stem cells. The transgenic medaka was established in which GFP transcripts with *olvas*3'UTR can be driven by *nos2*-promoter upon heat treatment. Since *olvas*3'UTR functions to stabilize and enhance the translation in a *cis*-acting manner in all types of germ cells, this system allows us to keep track of progenitor cells from *nos2*-expressing cells.

Right after heat treatment, Gs germ cells were marked by fluorescence. But, as time passed by, all types of germ cells in the germinal cradles were labeled with fluorescence (Figure 5). Furthermore, we could obtain eggs and embryos from the marked *nos2*-expressing cells for three months.

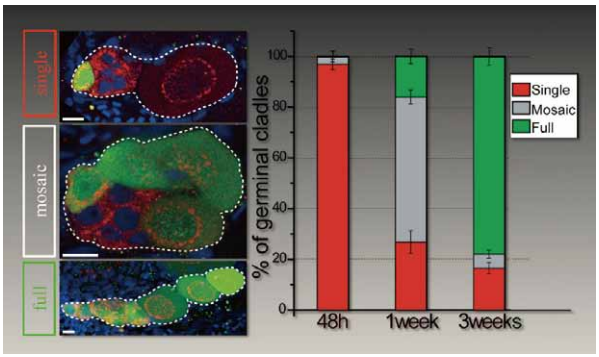

Figure 5. Clonal analysis of *nos2*-expressing cells in germinal cradles, proving the presence of germline stem cells.

These results clearly indicate that *nos2*-expressing germ cells in the germinal cradles contain germline stem cells that keep supplying eggs. This is the first demonstration of neo-oogenesis in adult ovary of vertebrates (Figure 6) (Nakamura et al., 2010 Science).

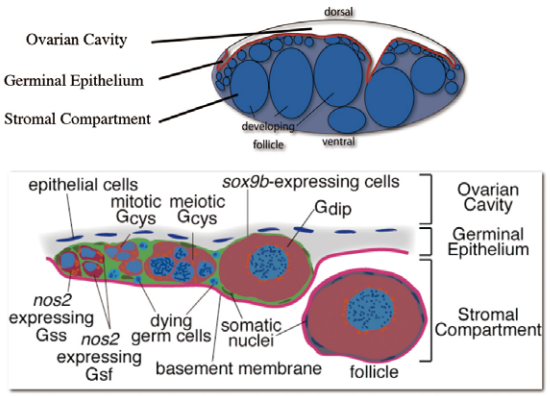

Figure 6. Upper figure indicates location of germinal epithelium in adult medaka ovary. Lower panel shows schematic representation of early oogenesis. Early oogenesis proceeds in the germinal cradles within the germinal epithelium.

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LABORATORY OF PLANT ORGAN DEVELOPMENT

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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*. We showed that the adaxial-specific expression of PHB is regulated by the action of microRNA165/166 (miR165/166) which targeted the *HD-Zip III* messenger RNAs. We also revealed that expression of *MIR165/166* genes is observed only in the abaxial side. One of the *MIR165/166* genes, *MIR165A*, is expressed preferentially in the abaxial epidermal cells (Figure 1). We also found that *MIR165A* is enough to suppress the expression of PHB in the cells located in the abaxial side. These results suggested that miR165/166 might suppress the expression of PHB in some non-cell-autonomous manner.

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. In the extreme cases, leaves are filamentous. This phenotype indicates that *ENF1* is involved in the fixation or maintenance of the position of the adaxial-abaxial boundary. We revealed that the *ENF1* gene encodes an enzyme associated with primary metabolism, and that *ENF1* is strongly expressed in leaf primordia although its expression was not found in the SAM. This indicates that *ENF1*-catalized metabolite and/or its derivatives affect the axis-dependent cell fate in leaf primordia. In contrast, another mutant, *enf2*, has leaves with an enlarged *FIL*-expression domain, and the *ENF2* gene encoded a plastid-localized unknown protein. Chloroplast development was repressed in a severe allele of the *enf2* mutant. Exogenous application of chloroplast development inhibitors to *Arabidopsis* seedlings mimics the defects of the *FIL*-expression pattern by *enf2* mutation. These results suggest that chloroplast development is required for normal differentiation of leaf tissues.

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. *prs wox1* double mutants completely lack the margin-specific cells in leaves, and interestingly, the abaxial side-specific epidermal cells, which are smaller than the adaxial side-specific epidermal cells, "invade" the adaxial side surface. The results 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.

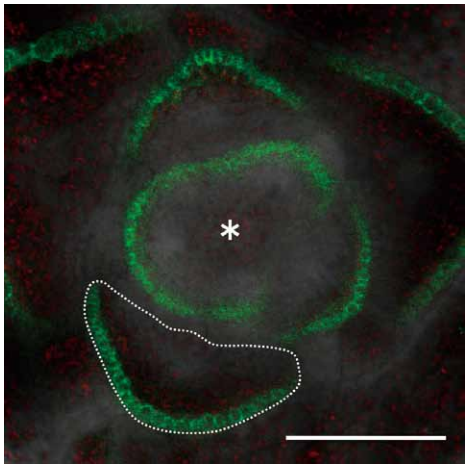

Figure 1. Expression domain of the *MIR165A* promoter:*GFPer* in Arabidopsis leaf primordia. Transverse section of the transgenic plant is shown. *MIR165A* is expressed mainly in the cells at the abaxial epidermis. GFP signal and autofluorescence of chloroplasts are indicated by green and red, respectively. White asterisk is the position of the SAM, and white dashed line outlines a leaf primordium. Scale bar is 100 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

To reveal how floral organs fix their forms through the developing processes, we analyzed mutants named *folded petals* (*fop*) (Figure 2). 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 anther in the flower buds. In *fop* mutants, petals can grow straight when the sepals are removed in the early stage of flower development. We also revealed that FOP proteins are related to wax/cutin synthesis or transport. Thus, we concluded that secreted wax/cutin on the petal epidermis might be important for the precise development of petals.

Figure 2. *fop* flowers (right) have folded petals (white arrow heads), while petals of wild type (left) elongate straightly.

II. Biochemical approach

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 floral buds of *Arabidopsis apetala1 cauliflower* double mutants and were analyzed by peptide sequence methods or LC-MS/MS methods. We chose about 30 peptides as candidates, and prepared synthetic peptides based on the obtained sequences. When applied to *Arabidopsis* seedlings, several peptides caused morphological defects in the SAM, vascular tissue, and root development. We are currently examining the mechanisms involved. We are also isolating small peptides from the apoplastic region in the heads of cauliflower (*Brassica oleracea* L. var. *botrytis*). Recently, we obtained several purified fractions of the apoplastic region, which affect axis-dependent leaf developments after exogenous application to *Arabidopsis* seedlings, and are analyzing the peptide sequences by LC-MS/MS methods.

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 a few days after germination, some of the newly generated rosette leaves changed to a filamentous structure lacking the adaxial-abaxial identity. This suggests a flow of signal(s) from the SAM to the leaf primordia has a role of fixing the abaxial-adaxial regions in that primordia. We are also examining the leaf serration mechanism by ablating some cells at the margin in serrated leaves of several plants.

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 InfraRed Laser Evoked Gene Operator (IR-LEGO) system, and showed gene expression in only a single cell of the root. Currently, we are endeavoring to examine cell-to-cell communication in the SAM using this system.

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〔Original papers〕

- Ishiguro, S., Nishimori, Y., Yamada, M., Saito, H., Suzuki, T., Nakagawa, T., Miyake, H., Okada, K., and Nakamura, K. (2010). The Arabidopsis *FLAKY POLLEN1* gene encodes a 3-hydroxy-3-methylglutaryl-coenzyme A synthase required for development of tapetum-specific organelles and fertility of pollen grains. Plant Cell Physiol. *51*, 896-911.
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〔Review paper〕

- Okada, K. (2010). Start of Arabidopsis research in Japan: a personal memoir of the seedling stage 1985-2000. J. Plant Research *123*, 267-273.

† a paper that was not listed in the 2009 Annual Report

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We have been studying the molecular and cellular mechanisms underlying the development of the vertebrate central nervous system (CNS), mainly using 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. Our special attention is now devoted to 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 their abnormal behaviors including a reduced response to ephrin-A2 and misprojection in the tectum without making clear target zones. Recently, we have generated *APC2*-deficient mice by a gene-targeting technique. We are now analyzing the defects in them to clarify the role 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 project their axons specifically to terminal nuclei of the accessory optic system (AOS) responsible for optokinetic reflex. We recently generated a knock-in mouse in which SPIG1 (SPARC-related protein containing immunoglobulin domains 1)-expressing cells are visualized with GFP, and found that SPIG1-positive RGCs project to the medial terminal nucleus (MTN), the principal nucleus of the AOS. Combination of genetic labeling and conventional retrograde labeling revealed that MTN-projecting ganglion cells are comprised of SPIG1^{+} and SPIG1^{-} ganglion cells distributed in distinct mosaic patterns in the retina. Furthermore, we revealed that SPIG1^{+} and SPIG1^{-} ganglion cells respond preferentially to upward motion and downward motion, respectively, by targeted electrophysiological recordings.

A key circuit module of DS ganglion cells is a spatially asymmetric inhibitory input from starburst amacrine cells. However, it was not known how and when this circuit asymmetry is established during development. We recently found that random or symmetric synaptic connections from starburst amacrine cells are established as early as postnatal day 6, and that inhibitory synaptic inputs are selectively reorganized over a 2-day period.

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 for the differentiation and distinct circuit formation of the two 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 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 functions as a receptor of VacA, a cytotoxin secreted by *Helicobacter pylori*: *Ptprz*-deficient mice are resistant to gastric ulcer induction by VacA. Although our understanding about physiological functions of Ptprz is thus progressing, our knowledge about its

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biochemical properties such as substrate specificity is still limited. We previously identified Git1, p190RhoGAP, and Magi1, as substrates for Ptprz by developing a new genetic method named "yeast substrate-trapping system". Recently, we found that Ptprz selectively dephosphorylates specific phospho-tyrosine residues in these substrate molecules. Alignment of the primary sequences surrounding the target phospho-tyrosine residues revealed considerable similarity. We performed kinetic analyses using various fluorescent substrate peptides with replacement at each position in series to determine the consensus substrate motif for Ptprz. The motif sequence thus obtained predicted paxillin as a novel substrate candidate. We verified that the site in paxillin is efficiently dephosphorylated by Ptprz in a cell-based assay.

IV. Brain systems for body-fluid homeostasis

Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluids. For Na homeostasis of the body, control of Na and water intake and excretion are of prime importance. It was suggested that the circumventricular organs (CVOs), where the blood-brain barrier is missing, are involved in monitoring body-fluid conditions. However, molecular and cellular mechanisms for sensing Na levels and osmolality of body fluids within the brain have long been an enigma. Our studies with *Na$_x$*-deficient mice revealed that Na$_x$, atypical sodium channel, is localized to the CVOs and serves as a sodium-level sensor of body fluids. *Na$_x$*-deficient mice do not stop ingesting salt when dehydrated, while wild-type mice avoid it.

Recently, we revealed that autoimmunity to Na$_x$ causes essential hypernatremia. Essential hypernatremia is clinically characterized by upward resetting of both the osmotic set point for vasopressin release and the threshold for thirst perception, resulting in persistent hypernatremia with a euvolemic state. Usually, structural abnormalities in the hypothalamic-pituitary area are detected. However, several cases of essential hypernatremia without demonstrable hypothalamic structural lesions have been reported.

We studied a case with clinical features of essential hypernatremia without demonstrable hypothalamic structural lesions. The patient was a 6.5-year-old Asian girl complaining of general fatigue and fluctuating drowsiness persisting for a week at the time of admission. Clinical tests revealed that she had marked hypernatremia with a serum Na concentration as high as 199 mM. Of note, she did not complain of any sensation of thirst despite her extreme hypernatremia. Intravenous infusion therapy with a series of hypotonic fluids resulted in a gradual decrease in her serum Na concentration. Further clinical tests revealed a solid tumor adjacent to the right adrenal gland. The tumor was surgically removed, on the assumption that the tumor was somehow related to her extreme hypernatremia; however, the patient's hypernatremia was not cured by the removal of the tumor. We therefore instructed her to drink 1500–2000 ml of water/day, however, her serum Na level has continued to fluctuate until now, 4 years after removal of the tumor.

The relationship between serum osmolality and the plasma vasopressin level obtained during a 4-year follow-up clearly showed that the normal increase of vasopressin secretion in response to serum hyperosmolality is lacking in this patient (Figure 1A). Western blotting with the patient's serum revealed that the patient developed autoantibodies, which detect both human and mouse Na$_x$ (Figure 1B and C). The histological diagnosis of the removed tumor was ganglioneuroma, predominantly composed of Na$_x$-positive Schwann-like cells, along with some ganglion cells (Figure 1D). As such, this neoplasia likely evoked an antitumor immune response, suggesting a paraneoplastic neurologic disorder characterized by neurologic dysfunction in the setting of a remote cancer.

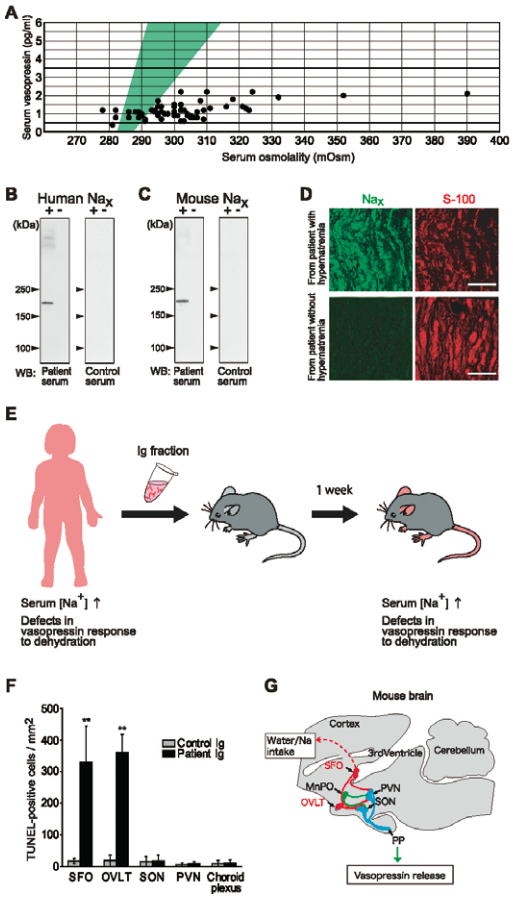

Figure 1. Autoimmunity to the sodium-level sensor in the brain causes essential hypernatremia. (A) Relationship between the serum osmolality and plasma vasopressin level obtained during a 4-year follow-up of the patient. The green area indicates the normal range. (B) Western blot analysis of the membrane extracts of C6 glioblastoma cells with (+) or without (–) expression of human Na$_x$ using serum from either the patient with hypernatremia (Patient serum) or a healthy human subject (Control serum). (C) Western blot analysis of the membrane extracts of C6 glioblastoma cells with (+) or without (–) expression of mouse Na$_x$. (D) Expression of Na$_x$ (green) and S-100 (red) in the ganglioneuroma removed from a patient with hypernatremia (upper). Na$_x$ expression was negative in a ganglioneuroma from a patient without hypernatremia (lower). S-100, a marker of Schwann cells. Scale bars, 100 μm. (E) Generation of animal model of the disease. Passive transfer of the immunoglobulin (Ig) fraction of the patient's serum reproduced her symptoms with abnormal reductions in water intake and vasopressin-release in wild-type mice. (F) Summary of terminal deoxyribonucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL) assays in tissue sections of the SFO, OVLT, SON, PVN, and choroid plexus from mice 3 days after the injection of control Ig or the patient's Ig. **p < 0.01 (compared with Control Ig in the same tissue region), by two-tailed t test; data are mean and SE of three independent experiments. (G) Neural pathways from Na$_x$-positive SFO and OVLT. MnPO, median preoptic nucleus; PP, posterior pituitary.

We therefore examined the pathophysiological effects of the autoantibody to Na_x by intravenously injecting wild-type mice with the patient's immunoglobulin (Ig) fraction (Figure 1E). After 1 week, we found that the mice took in significantly less water than those given the vehicle or control Ig under non-feeding conditions. After 24 hr of dehydration, the vasopressin level of the mice injected with the patient's Ig was elevated but significantly lower than that of the control mice given the vehicle or control Ig. Consistent with this impaired response, the amount of urine of the patient-Ig-injected mice during 24 hr of dehydration was significantly larger than that of the control mice. Of note, after absorption of the autoantibody with Na_x-peptide beads, the depleted patient's Ig preparation was almost ineffective.

Next, we examined the effect of the patient's Ig on salt intake behavior: the mice treated were allowed free access to both normal (Na-repleted) and Na-depleted food. During water restriction, the control mice progressively showed a preference for Na-depleted food. In contrast, those that received the patient's Ig did not show this normal behavioral response. After water restriction for 3 days, the plasma $[Na^+]$ of the mice given the patient's Ig reached over 160 mM, while it remained at a physiological level in the controls. On repletion with enough water, the preference for Na-depleted food of the control mice recovered to the normal level within 1 day, and importantly, their plasma $[Na^+]$ remained at the basal level during the test. In contrast, the mice given patient's Ig showed slightly but significantly higher levels of $[Na^+]$ in plasma even 1 week after water repletion. Thus, injection of the patient's Ig reproduced the persistent hypernatremia in mice as observed in the patient.

Immunohistochemical studies of the brain from the mice injected with the patient's Ig indicated that binding of patient's Ig lead to complement deposition and infiltration of inflammatory cells in Na_x-positive regions, suggesting that the complement-mediated cell death occurred in the CVOs where Na_x is expressed. Indeed, both apoptosis (Figure 1F) and necrosis were evident in the CVOs of the mice injected with the patient's Ig.

The subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) have projections to the supraoptic nucleus (SON) and paraventricular nucleus (PVN), which are responsible for the regulation of vasopressin production (Figure 1G). Na-level sensors and osmosensors are thought to be involved in the regulation of the activity of these projection neurons in the SFO and/or OVLT. Histological damage to the SFO and OVLT would be a reason for the dysregulation of vasopressin production/release. In addition, damage to the posterior pituitary, the site where vasopressin is released into the blood circulatory system, would also affect the release. This defect in the regulation of vasopressin appears to cause serious symptoms for the patient.

This study shows, for the first time, that a ganglioneuroma formed in the peripheral nervous system triggered an autoimmune channelopathy targeting Na_x, the Na-level sensor of body fluids in the brain, causing essential hypernatremia to develop. Pathogenetically, autoantibodies to Na_x likely induce persistent tissue damage within the Na_x-positive CVOs essential for systemic water/salt homeostasis through the activation of complement and infiltration of inflammatory cells.

Publication List

〔Original papers〕

- Chagnon, M.J., Wu, C.-L., Nakazawa, T., Yamamoto, T., Noda, M., Blanchetot, C., and Tremblay, M.L. (2010). Receptor tyrosine phosphatase sigma (RPTPσ) regulates, p250GAP, a novel substrate that attenuates Rac signaling. Cell. Signal. *22*, 1626-1633.
- Hiyama, T.Y., Matsuda, S., Fujikawa, A., Matsumoto, M., Watanabe, E., Kajiwara, H., Niimura, F., and Noda, M. (2010). Autoimmunity to the sodium-level sensor in the brain causes essential hypernatremia. Neuron *66*, 508-522.
- Nagakura, A., Hiyama, T.Y., and Noda, M. (2010). Na_x-deficient mice show normal vasopressin response to dehydration. Neurosci. Lett. *472*, 161-165.

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

- Nayak, G., Goodyear, R.J., Legan, P.K., Noda, M., and Richardson, G.P. Evidence for multiple, developmentally regulated isoforms of PTPRQ on hair cells of the inner ear. Dev. Neurobiol., 2010 Aug 16.
- Yonehara, K., Balint, K., Noda, M., Nagel, G., Bamberg, E., and Roska, B. Spatially asymmetric reorganization of inhibition establishes a motion-sensitive circuit. Nature, 2010 Dec 19.

DIVISION OF BRAIN BIOLOGY

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	TAKAFUJI, Mituse

#: 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 studied 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 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 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.

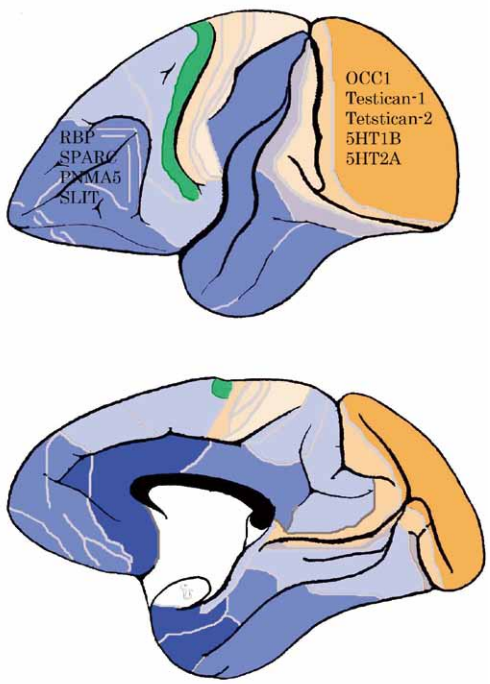

Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

II. Prefrontal-Enriched SLIT1 Gene Expression pattern in Old World Monkey Cortex that is established during Postnatal Development

This year, we reported enriched expression of the *SLIT1* gene in prefrontal and sensory association areas with the lowest expression level in the primary visual cortex. mRNA of *SLIT1*, an axon guidance molecule, was enriched in the higher-order association areas, but with developmentally related changes. *SLIT1* mRNA was mainly distributed in the middle layers of most cortical areas, abundantly in the prefrontal cortex and faintly in primary sensory areas. The lowest expression was in the primary visual area (V1) (Figure2). Analyses of other SLIT (*SLIT2* and *SLIT3*) mRNAs showed enriched expression in the higher-order association areas with a distinct laminar pattern. In contrast,

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

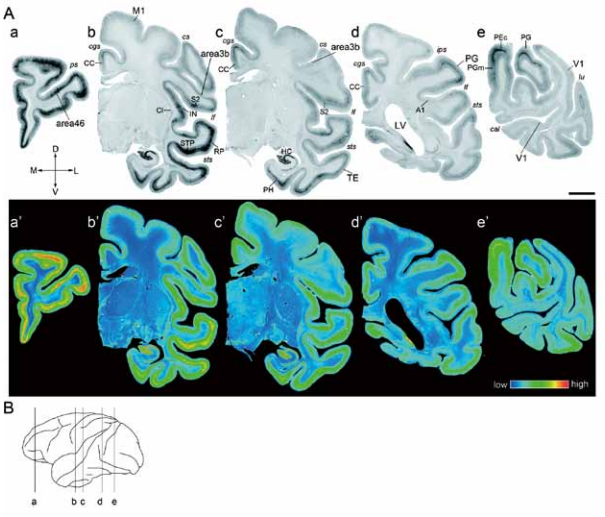

Figure 2. ISH Analysis of SLIT1 Gene in Macaque Brain.
(A) Coronal sections of a macaque monkey brain were obtained from the positions corresponding to a-e in the brain diagram shown in B. The representative six cortical areas (area 46, TE, PG, M1, area 3b and V1) are magnified in Figure 3. Pseudocolor representations of the same sections in a-e are shown in a'-e'. SLIT1 mRNA expression was observed in the entire cerebral cortex at variable levels. Note that the most intense signal was observed in the frontal pole section (a and a'). A1, primary auditory area (core region); M1, primary motor area; V1, primary visual area; CC, cingulate cortex; Cl, claustrum; HC, hippocampus; IN, insular cortex; LV, lateral ventricle; PEc, PE caudal part; PGm, PG medial part; PH, parahippocampal area; RP, rostral parabelt area; STP, superior temporal polysensory area; S2, secondary somatosensory area; cal, calcarine sulcus; cgs, cingulate sulcus; cs, central sulcus; ips, inferior parietal sulcus; lf, lateral fissure; lu, lunate sulcus; ps, principal sulcus; sts, superior temporal sulcus. Orientation of each section is indicated: D, dorsal; V, ventral; L, lateral; M, medial. Scale bar=5 mm. (B) Lateral view of the macaque neocortex. The lines indicate the planes sliced for the sections shown in A. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010)

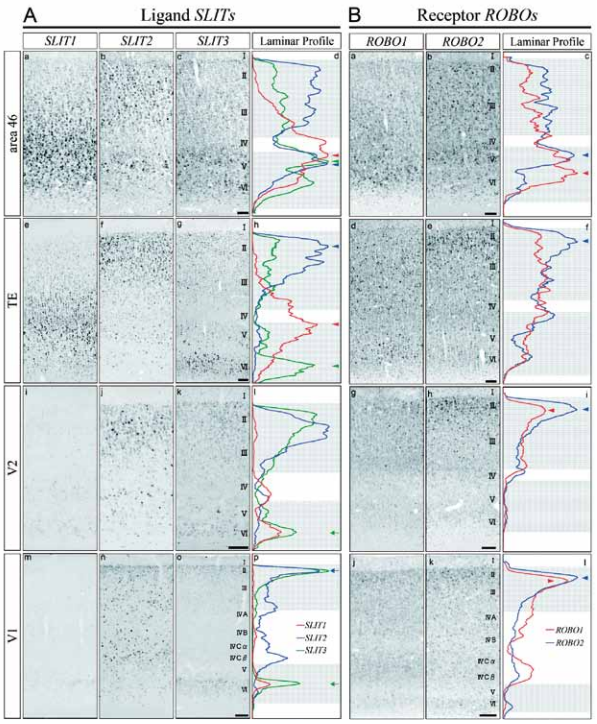

Figure 3. Differential Laminar Patterns of SLIT and ROBO genes in Macaque Cortex.
(A) ISH of SLIT1, SLIT2 and SLIT3 mRNAs in area 46 (a - c), TE (e - g), V2 (i - k) and V1 (m – o), respectively. The laminar profiles of the ISH signals are also shown. Red, blue and green lines indicate the profiles of SLIT1, SLIT2 and SLIT3 mRNAs respectively (d, h, l and p). The ISH signals of SLIT mRNAs were strongest in area 46 and weakest in V1. Their laminar patterns showed complementarity in TE. Arrowheads indicate the peaks of ISH signals for SLIT mRNAs in the association areas. Arrows indicate the peaks of the signals in the lower sensory areas (see in more detail in text) (B) ISH of ROBO1 and ROBO2 mRNAs in area 46 (a, b), TE (d, e), V2 (g, h) and V1 (j, k). The laminar profiles of the ISH signals are also shown. Red and blue lines indicate the profiles of ROBO1 and ROBO2 mRNAs (c, f, i and l), respectively. Arrowheads indicate the peaks of ISH signals for ROBO mRNAs. Scale bar=100 μm. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010)

the receptor Roundabout (*ROBO1* and *ROBO2*) mRNAs were widely distributed throughout the cortex (Figure 3). Perinatally, *SLIT1* mRNA was abundantly expressed in the cortex with modest area specificity. Downregulation of expression initially occurred in lower-order sensory areas around postnatal day 60 and followed in the association areas (Figure 4). Thus, prefrontal-enriched *SLIT1* mRNA expression results from a reduction in expression, specific for areas and layers. These results suggest that its role is altered during postnatal development, and that this is particularly important for prefrontal connectivity in the postnatal monkey cortex.

III. Differential expression patterns of occ1-related genes in adult monkey lateral geniculate nucleus

The extracellular matrix (ECM) plays important roles in the development and plasticity of the central nervous system. Last year, we reported that expression of *OCC1, testican-1, testican-2, testican-3, SPARC* and *SC1* mRNAs, which encode for the ECM protein family, exhibit distinct patterns in the adult macaque visual cortex in an activity-dependent manner (Takahata et al., Cereb Cortex. 19, 1937-1951, 2009). This finding suggests that OCC1-related proteins play crucial roles in the visual processing pathway. We therefore examined the mRNA expression patterns of occ1-related genes in the dorsal lateral geniculate nucleus (dLGN) of adult monkeys. testican-1 and testican-2 mRNAs were strongly expressed in both excitatory projection neurons and GABAergic interneurons in the dLGN. testican-3 mRNA expression, which is predominantly observed in GABAergic interneurons in the cortex, was restricted to excitatory projection neurons in the dLGN. SPARC mRNA was strongly and exclusively expressed in nonneuronal cells in the dLGN. Interestingly, the neuronal *SC1* mRNA expression was selectively observed in koniocellular layers of dLGN, while it is preferentially observed in blob regions of the primary visual area, suggesting a K-pathway preference of expression (Figure 5). Monocular inactivation experiments using tetrodotoxin injections demonstrated that the expression of *testican-1, testican-2* and *testican-3* mRNAs in

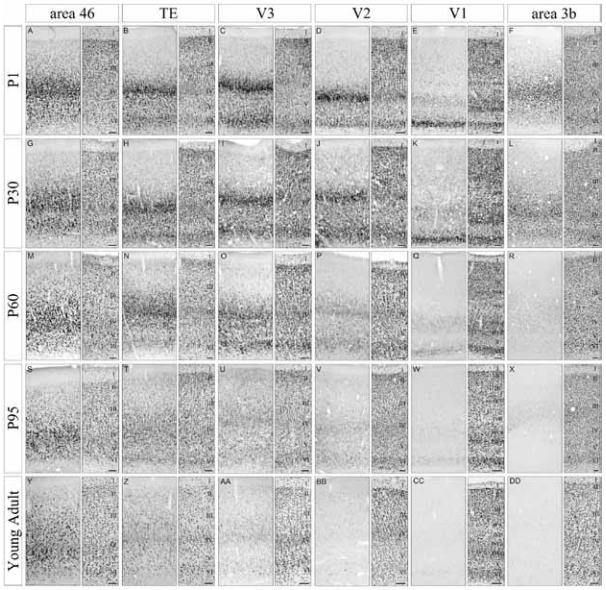

Figure 4. Postnatal Alteration of SLIT1 mRNA Expression in Various Cortical Areas.
The expression of SLIT1 mRNA in six cortical areas (area 3b, V1, V2, V3, TE and area 46) at five postnatal ages are shown. Coronal sections for ISH of SLIT1 mRNA (left panels) and the adjacent sections for cresyl violet staining (right panels) of the macaque neocortex are shown. (A-F) P1, (G-L) P30, (M-R) P60, (S-X) P95, (Y-DD) juveniles. Scale bar=100 μm. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010)

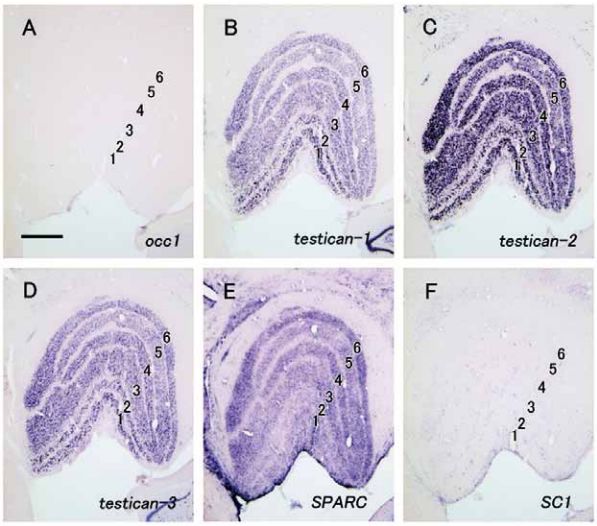

Figure 5. Normal expression patterns of occ1-related genes (A: occ1, B: testican-1, C: testican-2, D: testican-3, E: SPARC, F: SC1) in coronal sections of normal adult macaque dLGN. Scale bar = 1 mm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).

the dLGN are dependent on sensory activity (Figure 6). The differential expression patterns and activity dependence suggest that products of occ1-related genes may modulate visual processing and plasticity at the level of the dLGN, as well as V1.

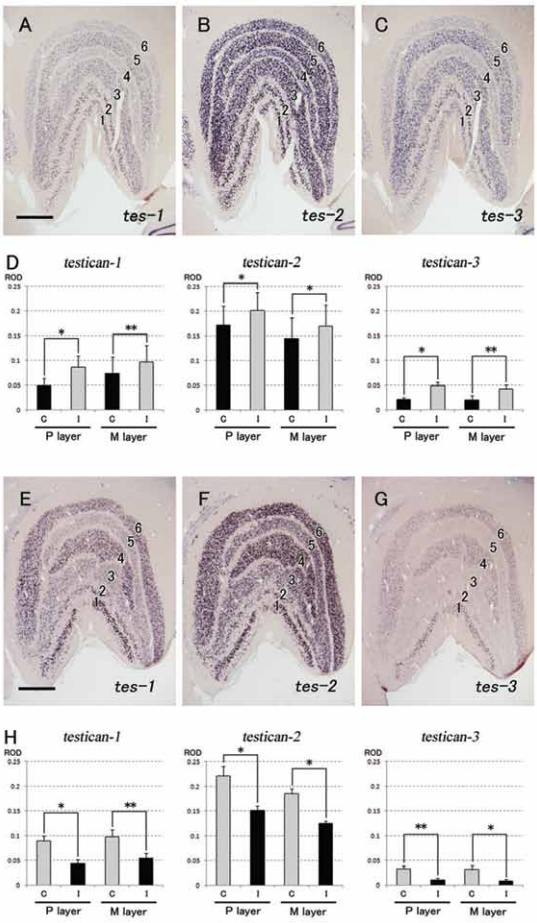

Figure 6. Significant decreases in transcripts of the three testican genes were observed after monocular inactivation in the dLGN. A-C, E-G: Coronal sections of ISH for testican-1 (A, E), testican-2 (B, F) and testican-3 (C, G) in the contralateral (A-C) or ipsilateral (E-F) dLGN to the inactivated eye. D, H: Statistical analysis of ROD in all three genes for each layer in the contralateral (D) or ipsilateral (H) dLGN to the inactivated eye. C is contralateral receiving layers (layer 1 for P layers and layers 4 and 6 for M layers), and I is ipsilateral receiving layers (layer 2 for P layers and layers 3 and 5 for M layers). */P < 0.05, **/P < 0.01 in paired Students' t-test (n = 4 each). Scale bar = 1 mm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).

Publication List

〔Original papers〕

- Watakabe, A., Komatsu, Y., Ohsawa, S., and Yamamori, T. (2010). Fluorescent in situ hybridization technique for cell type identification and characterization in the central nervous system. Methods *52*, 367-374.
- Takahata, T., Hashikawa, T., Tochitani, S., and Yamamori, T. (2010). Differential expression patterns of OCC1-related, extracellular matrix proteins in the lateral geniculate nucleus of macaque monkeys. J. Chem. Neuroanat. *40*, 112-122.
- Puig, M.V., Watakabe, A., Ushimaru, M., Yamamori, T., and Kawaguchi, Y. (2010). Serotonin modulates fast-spiking interneuron and synchronous activity in the rat prefrontal cortex through 5-HT1A and 5-HT2A receptors. J. Neurosci. *30*, 2211-2222.
- Sasaki, T., Komatsu, Y., Watakabe, A., Sawada, K., and Yamamori, T. (2010). Prefrontal-enriched SLIT1 expression in Old World monkey cortex established during the postnatal development. Cereb. Cortex *20*, 2496-2510.

DIVISION OF BRAIN CIRCUITS

Professor
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Postdoctoral Fellow: *MASAMIZU, Yoshito*
Visiting Scientists: *HIRA, Riichiro*
OHKUBO, Fuki
Technical Assistant: *SUGIYAMA, Tomomi*

Various firing patterns of many neurons represent information 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 has recently revolutionized the biological sciences. In particular, two of its features are proving to be very useful when compared with normal excitation modalities, namely imaging fluorescence signals from deep within living tissue and localized photochemical release of caged compounds.

I. Development of novel techniques to stimulate single inhibitory synapses

Recently, we developed two caged-GABA compounds. Two-photon excitation of the first caged GABA produced rapid activation of GABAergic currents in neurons in brain slices with an axial resolution of approximately 2 μm and enabled high-resolution functional mapping of GABA-A receptors. The second caged GABA, combined with an appropriate caged glutamate, allowed bimodal control of neuronal membrane potential with subcellular resolution using optically independent two-photon uncaging of each neurotransmitter. We used two-color, two-photon uncaging to fire and block action potentials from rat hippocampal CA1 neurons in brain slices with 720-nm and 830-nm light, respectively (Figure. 1). Thus, two-photon photolysis of caged GABA compounds should be a powerful tool for clarifying classical neurophysiological problems of synaptic function including dendritic integration, 'AND'-'OR' gate systems, diversity of GABA input points and others at the micrometer and even single-synaptic level.

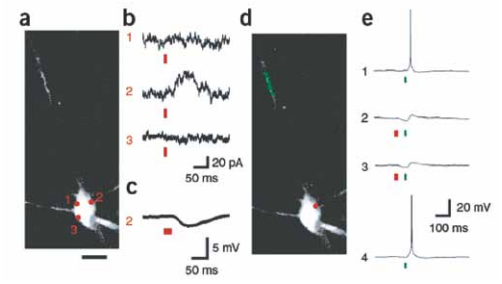

Figure 1. Two-photon excitation (at 830 nm) of GABA around the soma (red) inhibited the neural firing induced by two-photon excitation (at 720 nm) of glutamate at the dendritic locations (green, Kantevari et al., 2009)

II. Relationships between the synaptic connections and the geometry of dendritic spines

Dendritic spines of pyramidal neurons possess a variety of morphologies associated with synaptic strength and are distributed along the complicated structure of the dendritic branches. However, it has not been known whether the spine size and location (spine geometry) relate to the position of presynaptic cells innervating the spines. Here, we activated layer 2/3 pyramidal cells in the motor cortex by two-photon uncaging of glutamate and simultaneously performed two-photon calcium imaging of the dendritic spines of layer 5 pyramidal cells. We found that large spines were preferentially innervated by the cells on the ipsilateral side of the spines, whereas small spines were innervated by cells on both sides. The spines located distally from the parent soma were innervated exclusively by cells on the ipsilateral side of the spines. Our results indicate that synaptic connections are anisotropic and depend on spine geometry, which possibly increases the effectiveness of dendritic integration in information processing.

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. We are now combining a number of cutting-edge techniques to clarify the activity, distribution, and connections of the cortical neurons that are involved in sequential motor phases. The activities of the cortical neurons will be modulated by using 'optogenetic' tools 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〕

- Kantevari, S., Matsuzaki, M., Kanemoto, Y., Kasai, H., and Ellis-Davies, G.C.R. (2010). Two-color, two-photon uncaging of glutamate and GABA. Nat. Methods *7*, 123-125.
- Matsuzaki, M., Hayama, T., Kasai, H., and Ellis-Davies, G.C.R. (2010). Two-photon uncaging of γ-aminobutyric acid in intact brain tissue. Nat. Chem. Biol. *6*, 255-257.
- Obi, N., Momotake, A., Kanemoto, Y., Matsuzaki, M., Kasai, H., and Arai, T. (2010). 1-Acyl-5-methoxy-8-nitro-1,2-dihydroquinoline: A biologically useful phtolabile precursor of carboxylic acids. Tetrahedron Lett. *51*, 1642-1647.

DIVISION OF NEUROPHYSIOLOGY

Associate Professor
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NIBB Research Fellow: *MATSUNAGA, Wataru*

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 to research of the visual system, though we had previously been researching the salt-sensing system.

I. Psychobiological study of Medaka fish

One of our major subjects is the psychobiological study of medaka (*Oryzias latipes*). Medaka, as well as zebrafish, have many advantages for behavioral work. First, genetic examination of medaka is progressing at a rapid pace, like that of the mouse, 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; it is composed of the spinal cord, brainstem, cerebellum, and cerebrum. Thirdly, because they are fish, 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.

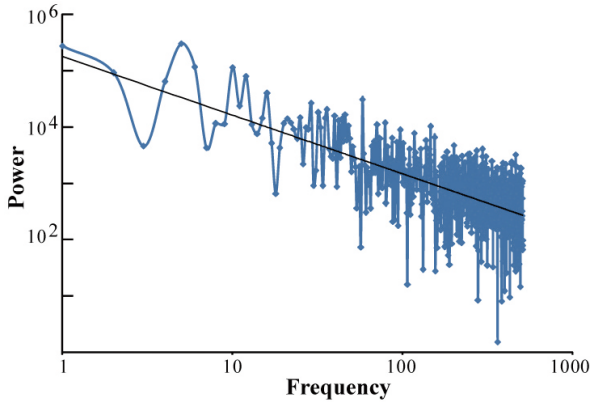

Figure 1. Typical pink noise. Pink noise or 1/f noise is a signal or process with a frequency spectrum such that the power spectral density is inversely proportional to the frequency.

This year, we examined a prey-predator interaction using medaka and the water flea (*Daphnia Magna*), and found that medaka was attracted to pink-noise motion (Figure 1) created by the water flea. These results suggest that the medaka brain includes a mathematical model of the water flea.

II. Psychophysical study of Human vision

Another of our major subjects is the psychophysical study 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, in which a moving object is perceived to lead a flashed object when both objects are aligned in actual physical space. The discoverer of this phenomenon, Dr. Nijhawan, has proposed that the human visual system uses motion signals to extrapolate the position of a moving object. The differential latencies hypothesis proposes that the flash-lag effect occurs simply because the visual system responds with a shorter latency to moving objects than to flash stimuli. Besides these two major models, various modified models have been proposed. The problem, however, has not yet been solved, and the debate continues. How does our brain decide the position of a moving object? This year, we proposed a simple conceptual model explaining the flash-lag effect (Delta model, Figure 2; Watanabe *et al*., 2010).

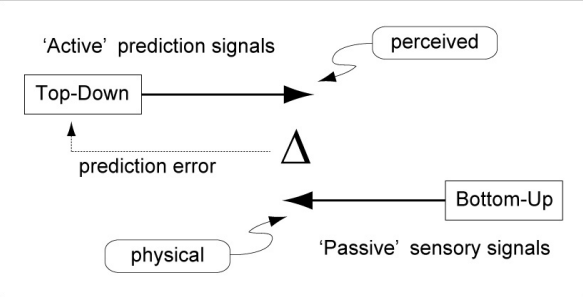

Figure 2. Delta model. Two types of signals (top-down and bottom-up) are assumed in this model. Top-down signals are hypothesized to be derived from a higher level of the visual system and represent the predictive visual information. Bottom-up signals are hypothesized to be derived from a lower level of the visual system and represent the original sensory information. Subtraction occurs between the two signals; the resultant prediction error (Δ) is input to a higher level of the visual system that operates the top-down signal to minimize the prediction error.

Publication List

〔Original papers〕

- Hiyama, T.Y., Matsuda, S., Fujikawa, A., Matsumoto, M., Watanabe, E., Kajiwara, H., Niimura, F., and Noda, M. (2010). Autoimmunity to the sodium-level sensor in the brain causes essential hypernatremia. Neuron *66*, 508-522.
- Matsunaga, W., and Watanabe, E. (2010). Habituation of medaka (*Oryzias latipes*) demonstrated by open-field testing. Behav. Processes *85*, 142-150.
- Watanabe, E., Matsunaga, W., and Kitaoka, A. (2010). Motion signals deflect relative positions of moving objects. Vision Res. *50*, 2381-2390.

LABORATORY OF NEUROCHEMISTRY

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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. In collaboration with Dr. Motoya Katsuki, Executive Director, National Institute of Natural Sciences, we observed that *D1R/D2R* DKO mice exhibited severe impairment in locomotion, no initiation of eating, and died by 4 weeks of age.

To investigate the role of D1R in locomotor control and eating behavior, we generated transgenic mice harboring tetracycline-regulated expression of the *D1R* gene on four different backgrounds, including wild type, *D1R* KO, *D2R* KO, and *D1R/D2R* DKO. Transgenic mouse lines showed doxycycline (Dox) controllable expression of transgenic *D1R* gene.

II. Locomotor activity controlled by D1R expression

To elucidate the effects of altered *D1R* expression, we applied Dox to the mice and monitored daily locomotor activity and food/water intake (Figure 1). 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 locomotor activity. When Dox was continuously applied for 28 days, in *D1R/D2R* DKO and *D1R* KO background, mice exhibited decreases in locomotion and food/water intake after transgene expression decreased to a certain level. This suggests that *D1R* is required for normal activity. Next, Dox was applied for only 14 days. In this case, transgene expression was suppressed to 22%, and then allowed to increase to the original level within 7 days after withdrawal of Dox administraion. Therefore, it is possible to know the effects of an increase of D1R expression. In *D1R/D2R* KO and *D1R* KO backgrounds, which have no endogenous *D1R* expression, withdrawal of Dox administration caused transient hyperactivity (Figure 2). During the process of change in locomotor activity after Dox withdrawal, transgene *D1R* expression gradually increased while locomotor activity fluctuated strikingly. These results indicate that the level of locomotor activity is not simply in proportion to the amount of D1R expression. Instead, increase of D1R expression from an abnormally low level is critical. To understand mechanisms of locomotor control through D1R, we are analyzing the relationship between D1R signaling and altered behavior. In addition, we are investigating whether or not there is a critical period in development for the regulation of locomotion and eating behavior by dopaminergic transmission.

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

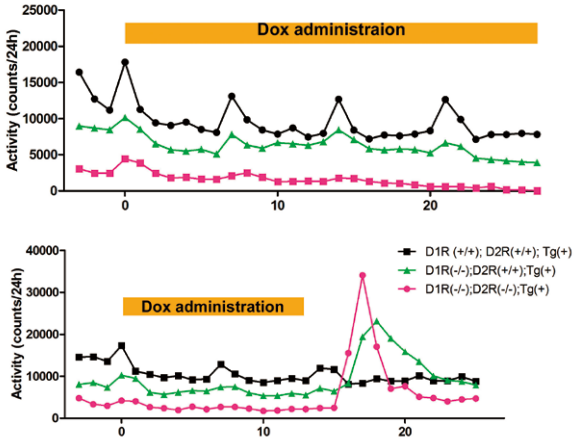

Figure 2. Locomotor activity of *D1R* transgenic mice that have *D1R/D2R* DKO and *D1R* KO backgrounds.

Publication List

〔Original paper〕

● Kusaka, M., Katoh-Fukui, Y., Ogawa, H., Miyabayashi, K., Baba, T., Shima,Y., Sugiyama, N., Sugimoto, Y., Okuno, Y., Kodama, R., Iizuka-Kogo, A., Senda, T., Sasaoka, T., Kitamura, K., Aizawa, S., and Morohashi, K.-I. (2010). Abnormal epithelial cell polarity and ectopic epidermal growth factor receptor (EGFR) expression induced in Emx2 KO embryonic gonads. Endocrinology *151*, 5893-5904.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

DIVISION of GENOME DYNAMICS †

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Gene amplification provides host organisms with an abundance of gene products. One example is the high copy number of rDNA (rRNA gene), which enables a large amount of protein production. On the other hand, drug-resistance genes can become amplified increasing their effectiveness. These two forms of gene amplification produce products that differ radically in structure. The former is a direct repeat product and the latter is an inverted repeat product. We previously clarified the mechanism of the former type of amplification and here have shed light on the latter.

We have carried out an amplification experiment using yeast on the assumption that double rolling circle replication (DRCR) is a core reaction in gene amplification. We induced DRCR by BIR (break induced replication) and obtained two gene amplification products, HSR (homogeneous staining region) and DMs (double minutes) (Watanabe and Horiuchi, *EMBO J*. 2005). To further confirm this result, we carried out an experiment in which DRCR was induced by two entirely different methods, the Cre-*lox* system and homologous recombination. We obtained the gene products HSR and DMs in either case respectively. Furthermore, using Chinese Hamster Ovary (CHO) cells, we induced DRCR by the Cre-*lox* system and obtained two gene products. In addition, scattered products, as are frequently observed in cultured cells, are obtained. Finally, we found that inversion occurred frequently in the inverted repeat structure of the products and is dependent on the DRCR process itself.

I. Construction of a new gene amplification system via DRCR (double rolling circle replication) using Cre-*lox* site-specific recombination

Previously, we developed a gene amplification system in *S. cerevisiae* that is based on DRCR, utilizing break-induced replication (BIR) (Watanabe and Horiuchi, *EMBO J*. 2005). This system produced two amplification products resembling the HSR and DMs of higher eukaryotes. If DRCR is an actual gene amplification mechanism in yeast, a quite different initiation reaction, which can induce DRCR, should produce amplification products resembling HSR and DMs. Thus, we tried to construct a new DRCR amplification system that is induced by another process, Cre-*lox* site-specific recombination. We first predicted that, if Cre recombination occurs between the two *lox* sites, one present on the replicated and the other on the un-replicated region, as shown in Figure 1 (a), the replication fork should switch the template from the parental (un-replicated) to the sister–chromatid (replicated) DNA strands. Furthermore, a

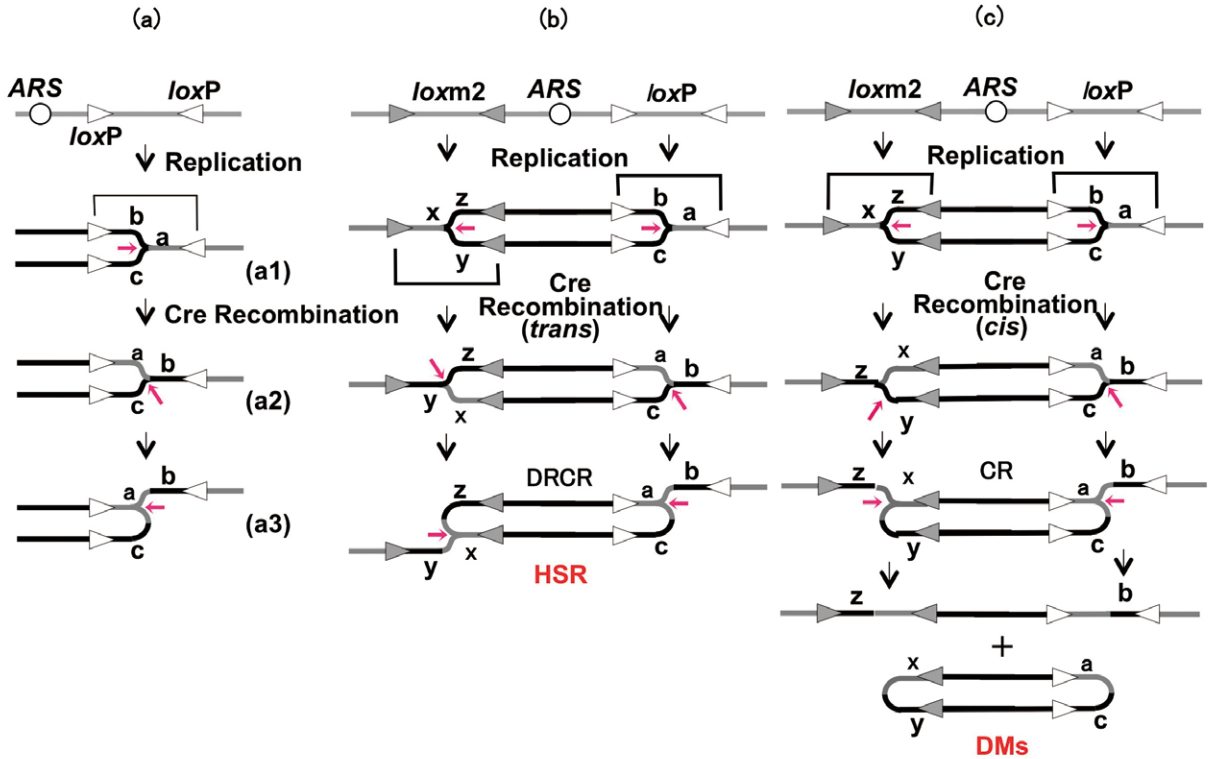

Figure 1. Cre-*lox* can initiate template switching, DRCR and CR (convergent replication).

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

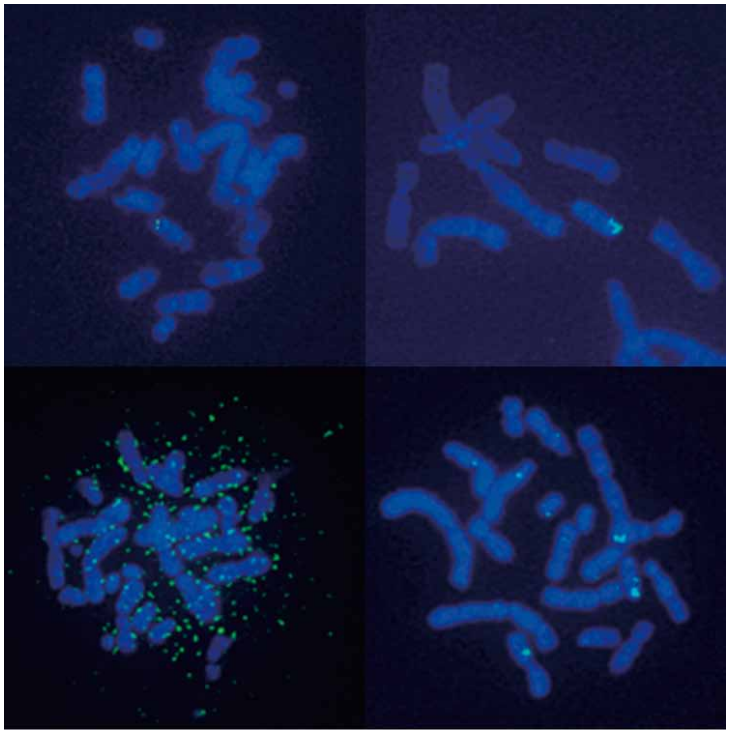

Figure 2. Three gene amplification products in CHO cells. (top left) control cell, (top right) HSR type, (bottom left) DMs type, (bottom right) Scattered type.

combination of the recombination processes, as shown in Figure 1 (b) and (c), could efficiently induce gene amplification through DRCR or CR (convergent replication). In fact, this system produced two kinds of gene product: highly amplified (>100 copies) chromosome HSR products and acentric multi-copy extra-chromosomal DMs products. The structures of these products resemble HSRs and DMs of higher eukaryotes, respectively. From previous and present results, we concluded that DRCR is indeed an amplification mechanism in budding yeast and can be naturally initiated if some structural requirements are satisfied.

Next, we constructed a similar amplification system in CHO cells, based on DRCR. This system also produced intra- and extra-chromosomal amplification products resembling HSRs and DMs (Figure 2. top right, bottom left). The amplified regions of HSR-type products undergo intensive rearrangement seen in mammalian gene amplification. Furthermore, the CHO system produces scattered-type amplification seen in cancer cells. This system can serve as a model for amplification of oncogene and drug-resistance genes, and may improve the productivity and ease of use of amplification systems that are widely used for making pharmaceutical proteins in mammalian cells (manuscript submitted for publication).

II. Mechanism of oncogene-type amplification under natural conditions

Site-specific recombination consists of two elements, a short specific sequence (*cis*-element) and a specific protein (*trans*-element) which recognizes the specific sequence and recombines efficiently between them. On the other hand, general recombination consists of a long non-specific sequence and several sets of recombination proteins (Rec proteins), which recognize homologous sequences and recombine between them. However, homologous recombination can take the place of site-specific recombination by replacing a short specific sequence with a long non-specific sequence. This indicates that if a *lox* sequence is replaced by a long sequence, homologous recombination can induce DRCR. Replacement of the sequence alone should initiate DRCR by itself, in the absence of any site-specific recombination protein factors. We confirmed this expectation as follows: we created a →←→← structure, called FAIR (Four Alternate Inverted Repeat) at the right end of chromosome VI and an amplification selective marker, *leu2d*, inserted within the FAIR structure. This yeast strain was plated on agar without leucine, Leu^+ clones grew out and their chromosomal structure analyzed. As expected, two types of amplification were observed, HSR- and DM -type, both with the expected repeated structure. The implications of these results are very important, namely, that if FAIR structures form under natural conditions, gene amplification will occur. In fact, there are studies in which FAIR structures were observed at an initial stage of drug-resistance gene amplification. Furthermore, there is data suggesting that the BFB cycle can be an initial step of amplification. From these previous and our present results, all steps of oncogene-type gene amplification can be deduced as follows: a double strand break or recombinational template switching spontaneously occurs on a chromosome, and the BFB cycle initiates. As a result, a di-centric chromosome is produced and ds-breakage occurs again. The chromosome structure after two cycles of BFB is a perfect FAIR structure. Thus, this can initiate DRCR and gene amplification starts.

Using budding yeast, we here demonstrate that the FAIR structure has the potential to induce DRCR. Thus, we believe that the basic mechanism of oncogene-type gene amplification has been determined. However, gene amplification may also be induced by the BFB cycle. Especially in higher eukaryotes, there are a large number of transposable elements, among which there should be FAIR structures. Thus, it is not surprising that DRCR initiates without any double-stranded breaks. It is very interesting to ask the question whether or not this type of gene amplification does indeed occur (manuscript in preparation).

III. Double rolling circle replication (DRCR) is recombinogenic.

Homologous recombination plays a critical role in maintaining genetic diversity as well as genome stability. Interesting examples showing hyper-recombination are found in nature. In chloroplast DNA (cpDNA) and the herpes simplex virus (HSV) genome, DNA sequences flanked by inverted repeats undergo inversion very frequently,

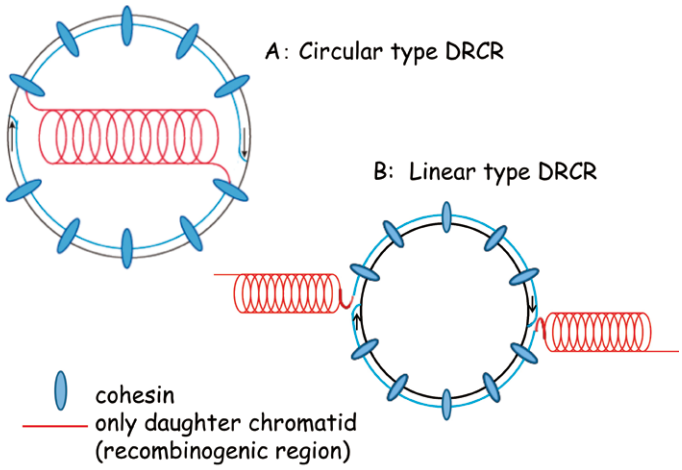

Figure 3. An "only daughter chromatid" model
A. circular-type DRCR, B. Linear-type DRCR

suggesting hyper-recombinational events. However, the mechanisms responsible for these events remain unclear. We previously observed very frequent inversion in a designed amplification system based on double rolling circle replication (DRCR). Utilizing the yeast 2μ plasmid, the genome of which is known to be replicated by DRCR and the gene amplification system in yeast, we demonstrate that DRCR is closely related to hyper-recombinational events. Inverted repeats or direct repeats inserted into these systems frequently caused inversion or deletion/duplication, respectively, in a DRCR-dependent manner. These results suggest that DRCR is involved in chromosome rearrangement associated with gene amplification and the replication of cpDNA and HSV genomes. We propose a model (Figure 3), termed the "only daughter chromatid" (red lines) model, in which DRCR inevitably produces only daughter chromatids and is markedly activated recombinationally (manuscript in press).

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	PLAVSKIN, Eugene
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All living organisms evolved from a common ancestor that lived more than 3.5 billion years ago, and the accumulation of mutations in their genomes has resulted in the present biodiversity. Traces of the evolutionary process are found in the genomes of extant organisms. By comparing the gene networks (and their functions) of different organisms, we hope to infer the genetic changes that caused the evolution of cellular and developmental processes.

I. Phylogeny and biogeography of land plants and insects

Phylogenetic trees with reliable, statistical confidences are the basis for evolutionary biology. We infer phylogenetic relationships of major lineages of land plants and insects.

The subfamily Apaturinae consists of 20 genera and shows disjunct distributions and unique host-plant associations. Most genera of this subfamily are distributed in Eurasia, South-East Asia and Africa, whereas the genera *Doxocopa* and *Asterocampa* are distributed mainly in South America and North America, respectively. Although Apaturinae larvae mainly feed on Cannabaceae, those of the genus *Apatura* are associated with *Salix* and *Populus* (Salicaceae), which are distantly related to Cannabaceae. Here, we infer the phylogeny of Apaturinae and reconstruct the history of host shifting and of colonization in the New World. We analyzed 9761 bp of nuclear and mitochondrial DNA sequence data, including the genes encoding *EF1a*, *Wg*, *ArgK*, *CAD*, *GAPDH*, *IDH*, *MDH*, *RpS5*, *COI*, *COII*, *ATPase8*, *ATPase6*, *COIII*, *ND3*, and *ND5* for 12 apaturine genera. We also inferred the phylogeny with six additional genera using mitochondrial sequence data alone. Within Apaturinae, two major clades are recovered in all the datasets. These clades separate the New World genera, *Doxocopa* and *Asterocampa*, indicating that dispersal to the New World occurred at least twice. According to our divergence time estimates, these genera originated during the Early Oligocene to the Early Miocene, implying that they migrated across the Bering Land Bridge rather than the Atlantic Land Bridge. The temporal estimates also show that host shifting to *Salix* or *Populus* in *Apatura* occurred more than 15 million years after the divergence of their host plants. Our phylogenetic results are inconsistent with the previously accepted apaturine genus groups and indicate that their higher classification should be reconsidered.

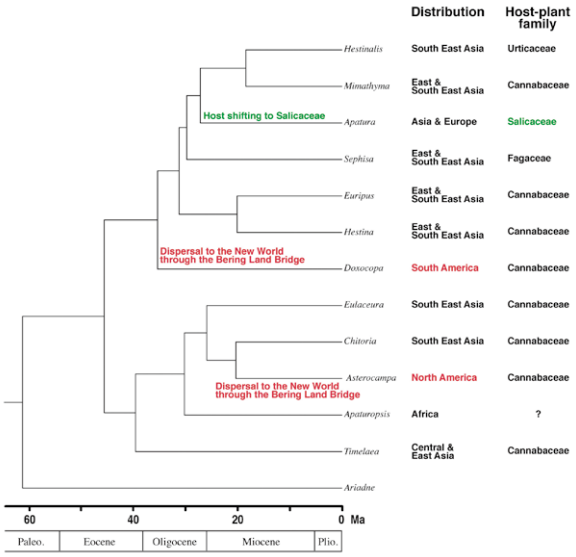

Figure 1. Evolution of Apaturinae

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

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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

Seed plants form shoot and root apical meristems containing multiple pluripotent stem cells in the sporophyte (diploid) generation, but do not form pluripotent stem cells in the gametophyte (haploid) generation. On the other hand, mosses, one of the basal groups of land plants, form stem cells in both gametophyte and sporophyte generations. No genes responsible for the initiation and maintenance of pluripotent stem cells in the moss gametophyte generation have been reported, and the common mechanisms and evolution of pluripotent stem cell formation in land plants are still unrevealed. We showed that *AINTEGUMENTA/PLETHORA/BABY BOOM (APB)* orthologs *PpAPB*s (*PpAPB1*, *2*, *3*, and *4*) regulate the formation of stem cells in the haploid generation in the moss *Physcomitrella patens*. Quadruple disruption mutants did not form any gametophores, indicating that *APB*s regulate gametophytic pluripotent stem cell formation. These *APB*s are transcriptionally regulated by auxin, and synergistically function with cytokinin signaling. 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*. Investigation of the effects of all six gene deletions is currently being undertaken by Yuji Hiwatashi. He also participated in collaborative works with Drs. Kenichiro Hayashi (Okayama Science University), Kazuhiko Nishitani (Tohoku University), and Koji Mikami (Hokkaido University) on the characterization and evolutionary significance of *ent*-kaurene synthases, xyloglucan endotransglucosylase/hydrolases, and a phosphatidylinositol phosphate kinase in *P. patens*, respectively.

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, expression patterns of class 1 KNOX genes suggest that the active site of auxin signaling is localized to the initiation site of the branch. This work was mainly done by Yuji Hiwatashi.

V. 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.

One of the key factors of reprogramming is the change in the epigenomic profile. In the differentiated cells, a gene expression profile that fits to the cell function is stably maintained. This stable maintenance is performed through chromatin modifications such as trimethylation of histone H3 at lysine 27 (H3K27me3) for gene repression and H3K4me3 for gene activation. In contrast, in the pluripotent stem cells, most genes are ready to be activated. In animal pluripotent stem cells, many genes with H3K27me3 also have H3K4me3, 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 using *P. patens*. We found that in young protonemata, which contain many pluripotent stem cells, more than 6,000 genes are in the bivalent state, and there are only a small portion of genes that have H3K27me3 but not H3K4me3. This epigenomic profile is almost identical to that in animal pluripotent stem cells, and we thus revealed that the epigenomic profile of pluripotent stem cells is similar in plants and animals. This study is mainly conducted by Takaaki Ishikawa and Yosuke Tamada.

VI. 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, contributes to the pink body coloration of the orchid mantis. Integuments of the mantis have an absorption peak at 534 nm, which is different from that of oxidized (440 nm) and reduced forms (495 nm) of xanthommatin solubilized in a neutral buffer. On the other hand, it well agrees with an absorption peak of a reduced and precipitated form of xanthommatin (533 nm). These results suggest that the coloration of the orchid mantis is formed by the change of specific chemical states of a component common to other mantis. We also found that integuments of the orchid mantis contain a large amount of uric acid, which serves for white coloration in other insects such as the silkworm. These results indicate that it is possible this unique coloration of the orchid mantis is formed by the combination of usual pigments. This work was mainly done by Hiroaki Mano.

VII. 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 in 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.

VIII. 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.

IX. 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 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〕

- Hayashi, K., Horie, K., Hiwatashi, Y., Kawaide, H., Yamaguchi, S., Hanada, A., Nakashima, T., Nakajima, M., Mander, L.N., Yamane, H., Hasebe, M., and Nozaki, H. (2010). Endogenous diterpenes derived from ent-kaurene, a common gibberellin precursor, regulate protonema differentiation of the moss *Physcomitrella patens*. Plant Physiol. *153*, 1085-1097.
- Mikami, K., Saavedra, L., Hiwatashi, Y., Uji, T., Hasebe, M., and Sommarin, M. (2010). A dibasic amino acid pair conserved in the activation loop directs plasma membrane localization and is necessary for activity of plant type I/II phosphatidylinositol phosphate kinase. Plant Physiol. *153*, 1004-1015.
- Ohshima, I., Tanikawa-Dodo, Y., Saigusa, T., Nishiyama, T., Kitani, M., Hasebe, M., and Mohri, H. (2010). Phylogeny, biogeography, and host-plant association in the subfamily Apaturinae (Insecta: Lepidoptera: Nymphalidae) inferred from eight nuclear and seven mitochondrial genes. Mol. Phylogenet. Evol. *57*, 1026-1036.
- Yokoyama, R., Uwagaki, Y., Sasaki, H., Harada, T., Hiwatashi, Y., Hasebe, M., and Nishitani, K. (2010). Biological implications of the occurrence of 32 members of the XTH (xyloglucan endotransglucosylase/hydrolase) family of proteins in the bryophyte *Physcomitrella patens*. Plant J. *64*, 645-656.

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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. Long-distance control of nodulation

Legume plants develop root nodules to recruit nitrogen-fixing bacteria called rhizobia. This symbiotic relationship allows the host plants to grow even in nitrogen poor environments. Since nodule development is an energetically expensive process, the number of nodules must be tightly controlled by the host plants. For this purpose, legume plants utilize a long-distance signaling known as autoregulation of nodulation (AON). AON signaling in legumes has been extensively studied over decades but the underlying molecular mechanism has remained largely unclear. We are trying to unveil the mechanism for AON at the molecular level.

1-1 Identification of *KLAVIER* that mediates long-distance negative regulation of nodulation and non-symbiotic shoot development

The previously isolated *klavier* (*klv*) mutant defective in long-distance negative regulation of nodulation exhibits a hypernodulating phenotype. We identified *KLAVIER* (*KLV*) as a causative gene of the *klv* hypernodulating mutant. *KLV* encoded a novel leucine-rich repeat receptor-like kinase (LRR-RLK) and mediated the systemic negative regulation of nodulation in *Lotus japonicus*. In leaves, *KLV* was predominantly expressed in the vascular tissues similar to another LRR-RLK gene, *HAR1*, which also regulates nodule number. A double mutant exhibited no additive effect on hypernodulation. This result indicated that *KLV* and *HAR1* function in the same genetic pathway that governs the negative regulation of nodulation. LjCLE-RS1 and LjCLE-RS2 represent potential root-derived mobile signals for the HAR1-mediated systemic regulation of nodulation. Overexpression of *LjCLE-RS1* or *LjCLE-RS2* did not suppress the hypernodulation phenotype of *klv* mutants, indicating that *KLV* is required and acts downstream of *LjCLE-RS1* and *LjCLE-RS2*.

In addition to the role of *KLV* in symbiosis, complementation tests and expression analysis indicated that *KLV* plays multiple roles in shoot development (Figure 1), such as maintenance of shoot apical meristem, vascular continuity, shoot growth, and promotion of flowering.

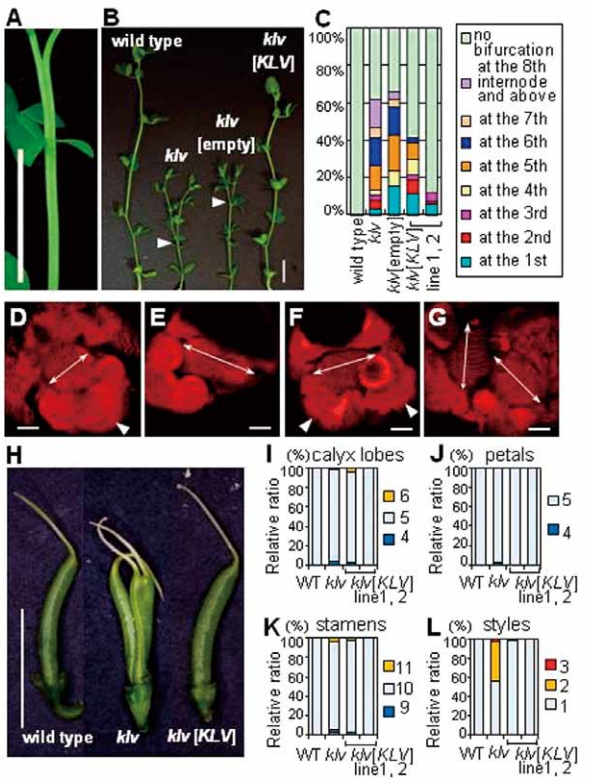

Figure 1. Morphological defects in the shoot apical meristem of *klv* and complementation of the *klv* bifurcation. (A) The bifurcated stem of a *klv* plant. (B) Shoot structures of uninoculated plants. *klv* [*KLV*] indicates a transgenic *klv* mutant introduced with the *KLV* gene, and *klv* [empty] indicates transgenic *klv* mutant with an empty vector. Each arrowhead indicates bifurcation of the stem. (C) Percentages of plants that show bifurcation. (D-G) Structures of SAMs in wild-type (D) and *klv* (E-G) plants at 4 DAG. Arrows indicate the SAM regions, and arrowheads indicate leaf primordia. (H) Close-up pictures of pistils. (I-L) Number of floral oragans.

Physical interaction analyses using transient expression in *Nicotiana benthamiana* revealed that KLV has the ability to interact with HAR1 and KLV itself. Together, these results suggest that the potential KLV-HAR1 receptor complex regulates symbiotic nodule development and that KLV is also a key component in other signal transduction pathways that mediate non-symbiotic shoot development.

1-2 *plenty*, a novel hypernodulation mutant

plenty is a novel hypernodulating mutant isolated by C^{6+} beam mutagenesis of the seeds of *L. japonicus* Miyakojima MG-20. A comparison of nodule numbers in *plenty* and wild type plants 3 weeks after inoculation with *Mesorhizobium loti* showed that the former had between 3 to 5 times more nodules. The relative range of the nodulation zone, defined as the length of the nodule-forming region to the length of the primary root, was approximately 4 times larger in the mutant than in the wild type. However, we never observed in *plenty* that nodules densely covered almost the entire root, as often seen in other hypernodulation mutants such as *har1*, *klv* or *tml*. Instead, *plenty* plants formed nodules on their lateral roots as well, while wild type plants suppressed the formation of nodules by autoregulation of nodulation (AON). Thus, it was suggested that the *plenty* mutant was defective in AON, though the magnitude of defects was not as great as in *har1*, *klv* or *tml*.

The *har1*, *klv* and *tml* mutant were reported that they formed a lot of small nodules. However, the size of the nodules was indistinguishable between wild type and *plenty* plants, even though the number of nodules increased in the *plenty* mutant. In contrast to the significant difference between wild type and *har1* plants, the *plenty* mutant did not differ from the wild type in the diameter of nodules and the nodule size distribution. Close-up pictures of nodules clearly indicate the size difference between the *plenty* mutant and other hypernodulation mutants (Fig. 2). This hypernodulation pattern of the *plenty* mutant was truly novel.

To locate the potential site of action of *PLENTY*, we conducted reciprocal grafting experiments with the wild type and *plenty* mutants. Grafting a *plenty* shoot onto a wild-type root led to nodulation in the wild-type; in contrast, grafting a wild-type shoot onto a *plenty* root resulted in an increased number of nodules, which was indistinguishable from that of *plenty* self-grafts. This root-determined hypernodulation of *plenty* indicates that unlike *HAR1* and *KLV*, *PLENTY* functions in the roots rather than in the shoots.

We further analyzed whether a root factor *PLENTY* and a shoot factor *HAR1* genetically interact with each other despite the different sites of action. We carried out reciprocal grafting using *plenty* and *har1-7* mutants. Shoot-regulated *har1-7* hypernodulation was obviously enhanced by grafting a *har1-7* shoot onto a *plenty* root, suggesting that *PLENTY* and *HAR1* might constitute the different AON signaling. This result was supported by *plenty*;*har1* double mutants that formed an additive number of nodules.

Based on these findings, PLENTY is presumed to be a root factor associated in an unknown AON pathway, whether it is local or shoot-mediated.

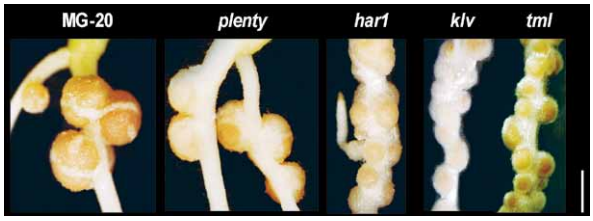

Figure 2. Close-up images of nodules. *L. japonicus* MG-20 (the wild type) and the *plenty* mutant are indistinguishable in the size of nodules. Other hypernodulation mutants form smaller nodules than MG-20 and the *plenty* mutant. Scale: 1mm.

II. Arbuscular mycorrhiza symbiosis

Mutualistic plant-fungal interaction; arbuscular mycorrhiza 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 molecules that conduct host-symbiont recognition were isolated from both host plant and AM fungi. These results are expected to accelerate molecular analysis of the AM signaling mechanism.

In the infection process of AM fungi, the host and the symbiont do not show obvious morphological changes, which makes it difficult to evaluate the development process of AM. In order to solve the problem, we established a molecular tool to facilitate the observation of AM fungal infection. We focused a protease gene *SbtM1* and a phosphate transporter gene *PT4*, that were highly induced during AM development and took advantage of them to established a molecular marker and visualize the infection process. These promoters and/or the open reading frames were fused with green fluorescence protein (GFP) gene or beta-glucuronidase (GUS) gene and these fusion constructs were introduced into *L. japonicus*. The transgenic plants would be good tools to observed infection process of AM fungi. We are screening AM mutants by genetic method to find novel AM signaling factors. We can analyze detailed phenotype of the symbiosis mutants and expect to find novel phenomena during AM development using these tools.

We are also conducting a screening of AM signaling factor with a different approach. Promoter analysis of *SbtM1* gene revealed AM response *cis* region in the promoter sequence. Using the *cis* sequence, we screened an AM response *trans* factor that controls transcription of AM induced genes by the yeast one hybrid system. Several candidate genes were isolated in this analysis. Reverse genetic approaches like TILLING and RNAi, and the *SbtM1* fusion constructs were used for selection of an AM response *trans* factor among the candidate genes.

III. Mathematical model of shoot apical meristem

The shoot apical meristem (SAM) of plants contains stem cells that have the ability to renew themselves and differentiate all aerial tissues such as stems and leaves. SAM

consists of a central zone (CZ) and its surrounding area named the peripheral zone (PZ) that is induced by an unknown signal from the CZ. Maintenance of the SAM essentially involves the interaction between WUS and CLV, in which WUS activates itself and CLV, and CLV inhibits WUS expression. Plants defective in *CLV* show enlarged SAM and stems with fasciation or dichotomous branching. In contrast, *wus* mutants generate a flattened SAM instead of the dome-shaped structure of the wild type, but produce many ectopic shoots across the SAM resulting in bushy plants.

Since it is not clarified how the SAM controls its proliferation and patterning, we constructed and analyzed a mathematical model that includes WUS-CLV feedback dynamics, spatial restriction of these dynamics, and area expansion by cell division. In numerical simulations, we can generate a phenotype similar to that of the wild-type plant, in which the SAM maintains a constant cell population because of the balance between cell proliferation by cell division and departure from the SAM to the outer region (Figure 3). In addition, numerical conditions corresponding to *CLV* defects result in a fasciated or dichotomously branched SAM. Furthermore, the defect in *WUS* causes proliferation of weak CZ spots. These numerical results are consistent with experimental observations in plants. Therefore, it is probable that this model captures the essence of SAM proliferation and patterning.

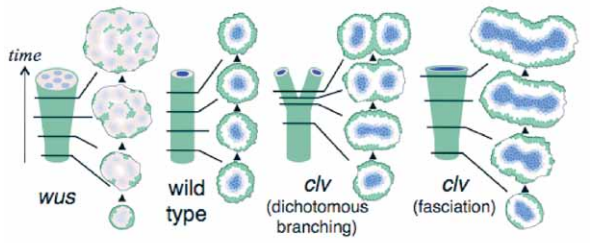

Figure 3. Numerical simulations under conditions of *wus* mutant, wild type, and *clv* mutants.

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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.

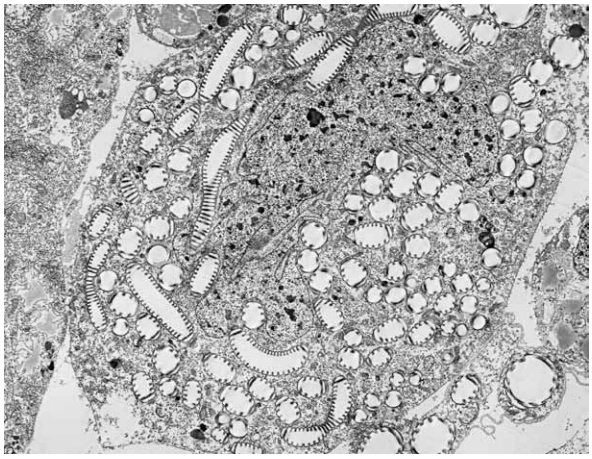

Figure 1. Transmission electron micrograph of the tracheole cell. Many cross-sections of the tracheoles can be observed within the cytoplasm of the tracheole cell. As the cell migrates, the tracheole is laid behind.

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.

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〔Original paper〕

● Kusaka, M., Katoh-Fukui, Y., Ogawa, H., Miyabayashi, K., Baba, T., Shima,Y., Sugiyama, N., Sugimoto, Y., Okuno, Y., Kodama, R., Iizuka-Kogo, A., Senda, T., Sasaoka, T., Kitamura, K., Aizawa, S., and Morohashi, K.-I. (2010). Abnormal epithelial cell polarity and ectopic epidermal growth factor receptor (EGFR) expression induced in Emx2 KO embryonic gonads. Endocrinology *151*, 5893-5904.

LABORATORY OF BIORESOURCES

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	HARA, Ikuyo
Secretary:	*SUZUKI, Tokiko*

Teleosts comprise about half of all vertebrate species and have adapted to a variety of environments, including sea-water, fresh water, the bottom of deep seas, small creeks and paddy fields. Analysis of their genome structure is important in order to understand the adaptation and diversification of this interesting group. 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 a comparative genomic analysis focusing mainly on fish chromosomes and gene evolution using medaka and other fishes, and identification of the causal gene of mutants for PGC migration. In addition to these activities, our laboratory is stepping forward to lead the National BioResource Project Medaka (NBRP Medaka).

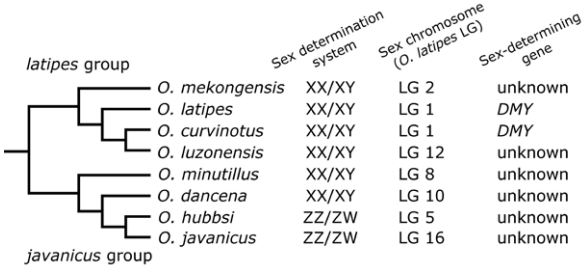

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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

The sex-determining gene *DMY* was identified on the Y chromosome of medaka, *Oryzias latipes*. However, this gene is absent in most *Oryzias* fishes, suggesting that closely related species have different sex-determining genes. We have recently demonstrated that, in the *javanicus* species group, *O. dancena* and *O. minutillus* have an XX/XY sex determination system, while *O. hubbsi* and *O. javanicus* have a ZZ/ZW system (Figure 1). Linkage analysis and FISH analysis showed that the sex chromosomes in these species were not homologous, suggesting independent origins of these sex chromosomes. Furthermore, *O. javanicus* and *O. hubbsi* have morphologically heteromorphic ZW sex chromosomes, in which the W chromosome has DAPI-positive heterochromatin. These findings suggest the repeated evolution of new sex chromosomes from autosomes in *Oryzias*, probably through the emergence of a new sex-determining gene.

II. Genetic dissection of migration of primordial germ cells in the 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 past 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. M-marker 2009, a new marker set for medaka mutant chromosome assign.

When the causal genes of mutants are cloned using candidate gene approaches or by chromosomal walking the first step involves assignment of the mutant to a specific chromosome. Bulked segregation analysis has been the most frequently used and effective method for assigning mutant loci to specific chromosomes. The bulk segregation analysis measures allele frequencies in pools of segregates that have been sorted according to phenotype. F2 wild type pool derived from two different strains has the same allele frequency throughout genome. However, because the mutant pool has only one allele in causal mutation there is a DNA marker which is derived solely from the mutant strain. Then we can assign the mutant phenotype to a specific chromosome locus by comparison of allele frequency of the pools. In medaka, the M-marker 2009 primer set, which consists of 48 PCR length polymorphism (PLP) markers (two markers per chromosome) have been used to map mutants using this method. M-marker 2009 markers were designed to amplify genomic regions containing insertion/deletion (in-del) polymorphisms between the Southern and Northern Japanese medaka strains that are most frequently used for position-based cloning of causal genes in mutants.

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

4-1 Establishment of core facility of NBRP 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). NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

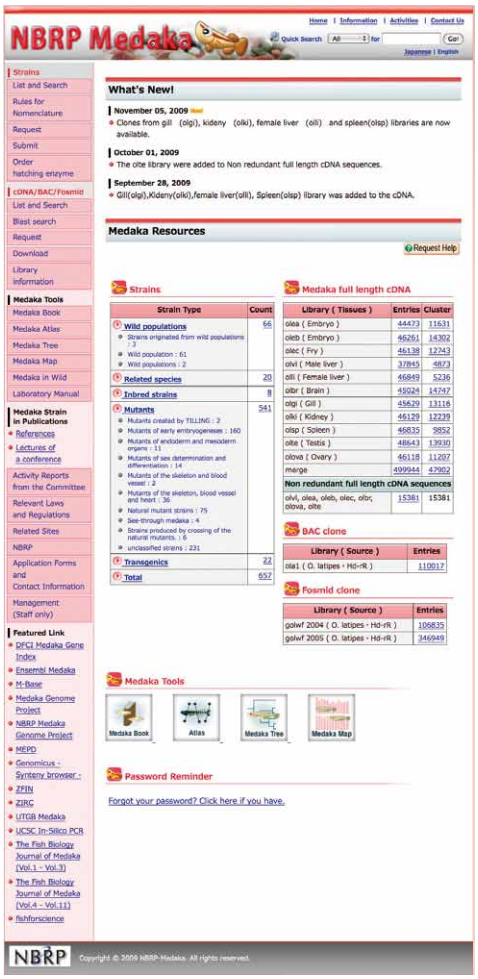

Figure 2. NBRP Medaka website

4-2 Establishment of polymorphism information of medaka inbred strains

One of the prominent characteristics of Medaka BioResources is the availability of inbred strains. These include several strain-specific characteristics such as vent of vertebrae during aging, acceleration of aging, shortened breeding duration, sensing of gravity, and maturation size etc. To analyze these strain-specific characteristics, the genome sequence data of each inbred line is crucial. The National Institute for Basic Biology and the National Institute of Genetics collaboratively conduct a genome sequencing project with a second generation sequencer for five representative inbred strains (Hd-rR-II1, HNI-II, Kaga, Nilan and HSOK) derived from Southern Japanese, Northern Japanese, China-West Korean and East Korean populations respectively. This data promotes quantitative trait loci (QTL) analysis using the inbred strains and facilitate the development of human disease models using the medaka system. In addition, the Ilumina GA sequence data from the Hd-rR-II1 strain enhance the reference genome sequence of medaka. Overall, this program brings medaka forward to a new level as a model system.

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LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrient is indispensable for cells to survive. Thus, the transmission of nutrient signals is also important. Tor (target of rapamycin) protein plays a central role in controlling cell growth in response to nutritional environments. Tor protein forms distinct Tor complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as translation initiation, ribosome biogenesis, and autophagy. On the other hand, TORC2, whose function is insensitive to rapamycin, is responsible for actin organization and cell integrity.

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.

I. Cell cycle at G2/M is regulated by TORC1.

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.

II. 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 (Figure 1).

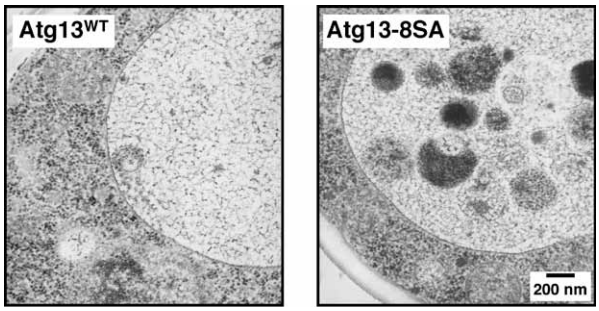

Figure 1. Induction of autophagy by expression of unphosphorylatable form of Atg13 (Atg13-8SA). Images of electron microscopy of $Atg13^{WT}$ (left) and Atg13-8SA (right) expressing cell.

III. TORC2 phosphorylates Ypk2 kinase 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 TORC2 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〕

- Kamada, Y., Yoshino, K., Kondo, C., Kawamata, T., Oshiro, N., Yonezawa, K., and Ohsumi, Y. (2010). Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol. Cell. Biol. *30*, 1049-1058.

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

- Yoshida, S., Imoto, J., Minato, T., Oouchi, R., Kamada, Y., Tomita, M., Soga, T., and Yoshimoto, H. A novel mechanism regulates H_2S and SO_2 production in *Saccharomyces cerevisiae*. Yeast. 2010 Oct 8.

〔Review articles〕

- Kamada, Y. (2010). Prime-numbered Atg proteins act at the primary step in autophagy. –Unphosphrylated Atg13 can induce autophagy without TOR inactivation. Autophagy *6*, 415-416.
- Kamada, Y., and Ohsumi, Y. (2010). The TOR-mediated regulation of autophagy in the yeast *Saccharomyces cerevisiae*. The Enzymes XXVIII, F. Tamanoi and M.N. Hall, eds. (Academic Press, Elsevier), pp. 143-165.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor:	*OHNO, Kaoru*
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	FUKAZAWA, Mitsue
	INAGAKI, 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).

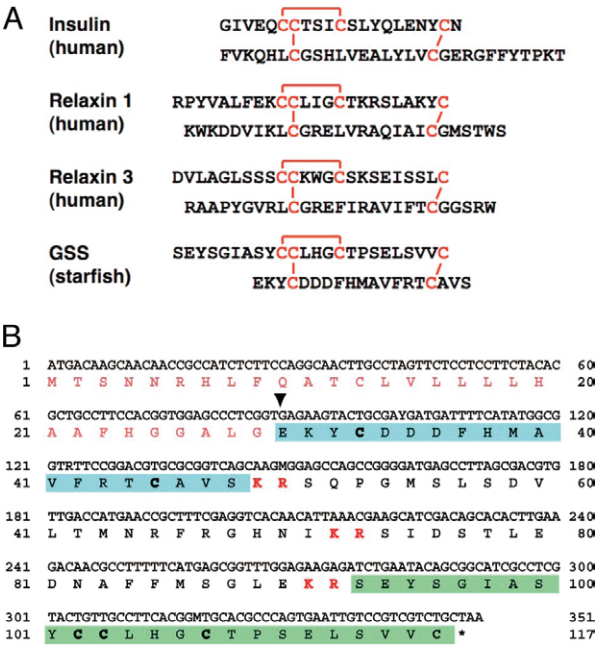


Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

Publication List

〔Original paper〕

- Fujiwara, A., Unuma, T., Ohno, K., and Yamano, K. (2010). Molecular characterization of the major yolk protein of the Japanese common sea cucumber (*Apostichopus japonicus*) and its expression profile during ovarian development. Comp. Biochem. Physiol. A Mol. Integr. Physiol. *155*, 34-40.

LABORATORY OF BIOLOGICAL DIVERSITY

TERADA Group

Assistant Professor: *TERADA, Rie*
Postdoctoral Fellow: *SHIMATANI, Zenpei*

Gene targeting (GT) mediated by homologous recombination (HR) is the most effective tool for generation of mutant plants for both studies of molecular genetics and molecular breeding. Since the first success of our *Waxy* GT in rice (*Oryza sativa* L.) based on a strong positive-negative (PN) selection, we have modified 15 individual rice genes into various forms, not only gene knock-out but also knock-in and a single nucleotide substitution at the target gene locus by our GT method.

I. Generation of blast fungus resistant rice by GT of *OsRac1*.

As an attempt to apply GT to molecular breeding, we have modified rice *OsRac1* to be constitutively active (CA) by amino acid substitution of the 19th glycine to valine (G19V). OsRac1, a homolog of mammalian Rac GTPase, plays an important role in the defense response of plants. Its GTPase activity is increased by G19V substitution through a single point mutation of G to T in the first exon of *OsRac1*. As shown in Figure 1A the point mutation was inserted into *OsRac1* by GT with vectors of pOsRac1A or pOsRac1B. Elimination of the positive marker, *hygromycin phospho transferase* (*hpt*) from targeted *OsRac1* by the Cre-*loxP* system created CA-*OsRac1* at the natural gene locus. Although single *loxP* remains in the first or third intron, it is expected to splice out. We have obtained 5 and 11 of the expected true GT (TGT) callus lines by pOsRac1A and pOsRac1B, respectively. G to T substitution was detected by sequence analysis in all 5 TGT lines by pOsRac1A and in 6 of 11 TGT lines by pOsRac1B, respectively. The substitution efficiency was 100% in TGT by pOsRac1A but was about 45% in TGT by pOsRac1B. Distance between the point mutation and *hpt* positive marker in the vector homology arm is thought to be an important factor for insertion efficiency of point mutation. This result was quite different from the GT at *Alcohol dehydrogenase* gene 2 (*Adh2*) where point mutations near to both ends of the vector homology arms were effectively integrated. Subsequently TGT callus lines were applied to Cre mediated *hpt* elimination. From both TGT with pOsRac1A and with pOsRac1B, each of the two expected marker-free lines were obtained. These 4 callus lines were regenerated to plants and are under analyses for fungal resistance.

II. Insertion of visual markers as fusion genes with *OsMADS* by GT.

Several markers have been developed for visualization of gene functions. We are progressing with visualization of *OsMADS* expression using knock-in targeting with visual markers for the study of flower development. *OsMADS* is a homolog of the *APETALA1* (*AP1*) gene and is predicted to work in the flower meristem under the Florigen Activation Complex, FAC, with Hd3a-14-3-3-OsFD1. We are inserting coding sequences of visual markers, luciferase and m-Orange, into the 3' TAG downstream of *OsMADS* as fusion proteins using our GT method. We constructed two knock-in targeting vectors named as p15L and p15Or (Figure 1B). We obtained 6 TGT lines having luciferase gene that connected to the coding sequence of *OsMADS* (Figure 1B, Table 1). We are now progressing with knock-in GT of the p15Or vector. These works are in collaboration with Professor Ko Shimamoto in the Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology (NAIST) and are supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan Grant-in-Aid for Scientific Research (S) (No. 19108005 to K.S.)

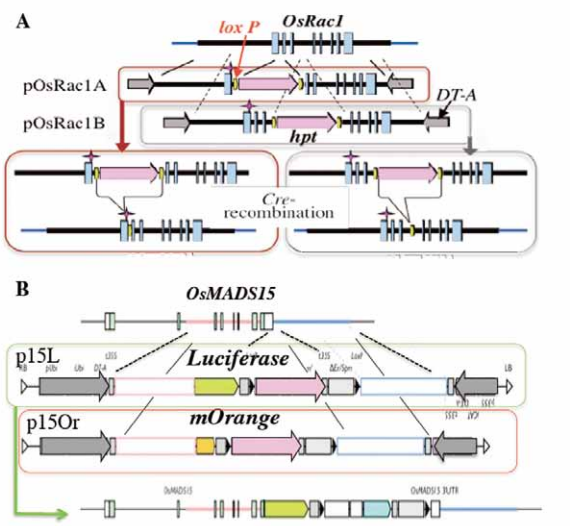


Figure 1. Generation of CA-*OsRac1 and* visualization of *OsMADS* by GT mediated gene modifications.

Targeted gene	PN selected Calli	TGT 5'+3'	Ratio of TGT/PN (%)
OsRac1 by pRac1A	94	5	5.5
OsRac1 by pRac1B	80	11	13.6
OsMADS by p15L	169	6	3.6

Table 1. GT of *OsRac1* and *OsMADS*

Publication List

〔Original paper〕

● Terada, R., Nagahara M., Furukawa K., Shimamoto, M., Yamaguchi, K., and Iida S. (2010). Cre-*loxP* mediated marker elimination and gene reactivation at the *waxy* locus created in rice genome based on strong positive-negative selection. Plant Biotechnology *27*, 29-37.

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

Assistant Professor: *HOSHINO, Atsushi*
Technical Assistant: *WATANABE, Seiko*

While genomic structures as well as their genetic information appear to transmit stably 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. 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.

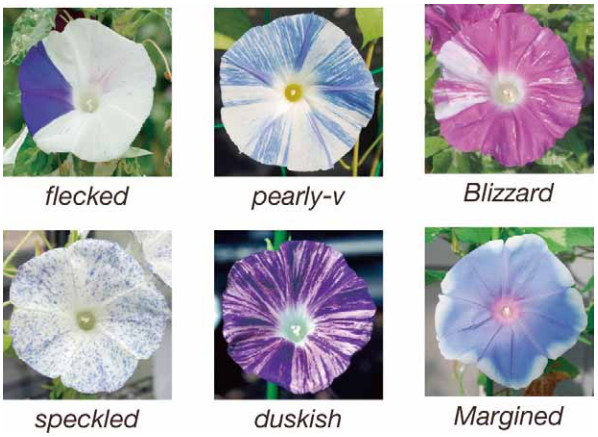


Figure 1. Flower phenotypes of Japanese morning glories.

II. Flower pigmentation patterns

Figure 1 represents examples of such mutants showing particular flower pigmentation patterns. Based on the molecular mechanisms conferring the particular patterns, these mutants can be classified into three groups. The first group includes the *flecked* and *speckled* mutants of *I. nil* that bloom variegated flowers with pigmented spots and sectors on whitish backgrounds. These mutations are caused by the insertions of certain groups of DNA transposons into the genes for flower pigmentation. Recurrent somatic mutations due to transposon excision from the genes result in pigmented spots and sectors on white backgrounds. In the second group, the *pearly-v* mutant of *I. tricolor* and the *duskish* mutant of *I. nil* also have variegated flowers, and epigenetic mechanisms are thought to regulate flower pigmentation. While the mutations in the two groups mentioned above are recessive, *Margined* and *Blizzard* of *I. nil* are dominant mutations. *Blizzard* and *Margined* mutants bloom pigmented corolla with irregular whitish spots and white edges, respectively. It was suggested that non-coding small RNA represses the expression of a pigmentation gene in the whitish parts of the corolla. We are currently characterizing detailed molecular mechanisms of the mutations in the latter two groups.

III. *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 started *de novo* genome sequencing of *I. nil* using high-throughput DNA sequencers. *I. nil* has a genome of about 800 Mbp, and we chose the Tokyo-kokei standard line for genome sequencing. We are collaborating with several laboratories of the National Institute for Genetics and Kyushu University.

IV. 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 increased to 200 lines and 117,000 clones by the end of 2010.

Figure 2. Breeding field and greenhouse for morning glory.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

Assistant Professor: TSUGANE, Kazuo

Although DNA transposons are one of the major components of plant genomes, their transposition is restricted genetically or epigenetically for genome stability. Because insertions of transposons have contributed to the creation of new genes and genome evolution, revealing the genome dynamisms driven by DNA transposons is the purpose of our research.

I. Transposition and target preferences of *nDart1* in rice genome

Gene tagging is a powerful tool for elucidating the function of rice genes, and foreign elements, such as T-DNA or maize DNA transposons *Ac*/*Ds* and *En*/*Spm*, and the endogenous retrotransposon *Tos17* have been systematically employed. A potential obstacle to these insertional mutants may be the concomitant occurrence of somaclonal variation associated with tissue cultures because tissue cultures are necessary to either introduce these foreign elements into rice calli or to activate dormant *Tos17* in the genome. While endogenous active DNA transposons, which are free from somaclonal variation because no tissue culture is involved in generating insertion mutants, have been extensively used for gene tagging in maize, snapdragon, petunia, and morning glories, only a few active endogenous DNA transposons, *mPing*, *nDart1*, *dTok*, and *nDaiZ* have been identified in rice. Of these active nonautonomous DNA elements, *nDart1* appears to be more suitable than the others for transposon tagging in rice because their transposition can be controlled under natural growth conditions, *i.e.*, the transposition of *nDart1* can be induced by crossing with a line containing an active autonomous *aDart* element and stabilized by segregating *aDart*. We have constructed mutant rice lines using DNA transposons in order to achieve functional genomics analysis in rice, a model plant for monocots and cereals.

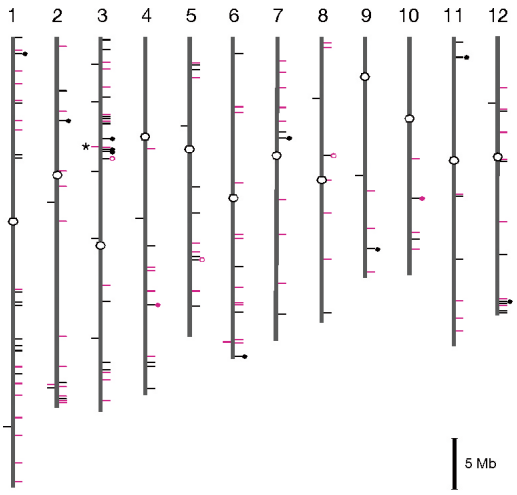


Figure 1. Localization of *nDart1-3* subgroup elements inserted in the genome of the *virescent* mutant and its progeny. The pink and black bars indicate *nDart1-0* and other *nDart1-3* subgroup elements, determined by comparing the results of nDart-TD.

Originally identified *nDart1-0* was found from the mutable *virescent* line as an insertion into the *OsClpP5* gene, *nDart1-0* related elements, and *nDart1-3* subgroups were also identified. In the sequenced Nipponbare genome, most of the *nDart1-3*-related elements reside in GC-rich regions, and no apparent consensus sequence is found in their insertion sites, including 8-bp target site duplications (TSDs). Since the GC content of genes (45%), especially exon (54%), is known to be higher than that of intergenic regions (43%) in rice, we can anticipate that the *nDart1*-related elements are likely to be inserted into genic regions. To assess whether *nDart1* is indeed suitable for gene tagging in rice, we have examined the transposition activities and target specificities of *nDart1* in a rice line containing an active autonomous *aDart1* element. We employed an amplified fragment length polymorphism (AFLP)-based transposon display (TD), by which we were able to visualize approximately 90% of the anticipated bands produced from the *nDart1*-related elements in the Nipponbare genome. Newly transposed *nDart1-3* subgroup elements were found to be integrated into various sites almost all over the genome (Figure1), and more than 60% of these sites were genic regions comprising putative coding and/or intron regions and their 0.5-kb 5'-upstream and 3'-downstream regions. Moreover, approximately two-thirds of these genic insertions were confined in the 0.5-kb 5'-upstream region (Figure2). These results are discussed with respect to the development of an efficient and somaclonal variation-free gene tagging system for rice functional genomics.

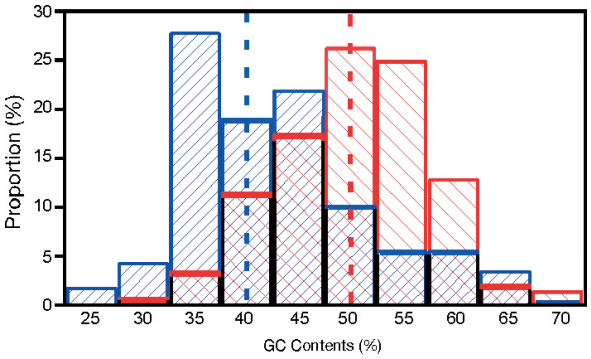


Figure 2. Average GC contents of both flanking 0.5-kb regions of the newly inserted the *nDart1-3* subgroup elements in progeny lines of mutable *virescent* rice. Red and blue histograms represent the GC contents of regions identified by *nDart1* insertions and 240 randomly selected control regions, respectively. The vertical broken lines indicate the intermediated value.

Publication List

〔Original paper〕

● Takagi, K., Maekawa, M., Y., Tsugane, K., and Iida, S. (2010). Transposition and target preferences of an active nonautonomous DNA transposon *nDart1* and its relatives belonging to the *hAT* superfamily in rice. Mol. Gen. Genomics *284*, 343-355.

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.

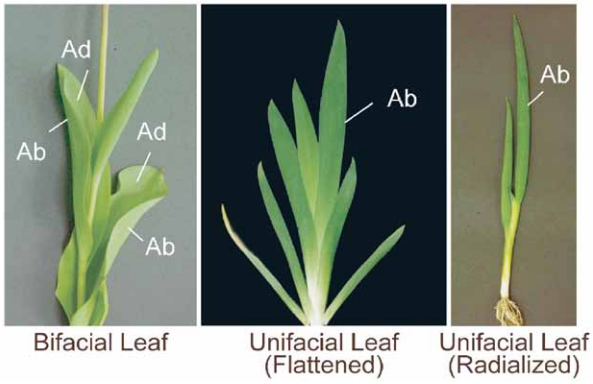


Figure 1. Bifacial and unifacial leaf structures. Ad, Adaxial side; Ab. Abaxial side.

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 (Yamaguchi and Tsukaya, 2010). 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 reveled that dominant abaxial activity leads to the unifacial leaf development (Yamaguchi et al., 2010).

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 that flattens 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, Yamaguchi et al., 2010).

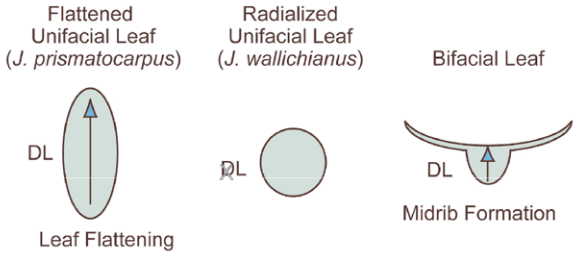


Figure 2. Mechanism of leaf blade flattening in unifacial leaves.

Publication List

〔Original papers〕

- Nakayama, H., Yamaguchi, T., and Tsukaya, H. (2010). Expression patterns of *AaDL*, a *CRABS CLAW* ortholog in *Asparagus asparagoides* (Asparagaceae), demonstrate a stepwise evolution of *CRC/DL* subfamily *YABBY* genes. Amer. J. Bot. *97*, 591–600.
- Nelissen, H., De Groeve, S., Fleury, D., Neyt, P., Bruno, L., Bitonti, M.B., Vandenbussche, F., Van Der Straeten, D., Yamaguchi, T., Tsukaya, H., Witters, E., De Jaeger, G., Houben, A., and Van Lijsebettens, M. (2010). Plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation. Proc. Natl. Acad. Sci. USA *107*, 1678-1683.
- Toriba, T., Suzaki, T., Yamaguchi, T., Ohmori, Y., Tsukaya, H., and Hirano, H. (2010). Distinct regulation of adaxial-abaxial polarity in anther patterning in rice. Plant Cell *22*, 1452–1462.
- Yamaguchi, T., Yano, S., and Tsukaya, H. (2010). Genetic framework for flattened leaf blade formation in unifacial leaves of *Juncus prismatocarpus*. Plant Cell *22*, 2141–2155.

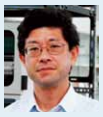
〔Original paper (E-publication ahead of print)〕

- Ikeuchi, M., Yamaguchi, T., Kazama, T., Ito, T., Horiguchi, G., and Tsukaya, H. ROTUNDIFOLIA4 regulates cell proliferation along the body axis in Arabidopsis shoot. Plant Cell Physiol. 2010 Sep 8.

〔Review article〕

- Yamaguchi, T., and Tsukaya, H. (2010). Evolutionary and developmental studies of unifacial leaves in monocots: *Juncus* as a model system. J. Plant Research. *123*, 35–41.

Division of Molecular Environmental Endocrinology



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IGUCHI, Taisen

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WATANABE, Hajime

Assistant Professors: *MIYAGAWA, Shinichi*
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Visiting Scientists: *HIRAKAWA, Ikumi*
TOHYAMA, Saki
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HAYASHI, Tomoko
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. In 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.

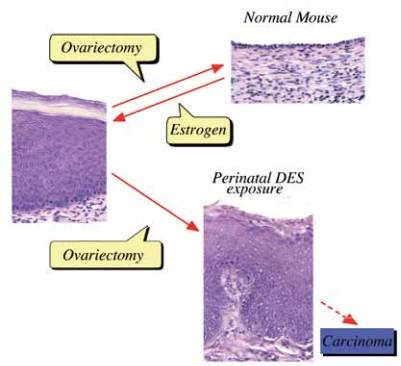

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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 diethylstilbestrol (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 estrogen receptor α (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.

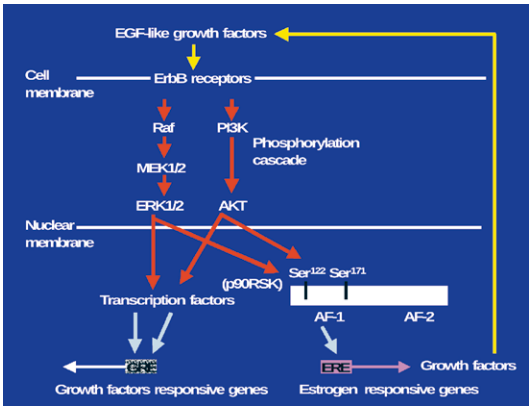

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

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.

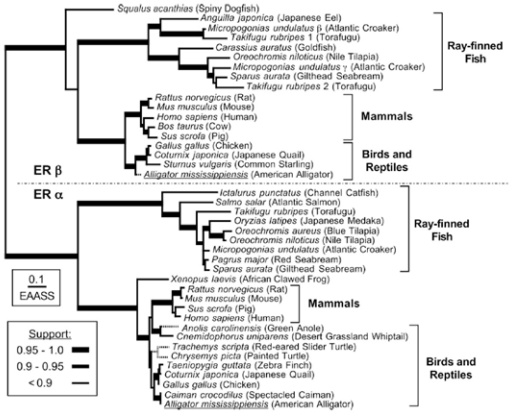

Figure 3. Evolutionary relationships of estrogen receptor sequences.

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

Vertebrates show diverse sexual characters 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 classical androgens as ligands, emerged before the Chondrichthyes-Osteichthyes split and *AR* gene was duplicated during a teleost-specific gene duplication event.

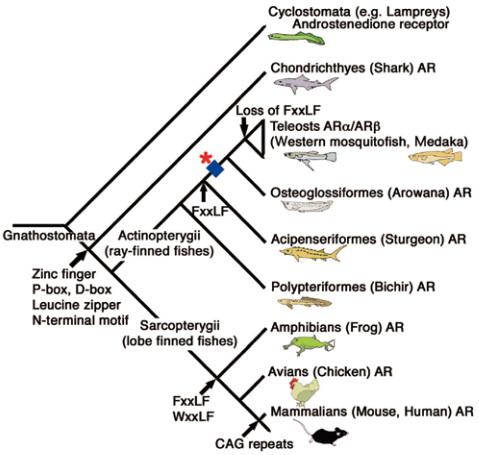

Figure 4. Evolutionary relationships of androgen receptor sequences.

IV. Male production by juvenile hormones 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 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

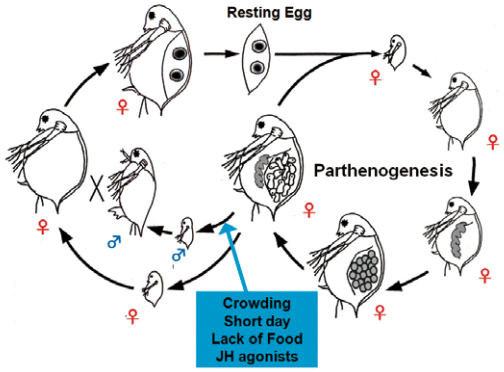

Figure 5. A life cycle of *Daphnia*.

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 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,* 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.

V. 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 Calorina, San Diego Zoo, USA, and the Asa Zoo in Hiroshima.

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Division of Environmental Photobiology

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MINAGAWA, Jun

Postdoctoral Fellow: *TAKIZAWA, Kenji*
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 studies of model green algae 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.

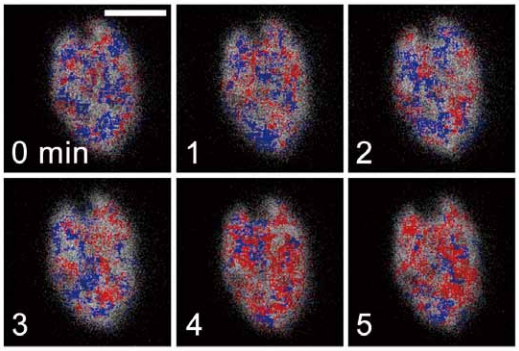

Figure 1. Visualization of the progress of a state 1-to-2 transition by means of FLIM. Blue and red dots correspond to 170 and 250 psec lifetime components, respectively.

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 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.

This year, 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.

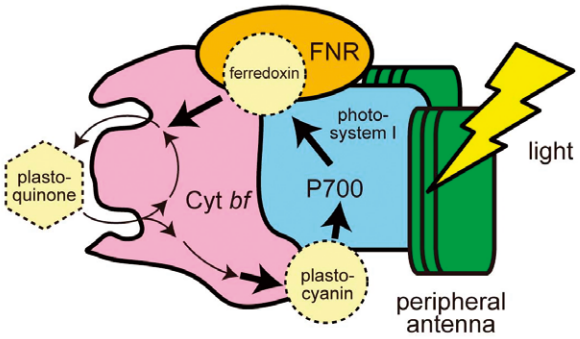

Figure 2. Cyclic electron flow by way of the CEF supercomplex.

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.

This year, 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.

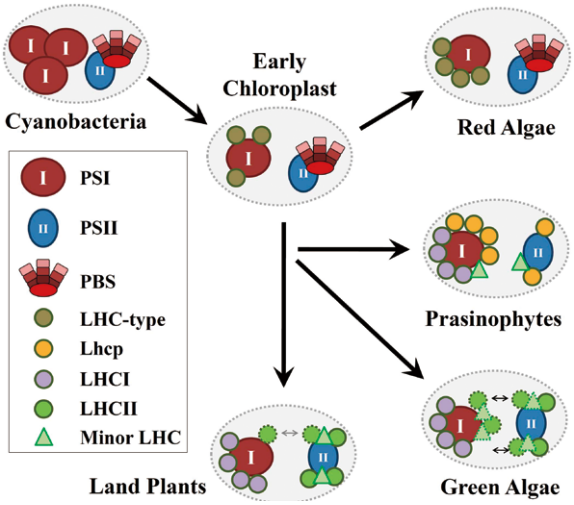

Figure 3. Evolutionary model of LHC affinity in photosynthetic eukaryotes as revealed by biochemical study of the LHC systems in *O. tauri*.

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DIVISION OF PLANT DEVELOPMENTAL GENETICS (ADJUNCT) †

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The leaf is the fundamental unit of the shoot system, which is composed of the leaf and stem. The diversity of plant forms is mostly attributable to variation of leaf and floral organs, which are modified leaves. Moreover, leaf shape is sensitive to environmental stimuli. The leaf is therefore the key organ for a full understanding of plant morphogenesis. The genetic control of the development of leaf shapes, however, has remained unclear. Recently, studies of leaf morphogenesis reached a turning point after our successful application of the techniques of developmental and molecular genetics using the model plant *Arabidopsis thaliana* (L.) Heynh. (Tsukaya 2008).

I. Mechanisms of leaf development

Focusing on the mechanisms that govern the polarized growth of leaves in *Arabidopsis thaliana,* we have identified four genes for polar-dependent growth of leaf lamina: the *ANGUSTIFOLIA* (*AN*) and *AN3* genes, which regulate the width of leaves, and the *ROTUNDIFOLIA3* (*ROT3*) and *ROT4* genes, which regulate the length of leaves. *AN* and *ROT3* genes control cell shape while *AN3* and *ROT4* genes regulate cell numbers in leaves. In addition to polar-dependent leaf shape control, we have focused on the mechanisms of organ-wide control of leaf size, which are reflected in the 'compensation' phenomenon (reviewed in Tsukaya 2008). Additionally, the accumulation of knowledge on the basic mechanisms of leaf shape control has enabled us to conduct Evo/Devo studies of the mechanisms behind leaf-shape diversity. Below is an overview of our research activities and achievements during 2010.

1-1 Polar growth of leaves in *A. thaliana*

ROT4 is a member of the *RTFL/DVL* gene family that encodes peptides. Overexpression of ROT4 is known to cause stunted leaves due to decrease of number of cells in the lamina. In addition, we found that pedicels and secondary inflorescences bend abnormally by the constitutive over-expression of ROT4 peptide in Arabidopsis. Detailed examination of the morphology revealed that the bending was caused by an abnormal protrusion of the inflorescence stem, suggesting that ROT4 might be somehow involved in positional value determination (Ikeuchi et al. 2011). This idea is further supported by analyses of chimeric expression of ROT4 in the lamina: sectors over-expressing ROT4 showed altered positioning of leaf blade/leaf petiole boundary. We also identified an important domain in the ROT4 peptide by a series of deletion experiments (Ikeuchi et al. 2011).

1-2 Evolution of establishment mechanisms of leaf polarities in monocots

We have recently started to attempt an understanding of the genetic basis of the development of unifacial leaves that are known only from monocot clades. Our analyses indicated that the unifacial character might be due to overall changes in all polarities. Understanding the differences in the genetic mechanisms for the establishment of unifacial and normal bifacial leaves will provide good clues as to how leaf-shape is diversified.

For such purposes, comparative molecular-genetic and anatomical analyses between unifacial and bifacial leaf development have been undertaken using members of the genus *Juncus* (Yamaguchi and Tsukaya, 2010). Interestingly, molecular characterization of unifacial leaves of *Juncus* revealed that they have only abaxial identity in the leaf blades, lack leaf margins, and possess flattened leaf lamina. This finding is very surprising, because laminar growth occurs at the adaxial-abaxial junction in bifacial leaves such as Arabidopsis and snap dragon.

Detailed analyses of *Juncus* species revealed that the flattened leaf lamina in the unifacial leaves in *Juncus* is, at least in part, dependent on function of the *DL* gene that acts to thicken the lamina (Yamaguchi et al. 2010). We also isolated several interesting mutants of *Juncus* that exhibit abnormalities in leaf polarity have already been isolated.

1-3 Size control of leaves and mechanisms of compensation

We recently showed that the meristematic region in the leaf primordia is spatially and temporally regulated (Kazama et al. 2010) and *SPT* controls the size of the meristematic region (Ichihashi et al., 2010). Leaf size depends on not only meristematic activity but also the cell enlargement process that follows. How are cell proliferation and cell enlargement coordinated in leaf morphogenesis?

To answer this, a new tool for studies of the coordination system, heat-shock-inducible, chimeric expression systems of *KRP2* or *AN3,* was established. Using this (Figure 1), we found that *an3*-dependent compensation is a non-cell-autonomous process, and that *an3* cells seem to generate and transmit an intercellular signal that enhances post-mitotic cell expansion. This is the first proof of an organ-level, cell non-autonomous integration system between cell proliferation and cell expansion.

In addition, we revealed genetic pathways of leaf serration (Kawamura et al. 2010); an unexpected role of *AN3* on dorsoventral identification in leaves (Horiguchi et al. 2011); and brassinosteroid- and auxin- related regulation of shade avoidance syndrome in leaves (Kozuka et al. 2010). We also

†: This laboratory was closed on 31 March, 2010.

Figure 1. AN3 leaf chimera. Green signal represents *AN3::3xGFP* cells while the other areas are composed of *an3* mutant cells. Modified from Kawade et al. (2010).

found that plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation (Nelissen et al., 2010).

II. Biodiversity in plants

This year we reported two new species from Asian countries: *Pipthospata repens* and *Phaius hekouensis* (Okada and Tsukaya 2010; Tsukaya et al. 2010).

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- Tsukaya, H. (2010). Leaf development and Evolution. J. Plant Res. *123*, 3-6.
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Laboratory of Photoenvironmental Biology †

Professor (Adjunct)
WATANABE, Masakatsu

Photosynthetic microorganisms, such as cyanobacteria and flagellate algae, respond to light in order to locate themselves in appropriate photoenvironments. Our research is aimed at the elucidation of the photoreceptive and signal transduction mechanisms of light responses in microorganisms. This approach has led us to the discovery, characterization, and application of a remarkably unique light sensor molecule as described below.

I. Photoactivated Adenylyl Cyclase (PAC), an algal photoreceptor protein with intrinsic effector function to produce cAMP

In 2002, we found a novel blue-light receptor with an effector role in *Euglena* (Iseki *et al*., Nature *415*, 1047-1051, 2002): *Euglena gracilis*, a unicellular flagellate, which shows blue-light type photomovements (Figure 1). The action spectra indicate the involvement of flavoproteins as the photoreceptors mediate them. The paraflagellar body (PFB), a swelling near the base of the flagellum, is thought to be a photosensing organelle responsible for photomovements. To identify the photoreceptors in the PFB, we isolated PFBs and purified the flavoproteins therein. The purified flavoprotein (ca. 400 kDa), with noncovalently bound FAD, seemed to be a heterotetramer of α- and β-subunits. Predicted amino acid sequences for each of the subunits were similar to each other and contained two FAD-binding domains (BLUF: sensor of blue light using FAD) (F1 and F2) each followed by an adenylyl cyclase catalytic domain (C1 and C2). The flavoprotein showed adenylyl cyclase activity, which was elevated by blue-light irradiation. Thus, the flavoprotein (PAC: photoactivated adenylyl cyclase) can directly transduce a light signal into a change in the intracellular cAMP level without any other signal transduction proteins (Figure 2).

Figure 1. *Euglena gracilis*, a unicellular flagellate alga. It swims forward (to the left) by shaking its flagellum, the protruding whip-like structure. Flagellar motion is controlled by ultraviolet to blue light signals sensed by the photoreceptor molecules in the "real eye" located adjacently to the basal part of the flagellum, so that the cell can locate itself in appropriate light environments for its survival. The orange, so-called, "eyespot" is not the "real eye" but a light shade enabling the cell to recognize the light's direction.

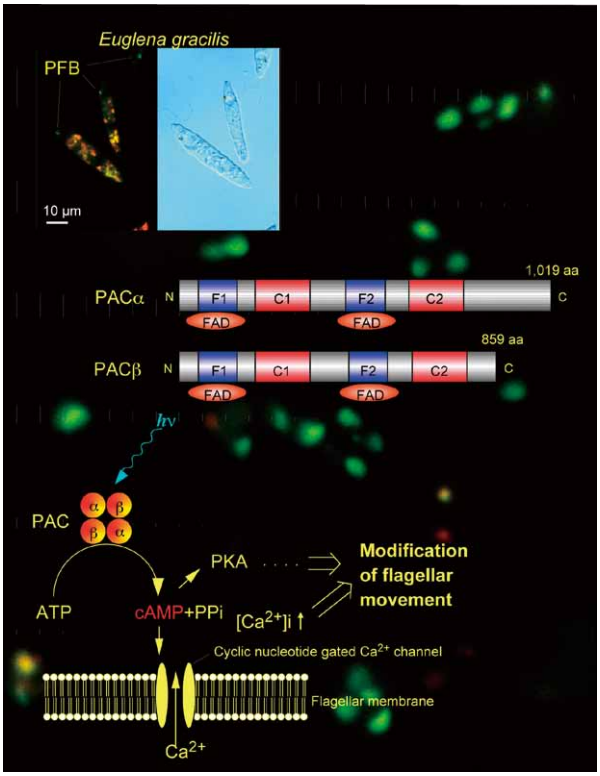

Figure 2. Domain structure of Photoactivated Adenylyl Cyclase (PAC) and its two possible action mechanisms to mediate photoavoidance behavior in *Euglena gracilis*. The green spots in the background are fluorescence microscopical images of isolated paraflagellar bodies (PFBs), the "real eyes".

A unique function such as this is best suited not only for the rapid control of the flagellar motion of the *Euglena* cell but also for a variety of biotechnological photocontrol of cAMP-controlled biological functions, including neuronal functions and developmental processes in a variety of organisms in which PAC can be heterologously expressed. For example, in collaboration with Max-Planck-Institut für Biophysik (Frankfurt) and other German groups, expression of PAC in cells was performed, which allowed the manipulation of cAMP with exquisite spatiotemporal control. We functionally expressed PACs in two popular expression systems, *Xenopus laevis* oocytes and HEK293 cells. Moreover, transgenic *Drosophila melanogaster* flies demonstrated functional PAC expression by showing blue light–induced behavioral changes (Schröder-Lang, S. *et al*., Nat. Meth. *4*, 39-42, 2007)

II. Differentiation of sensitivity of the photoreceptive flavin-binding domains of α- and β-subunits of PAC

In our previous report, we demonstrated that a recombinant version of the PACαF2 domain displays blue light-induced photocycle similar to those of prokaryotic BLUFs (Ito *et al*., 2005, Photochem. Photobiol. Sci., 4, 762-769). Here, we further examine the recombinant PACβF2 domain, which

†: This laboratory was closed on 31 March, 2010.

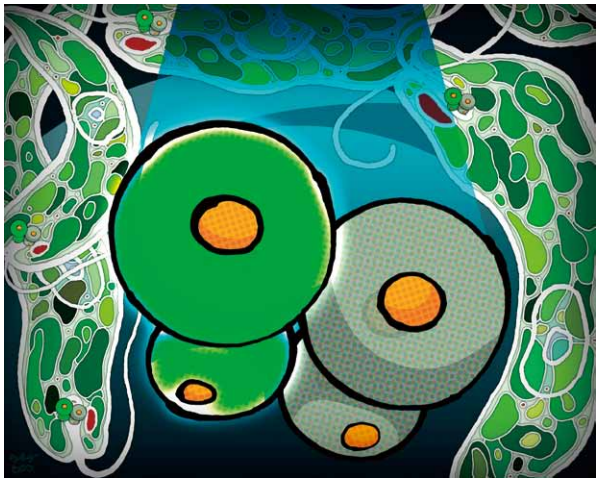

Figure 3. A schematic illustration of the putative $\alpha_2\beta_2$ heterotetrameric structure (center) of the PAC molecule of *Euglena* cells (periphery).

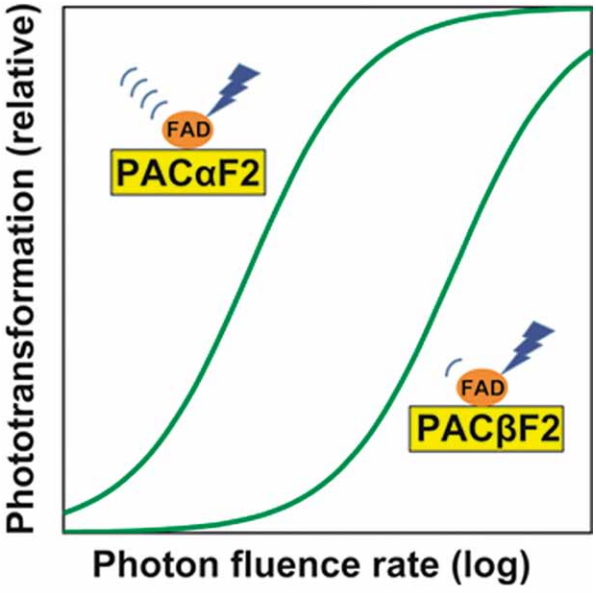

Figure 4. A schematic illustration of the differentiated light sensitivities between the homologous flavin-binding domains of the α and the β subunits of the PAC molecule. The former is estimated to be about 20 times more sensitive than the latter.

like PACαF2, exhibits blue light-induced photocycle. The estimated quantum efficiency for the phototransformation of PACβF2 was 0.06–0.08, and the half-life for dark relaxation was 3–6 s while the corresponding values for the PACαF2 were 0.28–0.32 and 34–44s. The remarkable differences between PACαF2 and PACβF2 may be related to the sensitivity of the photoactivation. In PACαF2, amino acid position 556, which is equivalent to Trp104 in the BLUF domain of the purple bacterial AppA protein, is occupied by a Leu residue, while in PACβF2, the equivalent BLUF domain site is conserved as Trp560. Amino acid substitution at this site in PACβF2-Trp560Lue markedly increased the estimated quantum efficiency (0.23) and accelerated the half-life of the dark-relaxation (2s). These results indicate that Trp560 in PACβF2 plays a main role in suppressing quantum efficiency.

Publication List

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LABORATORY OF GENOME INFORMATICS

Assistant Professor
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Postdoctoral Fellow: *KAWAI, Mikihiko*

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 these 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. The latter feature is especially useful when the user's interest is focused on some taxonomically related organisms.

We have continued to develop and enhance the database functionality. Each orthologous group entry is assigned functional annotation and external database links that are a summarization of the information assigned to the individual genes belonging to that group. Phenotypic properties of each genome are stored and can be used for specifying a set of genomes for phylogenetic pattern analysis. MyMBGD mode, which allows users to add their own genomes to MBGD, accepts raw genomic sequences without any annotation. The database now contains well over 1000 published genomes including 23 eukaryotic microbes and 4 multicellular organisms.

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

II. Enhancement of the algorithm for identifying 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 combine closely related genome comparison with distantly related genome comparison, the algorithm accepts ingroup/outgroup specification for each input genome, and considers taxonomic information partially during ortholog grouping. The resulting table has a nested structure when a duplication event occurs within the ingroup lineage. To improve the scalability of the algorithm for comparison of thousands of genomic sequences, we have developed an efficient method to update the clustering result incrementally. This method is especially useful for a user who wants to compare his/her own original genomes with the published genomes available in the MBGD server, where the orthologous relationship among the published genomes has already been calculated and is available.

We are also extending the algorithm for handling metagenomic data. In contrast to usual comparative genome analyses, in metagenomic analysis, the source organism of each metagenomic sequence is not known; instead, taxonomic position of the source organism of each metagenomic sequence should be inferred. For this purpose, we have extended the DomClust algorithm to infer taxonomic position for each metagenomic sequence by mapping 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. For example, “Neighborhood gene clustering” identifies a set of genes that are located in the vicinity of each other in both the ortholog table and the genomic sequence, and assigns the same color to each group. “Phylogenetic pattern clustering” performs hierarchical cluster analysis based on the dissimilarity between phylogenetic patterns, and reorders the ortholog table according to the clustering result. 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.

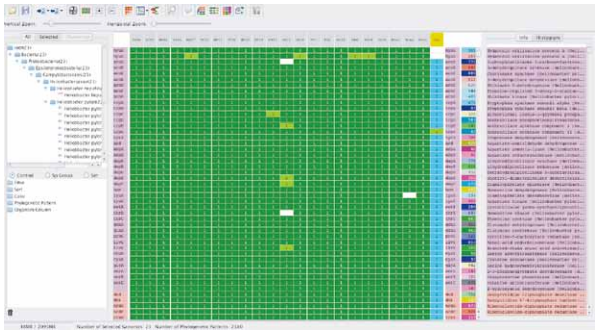


Figure 1. The RECOG client program showing orthologous relationships among 22 *Helicobacter pylori* strains including four Japanese strains determined in our project. *Helicobacter hepaticus* is also added as an outgroup species in the rightmost column.

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*. As a result, we were able to identify several genes that characterize the East Asian strains. For example, almost all of the molybdenum-related genes, which are related to the catalysis of two-electron redox reactions, are disrupted specifically in the East Asian strains (Figure 2).

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.

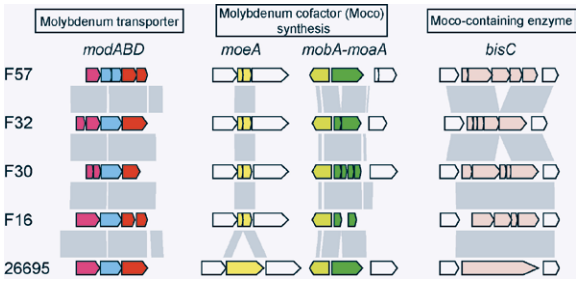


Figure 2. Systematic disruption of the molybdenum-related genes among the Japanese strains (F57, F32, F30, F16). The strain 26695 is a European strain, which has intact genes.

Publication List

〔Original paper〕

● Uchiyama, I., Higuchi, T., and Kawai, M. (2010). MBGD update 2010: toward a comprehensive resource for exploring microbial genome diversity. Nucleic Acids Res. *38*, D361-D365.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

Associate Professor
NONAKA, Shigenori

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

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).

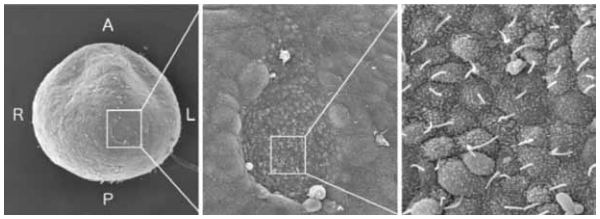


Figure 1. Left: ventral view of a 7.5-day mouse embryo. Middle: the node. Right: node cilia.

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.

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 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 are also developing another light-sheet microscope for wider application of living samples.

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.

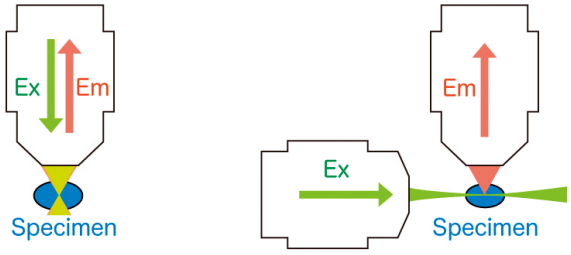


Figure 2. Principle of light-sheet microscopy. Left: light path of conventional fluorescent microscopes using single objective lens for both illumination (Ex) and detection (Em). Right: light-sheet microscopes including DSLM, where illumination light is limited to the focal plane of the detection objective.

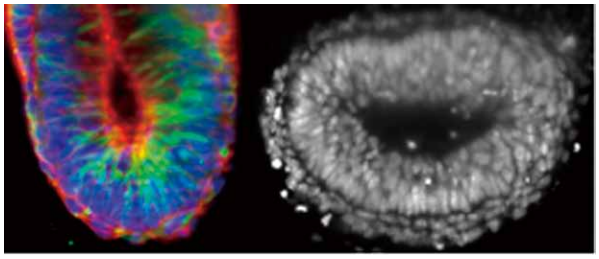


Figure 3. 6.5-day mouse embryos visualized by DSLM. Left: an optical transverse section of a fixed embryo. Right: cross section of a living one expressing GFP in the nuclei.

Publication List

〔Original papers〕

- Hashimoto, M., Shinohara, K., Wang, J., Ikeuchi, S., Yoshiba, S., Meno, C., Nonaka, S., Takada, S., Hatta, K., Wynshaw-Boris, A., and Hamada, H. (2010). Planar polarization of node cells determines the rotational axis of node cilia. Nat. Cell Biol. *12*, 170-176.
- Hirota, Y., Meunier, A., Huang, S., Shimozawa, T., Yamada, O., Kida, Y.S., Inoue, M., Ito, T., Kato, H., Sakaguchi, M., Sunabori, T., Nakaya, M., Nonaka, S., Ogura, T., Higuchi, H., Okano, H., Spassky, N., and Sawamoto, K. (2010). Planar polarity of multiciliated ependymal cells involves the anterior migration of basal bodies regulated by non-muscle myosin II. Development *137*, 3037-3046.

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
	TANIGUCHI-SAIDA, Misako
Technical Assistant:	*ASAO, Hisayo*
Secretary:	*ICHIKAWA, Mariko*

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

Representative Instruments

Genomics

The advent of next-generation sequencing technologies is transforming today's biology by ultra-high-throughput DNA sequencing. Utilizing the SOLiD 4 System (Applied Biosystems), the Functional Genomics Facility is committed to joint research aiming to exploring otherwise inaccessible new fields in basic biology. SOLiD 4 can output data up to 100 G/run.

During 2010 we started 14 next-generation sequencing 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 human) 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.

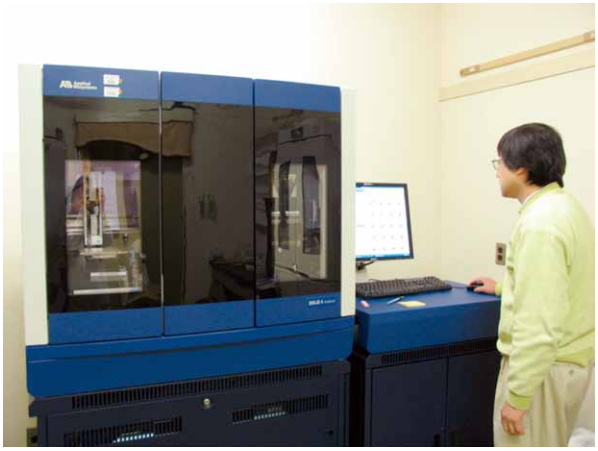


Figure 1. Next-generation sequencer SOLiD 4

Proteomics

Three different types of mass spectrometers and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2010, we analyzed approximately 400 samples by mass spectrometers and 80 samples by 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

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

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

Technical Assistants: *HASHIYAMA, Tomomi*
SUZUKI, Miyuzu
Visiting Scientists: *GALLOT, Aurore*
SRINIVASAN, Dayalan

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 muticellular 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 an 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.

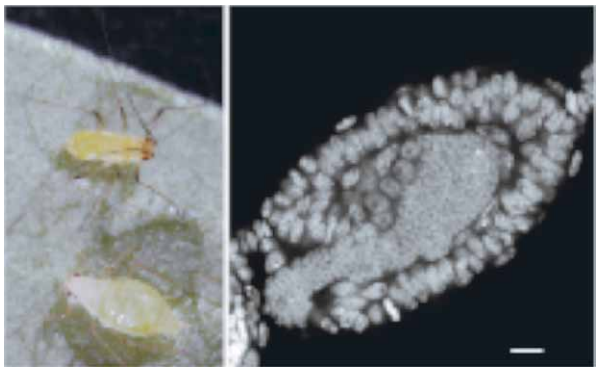


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo in which symbionts are infecting (Right). Scale bar = 20um.

Publication List

〔Original papers〕

- Huang, T.-Y, Cook, C.E., Davis, G.K., Shigenobu, S., Chen, R. P.-Y., and Chang, C.-C. (2010). Anterior development in the parthenogenetic and viviparous form of the pea aphid, *Acyrthosiphon pisum*: hunchback and orthodenticle expression. Insect Mol. Biol. *19*, 75-85.
- Legeai, F., Shigenobu, S., Gauthier, J., Colbourne, J., Rispe, C., Collin, O., Richards, R., Wilson, A., and Tagu, D. (2010). AphidBase: A centralized bioinformatic resource for annotation of the pea aphid genome. Insect Mol. Biol. *19*, 5-12.
- Nakabachi, A., Shigenobu, S., and Miyagishima, S. (2010). Chitinase-like proteins encoded in the genome of the pea aphid, *Acyrthosiphon pisum*. Insect Mol. Biol. *19*, 175-185.
- Niwa, R., Namiki, T., Ito, K., Shimada-Niwa, Y., Kiuchi, M., Kawaoka, S., Kayukawa, T., Banno, Y., Fujimoto, Y., Shigenobu, S., Kobayashi, S., Shimada, T., Katsuma, S., and Shinoda, T. (2010). *Non-molting glossy/shroud* encodes a short-chain dehydrogenase/reductase that functions in the "Black Box" of the ecdysteroid biosynthesis pathway. Development *137*, 1991-1999.
- Price, D.R.G., Tibbles, K., Shigenobu, S., Smertenko, A., Russel, C.W., Douglas, A.E., Fitches, E., Gatehouse, A.M.R., and Gatehouse, J.A. (2010). Sugar transporters of the major facilitator superfamiliy in aphids; from gene prediction to fucntional characterization. Insect Mol. Biol. *19*, 97-112.
- Shigenobu, S., Bickel, R.D., Brisson, J.A., Butts, T., Chang, C., Christiaens, O., Davis, G.K., Duncan, E.J., Ferrier, D.E.K., Iga, M., Janssen, R., Lin, G., Lu, H., McGregor, A.P., Miura, T., Smagghe, G. Smith, J.M., van der Zee, M., Velarde, R., Wilson, M.J., Dearden, P.K., and Stern, D.L. (2010). Comprehensive survey of developmental genes in the pea aphid, *Acyrthosiphon pisum*: frequent lineage-specific duplications and losses of developmental genes. Insect Mol. Biol. *19*, 47-62.
- Shigenobu, S., Richards, S., Cree, A.G., Morioka, M., Fukatsu, T., Kudo, T., Miyagishima, S., Gibbs, R.A., Stern, D.L., and Nakabachi, A. (2010). A full-length cDNA resource for the pea aphid, *Acyrthosiphon pisum*. Insect Mol. Biol. *19*, 23-32.
- The International Aphid Genomics Consortium. Genome Sequence of the Pea Aphid *Acyrthosiphon pisum*. PLoS Biol. *8*, e1000313.

Spectrography and Bioimaging Facility

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

Technical Staff: *HIGASHI, Sho-ichi*
TANIGUCHI-SAIDA, Misako
Technical Assistants: *ENDOU, Seiichiro*
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.

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) 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 last April.

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 5 Individual Collaborative Research projects, including applications for higher plants and small fish.

Publication List on Collaboration

〔Original papers〕

- Arimoto-Kobayashi, S., Sano, K., Machida, M., Kaji, K., and Yakushi, K. (2010). UVA activation of N-dialkylnitrosamines releasing nitric oxide, producing strand breaks as well as oxidative damages in DNA, and inducing mutations in the Ames test. Mut. Res. *691*, 47-54.
- Hayashi, Y., Kobira, H., Yamaguchi, T., Shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y., and Kitano, T. (2010). High temperature causes masculinization of genetically female medaka by elevation of cortisol level. Mol. Reprod. Dev. *77*, 679-686.

● *Research activity by Y. Kamei*

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

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.

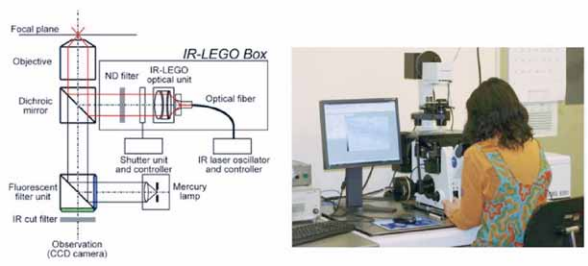


Figure 1. Infrared laser evoked gene operator (IR-LEGO) microscope system at NIBB

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.

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). On the other hand, the heated area was limited to a small volume about as large as a typical cell.

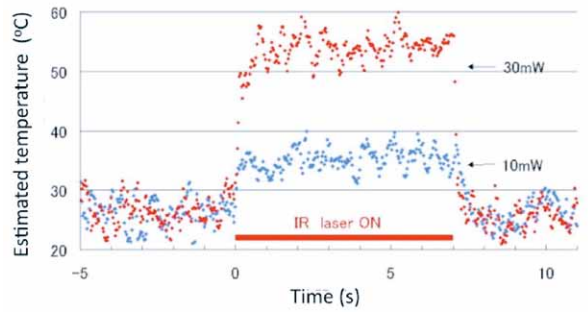


Figure 2. Time course of temperature at IR-laser focus
IR laser was irradiated to EGFP expressing bacterium by IR-LEGO microscope. Fluorescent intensity change was used to estimate temperature shift. Temperature was raised by laser irradiation and kept at a constant level during the irradiation.

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).

Studies for 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. Applying to a mutant and its rescue transgenic strain; hsp-cre and rescue gene which is sandwiched by loxP sequence, we will achieve single-cell knockout experiments in living organism, 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.

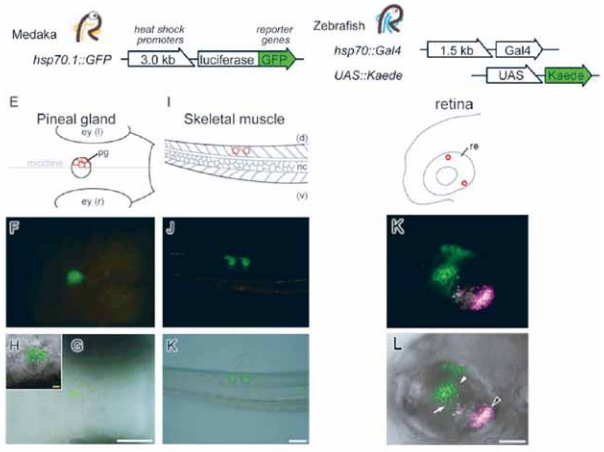


Figure 3. Local gene expression in fish by IR-LEGO
GFPs were expressed in various tissue in medaka and zebrafish. IR-LEGO can induce local gene expression by heat shock response in many organisms.

Publication List

〔Original papers〕

- Hayashi, Y., Kobira, H., Yamaguchi, T., Shiraishi, E., Yazawa, T., Hirai, T., Kamei, Y., and Kitano, T. (2010). High temperature causes masculinization of genetically female medaka by elevation of cortisol level. Mol. Reprod. Dev. *77*, 679-686.
- Ishikawa, T.#, Kamei Y.#, Otozai, S., Kim, J., Sato, A., Kuwahara, Y., Tanaka, M., Deguchi, T., Inohara, H., Tsujimura, T., and Todo, T. (2010). High-resolution melting curve analysis for rapid detection of mutations in a Medaka TILLING library. BMC Mol. Biol. *11*, 70. (#: equally contribution)
- Oda, S., Mikami, S., Urushihara, Y., Murata, Y., Kamei, Y., Deguchi, T., Kitano, T., Fujimori, K.E., Yuba, S., Todo, T., and Mitani, H. (2010). Identification of a functional Medaka heat shock promoter and characterization of its ability to induce in vitro and in vivo exogenous gene expression in Medaka. Zool. Sci. *27*, 410-415.

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

The 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.

Figure 1. Biological Information Analysis System

Most of the PCs in each laboratory, as well as all of the above-mentioned service machines, are connected by a local area network, which is linked to the high performance backbone network ORION connecting the three research institutes in Okazaki. Many local services, including sequence analysis services, file sharing services, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (http://www.nibb.ac.jp/).

Research activity by I. Uchiyama

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p.71).

NIBB BioResource Center

Head
IGUCHI, Taisen

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

Professor(Concurrent)	*SASAOKA, Toshikuni*
Associate Professors:	*WATANABE, Eiji*
	*SASAOKA, Toshikuni**
	TANAKA, Minoru
	NARUSE, Kiyoshi
Technical Staff:	*HAYASHI, Kohji*
	NOGUCHI, Yuji
Postdoctoral Fellow:	*YAMANAKA, Megumi*
Technical Assistants:	*KAWAMURA, Motofumi*
	INADA, Yosuke
	KAJIWARA, Yuya
	MATSUMURA, Kunihiro
	ICHIKAWA, Yoko
	TAKAGI, Yukari
	SUGINAGA, Tomomi

Figure 1. The center facility for transgenic animals 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 analyzemutant, 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. Technical staff washing cages for mice

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

In 2010 (from January 1 to December 31) 4,475 mice and 310 fertilized eggs were brought into the facility in the Yamate area, and 42,005 mice (including pups bred in the facility) and 40 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 2010 (from January 1 to December 31) 142 mice were brought into the facility in the Myodaiji area, and 2,129 mice (including pups bred in the facility) were taken out.

Figure 3. Breeding equipment for mice in transgenic studies using recombinant viruses

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 opitimal conditions, removing biohazard risk. 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 five mutant lines and over ten 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 2010 (from January 1 to the date as of December 21), 4,170 medaka and zebrafish (330 eggs, 1,310 embryos and 2530 adults) were brought to the facility and 53,077 medaka and zebrafish (51,698 fertilized eggs, 180 embryos and 1,199 adults, including animals bred in the facility) were taken out. In the laboratory for chick embryos 2,230 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 2010 we shipped 191 independent medaka strains, 349 cDNA/BAC/Fosmid clones, and 305 amples of hatching enzyme to the scientific community worldwide.

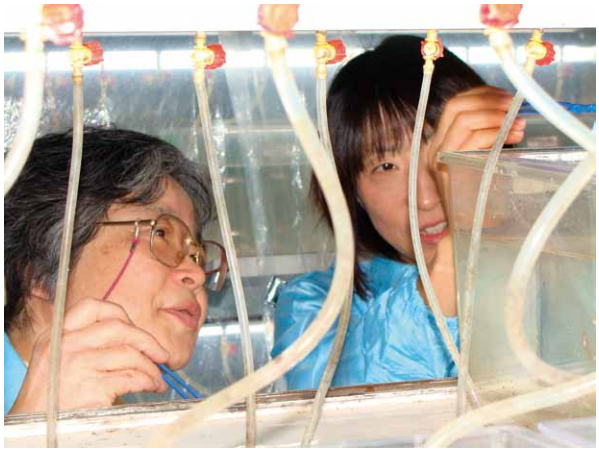


Figure 4. Technical and lab staff taking care of fish tanks.

III. Research activities

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

Model Plant Research Facility

● ***Plant Culture Laboratory***

Technical Staff: *KAJIURA-KOBAYASHI, Hiroko*
NANBA, Chieko
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 other several 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 50 researchers of 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 equipped. 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, a pumpkin *Cucurbita maxima*, 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.

Figure 5. A green house with air-conditioning was built in 2010 and mainly used for cultivation of *Lotus japonicus*.

● ***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 National BioResource Project (NBRP) Morning glory, and collaborates with the core organization center, Kyushu University. Morning glories are grown and propagated using the facilities and the equipment in the NIBB BioResource Center. We collected a new BAC library consisting of 27,648 clones and 6 mutant lines, updated the EST database, and provided 6 DNA clones and 6 mutant lines to both local and foreign biologists this year.

Research activity by the assistant professor A. Hoshino is shown in the laboratory page (p.59).

Cell Biology Research Facility

Assistant Professor: *HAMADA, Yoshio*
Technical Assistants: *SUGINAGA, Tomomi*
TAKESHITA, Miyako

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 activity by the assistant professor Y. Hamada, the principal investigator of the Laboratory of Cell Sociology, is shown in the laboratory page (p.14).

Figure 6. Technical staff operating a biosafety cabinet.

Center for Radioisotope Facilities (Okazaki Research 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 2010 to December 2010 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 Figure 1 and Table 2. The annual changes of registrants and the number of totals per year are shown in Figure 2.

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

The Myodaiji-Area consists of two controlled area, CFBI (Common Facilities Building I)-branch and LGER (Laboratory of Gene Expression and Regulation)-branch. Since LGER-branch users decreased over the years, it was closed on August 31st in accordance with the law (Figure 3). The CFBI-branch was renamed to the Myodaiji-area of CRF.

	Myodaiji-Area	Yamate-Area
registrants	114	77
users	61	34

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2010

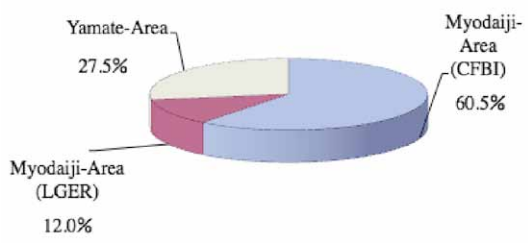


Figure 1. Percentage of users going in and out of each controlled area in 2010

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch		
users	2086	347	889	3322
visitors	190	105	143	438
total	2276	452	1032	3760

Table 2. Users and visitors who entered each controlled area in 2010

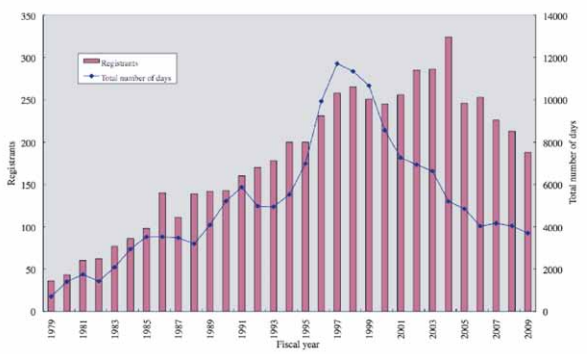


Figure 2. Annual changes of registrants and number of totals per fiscal year

	Myodaiji-Area		Yamate-Area	total
	CFBI-branch	LGER-branch*		
^{125}I Received	37000	–	1130	38130
^{125}I Used	1850	–	1221	3071
^{45}Ca Received	0	0	–	0
^{45}Ca Used	0	0	–	0
^{35}S Received	1073000	0	0	1073000
^{35}S Used	1164036	0	0	1164036
^{32}P Received	1310000	0	248750	1558750
^{32}P Used	1318000	0	170990	1488990
^{14}C Received	277870	0	0	277870
^{14}C Used	173857	0	0	173857
^{3}H Received	666250	0	2812000	3478250
^{3}H Used	122943	0	2319604	2442547
^{22}Na Received	3700	–	–	3700
^{22}Na Used	2979	–	–	2979

*LGER was closed at August 2010.

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2010

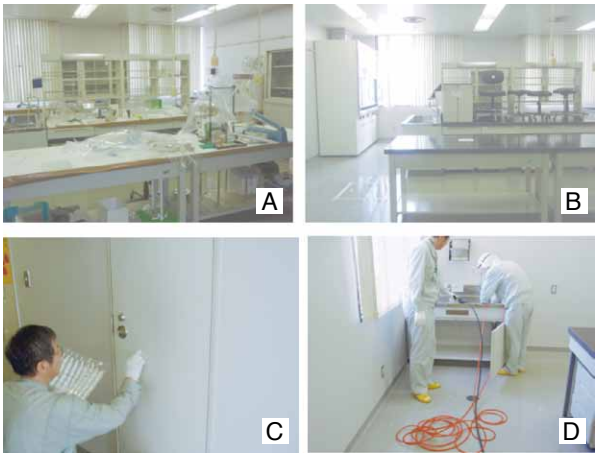


Figure 3. Cleaning controlled area, LGER-branch, for closing
A: Before cleaning of room No.G603
B: After cleaning of room No.G603
C: Checking radiation contamination
D: Cleaning the drainpipe

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 international cooperation and public relations, however those aspects of the department became the "Office of Public Relations and International Cooperation" in April 2009.

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 and International Cooperation)

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 AND INTERNATIONAL COOPERATION

Chair(Public Relations)
FUJIMORI, Toshihiko
Assistant Professor (Specially appointed):
Technical Assistants:

Chair(International Cooperation)
YOSHIDA, Shosei

KURATA, Tomoko
OTA, Misaki
ADACHI, Shoko
TAKAHASHI Ritsue
OTA, Kyoko
KAWAGUCHI, Colin

The Office of Public Relations and International Cooperation, 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 Office of Public Relations and International Cooperation also manages the planning and administration of the international symposiums and international training courses held at NIBB.

The main activities of the office in 2010

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) Supporting international conferences

On-line registration, web page construction, brochure editing, hotel reservation, banquet and party arrangement, photography and video recording, etc., for international conferences held at NIBB (Table 1)

3) Management of education related programs

The Fifth International Practical Course (January and February, 2010)

4) Updating and maintenance of the NIBB web page

5) Editing of publications, production of posters and leaflets

Design and distribution of posters for international conferences and advertisements for the graduate school's entrance examination. 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).

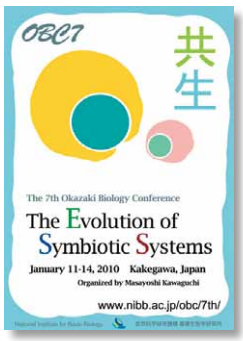
6) Organization of scientific outreach programs

Planning NIBB open house (October, 2010), Summer program for high school students (August, 2010), etc.

7) Aiding visitors (in collaboration with the Technical Division)

Name	*Date*	*Title*	*Organizer*
7th Okazaki Biology Conference	January 11-14	The Evolution of Symbiotic Systems	M. Kawaguchi J. Lake
56th NIBB Conference	March 12-14	Neocortical Organization	T. Yamamori
57th NIBB Conference	October 14-16	The Dynamic Genome	T. Horiuchi
2nd NIBB-MPIPZ joint symposium	November 16-18	Plant Science Communication 2010	M. Kawaguchi M. Hasebe K. Okada

Table 1. International conferences managed by the Office of Public Relations and International Cooperation in 2010



7th Okazaki Biology Conference

56th NIBB Conference

Summer program for students

2nd NIBB-MPIPZ joint symposium

Open Campus 2010

Figure 1. Examples of posters, pamphlets and abstract books produced by the Office of Public Relations and International Cooperation in 2010

TECHNICAL DIVISION

Head
FURUKAWA, Kazuhiko

Common Facility Group

Chief: *MIWA, Tomoki*

● ***NIBB Core Research Facilities***

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

● ***NIBB Bioresource Center***

Subunit Chief:	*HAYASHI, Kohji*
	NANBA, Chieko
Technical Staff:	*NOGUCHI, Yuji*
Technical Assistants:	*ICHIKAWA, Yoko*
	TAKAGI, Yukari
	SUZUKI, Keiko
	TAKESHITA, Miyako

● ***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*
Technical Staff:	*MOROOKA, Naoki*

● ***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.

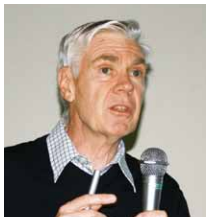
The Technical Division hosts an annual meeting for technical engineers who work in various fields of biology at universities and research institutes throughout Japan. At this meeting, the participants present their own activities and discuss technical problems. The proceedings are published after each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

The Seventh Okazaki Biology Conference "The Evolution of Symbiotic Systems"

Organizer: Masayoshi Kawaguchi (NIBB)	Co-Organizer: James Lake (UCLA)
January 11 (Mon)-14 (Thu), 2010	

Symbiosis refers to close and often long-term interactions between species. These interactions involve dynamic changes in the genomes, metabolisms, and signaling networks of the symbiotic partners. A unified understanding of these interactions is required when studying symbiotic organisms. To emphasize the enormous variety of symbiotic consortia and the underlying commonalities that relate these systems, the 7th Okazaki Biology Conference (OBC) on "The Evolution of Symbiotic Systems", was held in January at YAMAHA Resort Tsumagoi, Kakegawa. At the conference we had 35 participants, including 13 researchers from abroad. Following a plenary lecture by James Lake, a co-organizer of the conference, we had 31 research presentations that included 3 talks on newly developing fields given by young scientists. The topics were fantastically diverse from an early prokaryotic endosymbiosis, the evolution of plastids -including the apicoplast of parasitic human malaria, diversity of cultivable and uncultivable endosymbionts, partner shifts in bacterial and mycorrhizal symbioses, interdependent genomes that generate evolutionary novelty, marine symbioses from reef-building corals to crabs that live on sea-floor hydrothermal deposits, plant-pollinator interactions, insect-microbe interactions in aphids and termites, plant-microbe interactions using model legumes, and artificial symbiotic systems. Artificial systems composed of a small set of living organisms looked very promising because they can reduce complexity and provide a platform to address many important questions about natural systems, for example, what are the potential origins of symbioses? What determines the persistence of symbioses? Participants very much enjoyed the discussions and the opportunity to interact with researchers from different fields of symbiosis study. After the conference, six topics from among these various subjects were introduced as a Multi-author Review in the international journal Cellular and Molecular Life Sciences.

One of the important aspects of symbiosis is the generation of novel adaptive traits trough cooperation. Working together, symbiotic organisms can sometimes accomplish biological feats that neither can achieve alone. We hope that this conference will trigger new integrated symbiosis research that achieves what none of the single aspects can achieve alone.



Organizer and Co-organizer

Speakers

Arnold, Michael L. (Univ. Georgia), Baldwin, Ian T. (Max Planck Inst. Chem. Ecol.), Bordenstein, Seth R. (Vanderbilt Univ.), Delwiche, Charles (Univ. Maryland), Engel, Annette Summers (Louisiana State Univ.), Lake, James (UCLA), Norris, Dale M. (Univ. Wisconsin), Reddick, Lovett Evan (Univ. Tennessee), Rumpho, Mary E. (Univ. Maine), Sato, Shigeharu (MRC Natl. Inst. Med. Res.), Shou, Wenying (Fred Hutchinson Cancer Res. Center), Weis, Virginia M. (Oregon State Univ.)
Fukatsu, Takema (AIST), Hayashi, Makoto (Natl. Inst. Agrobiol. Sci. (NIAS)), Hongoh, Yuichi (Tokyo Inst. Tech.), Hosoda, Kazufumi (Osaka Univ.), Jenke-Kodama, Holger (Okinawa Inst. Sci. Tech.), Kawaguchi, Masayoshi (NIBB), Kucho, Ken-ichi (Kagoshima Univ.), Maruyama, Tadashi (Jap. Agency Marine-Earth Sci. Tech. (JAMSTEC)), Minamisawa, Kiwamu (Tohoku Univ.), Nakajima, Toshiyuki (Ehime Univ.), Okazaki, Shin (Nara Women's Univ.), Saeki, Kazuhiko (Nara Women's Univ.), Shigenobu, Shuji (NIBB), Shinzato, Chuya (Okinawa Inst. Sci. Tech.), Suganuma, Norio (Aichi Univ. Edu.), Takabayashi, Junji (Kyoto Univ.), Takeda, Naoya (NIBB), Tanaka, Kan (Chiba Univ.), Yokoyama, Jun (Yamagata Univ.), Yamaguchi, Haruyo (Univ. Tsukuba)

56th NIBB Conference
Neocortical Organization

Organizing Chair: Tetsuo Yamamori

March 12 (Fri)-14 (Sun), 2010

The neocortex is a unique structure in mammals and thought to be the center that directs their perceptions and behaviors. As such it has been a main focus in neuroscience for over a hundred years. However, only recently have we been able to study the mechanisms for the formation and function of the neocortex at the molecular level. I therefore decided that it was a good time to organize a meeting in such a way that it could serve as a catalyst for interaction between the following two aspects of research on Neocortical Organization. One was as a venue for studies on the functional and genetic organization of the neocortex. The other was that it would also serve as an opportunity for interaction between leading Japanese and international researchers who are carrying out cutting-edge research in the field.

The symposium was organized into the following five sessions.

Session 1: Formation of Cortical Neural Specificity
Session 2: Functional Organization of Cortical Networks in the Visual and Auditory Systems
Session 3: Functional Organization of Cortical Networks in Primates and Humans
Session 4: Motion Control and the Anatomical and Physiological Basis
Session 5: Higher Cognition and Representation

The symposium was a success, and I would like to share the concluding remarks as presented by Prof. Michael Stryker (http://www.nibb.ac.jp/conf56/report.html) during the final session of the symposium. Here, I only cite his last two remarks.

"International meetings can be enormously useful, as well as pleasant. This meeting gives us much more than a similar investment of time spent reading one another's papers. For one, we have the chance to ask and answer questions about points we may have misunderstood, or points for which we don't find the published evidence convincing. The result of such discussions may be more experiments, a different analysis, or sometimes just a clarification. In addition, we get to hear and understand and discuss plans for future directions, which can improve all our future work. We also learn about new tools that our colleagues have created. Finally, the Poster Sessions in particular give us foreigners the opportunity to meet many brilliant and energetic young Japanese student scientists, and they to meet us, forming a basis for future scientific exchanges and postdoctoral fellowships.

Finally

Let us end with an expression of thanks to the National Institute of Basic Biology and to Professor Yamamori for this stimulus to progress in neuroscience, and for this celebration of Community between Japanese and Western science.

Arigato

Michael Stryker, Professor, UCSF"

(Tetsuo Yamamori)

Speakers

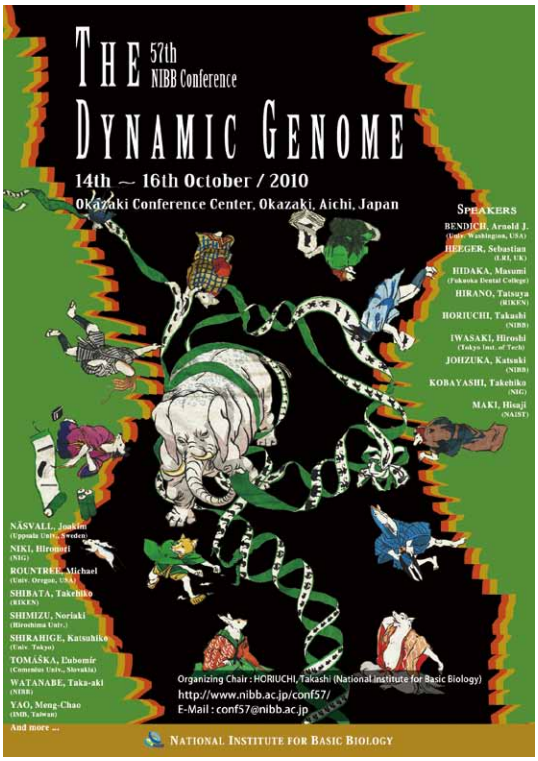
Harris, Kenneth (Imperial London College), Hensch, Takao (Harvard Univ.), Kennedy, Henry (INSERM, U846), Macklis, JeffreyD. (Masschusetts General Hospital), Rubenstein, JohnL.R. (UCSF), Stryker, Michael (UCSF), Sur, Mriganka (MIT), Watanabe, Takeo (Boston Univ.)
Fujita, Ichiro (Osaka Univ.), Isa, Tadashi (NIPS), Kawaguchi, Yasuo (NIPS), Kawato, Mitsuo (ATR), Komatsu, Hidehiko (NIPS), Mori, Kensaku (Univ.of Tokyo), Nambu, Atsushi (NIPS), Osumi, Noriko (Tohoku Univ.), Sakai, Kuniyoshi (Univ.of Tokyo), Sakano, Hitoshi (Univ.of Tokyo), Taira, Masato (Nihon Univ.), Takada, Masahiko (Kyoto Univ.), Tanaka, Keiji (RIKEN), Tanifuji, Manabu (RIKEN), Tannji, Jun (Tamagawa Univ.), Tsumoto, Tadaharu (RIKEN), Yamamori, Tetsuo (NIBB), Yoshimura, Yumiko (NIPS)

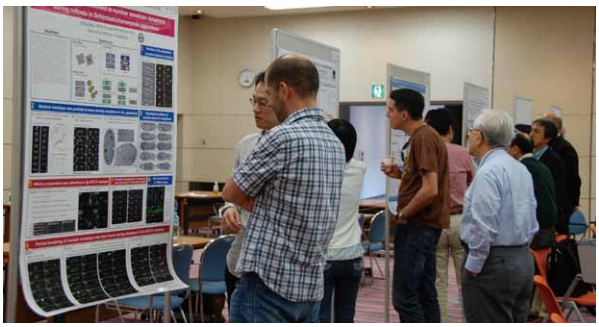
57th NIBB International Conference
The Dynamic Genome

Organizer: Takashi Horiuchi

October 14 (Thu)-16 (Sat), 2010

There are two forms of environmental change that affect living organisms. One is environmental change which organisms regularly experience, such as fluctuations in temperature, availability of food sources, etc. and the other are those they have not experienced before. In the former case, organisms rely on their program or genetic network which has been constructed during evolution to adapt to the changes and survive. On the other hand, when they are exposed to environmental changes they have never developed responses for (for example new pesticides), they may be virtually eradicated or only a very small percentage of them survive by developing tolerance to the severe environment. The mechanisms induced in this case are dynamic genome changes, such as recombination and mutation which probably play a central role in biological evolution. Thus, with this in mind, we titled this conference "The Dynamic Genome", and we provided an opportunity for a discussion with more than forties international researchers active in this field. These discussions covered more complex process, such as gene amplification and mechanisms involved in proteins, such as cohesin and condensin, and gene evolution.





Speakers

Bendich, Arnold (Univ. Washington), Heeger, Sebastian (London Res. Inst.), Näsvall, Joakim (Uppsala Univ.), Rountree, Michael (Univ. Oregon), Tomaska, Lubomir (Comenius Univ.), Yao, Meng-Chao (Inst. Mol. Biol., Taiwan)
Hidaka, Masumi (Fukuoka Dental Col.), Hirano, Tatsuya (RIKEN), Horiuchi, Takashi (NIBB), Iwasaki, Hiroshi (Tokyo Inst. Tech.), Johzuka, Katsuki (NIBB), Kobayashi, Takehiko (NIG), Maki, Hisaji (NAIST), Niki, Hironori (NIG), Sekiguchi, Mutsuo (Fukuoka Dental Col.), Shibata, Takehiko (RIKEN), Shimizu, Noriaki (Hiroshima Univ.), Shirahige, Katsuhiko (Univ. Tokyo), Watanabe, Taka-aki (NIBB)

The Second NIBB-MPIPZ Joint Symposium Plant Science Communications 2010

Organizers: Masayoshi Kawaguchi, Mitsuyasu Hasebe, Kiyotaka Okada

November 16 (Tue)-18 (Thu), 2010

Based on agreements on international academic collaboration with the Max-Planck Institute for Plant Breeding Research (MPIPZ, Germany) and the Temasek Life Sciences Laboratory (TLL, Singapore) formalized in 2009 and 2010, respectively, the 2nd international joint symposium titled "Plant Science Communications 2010" was held from the 16th through 18th of November 2010 at the Okazaki Conference Center. It included 11 speakers, mainly Principal Investigators (PIs), from MPIPZ and 5 PIs from TLL plant science laboratories as well as 8 speakers from NIBB plant science laboratories. In addition, 6 select researchers from JSPS Machida Plant Meristem Project joined and spoke at the symposium. The number of participants was more than a hundred.

The symposium was successfully managed by young assistant professors from plant science laboratories in NIBB. There were lively discussions of the latest results during and after the talks. The topics were focused on development, natural variation, plant-microbe interaction and epigenetics. It was impressive that large-scale analyses using next-generation sequencing technology are becoming a standard in the research done at MPIPZ. The poster session included 47 posters by young plant scientists, and active discussions until the end of the session. An NIBB Lab Tour Session was organized for the morning of the second day to provide time for discussion of future collaborations between the attendants and NIBB researchers. The representatives of the three institutes, NIBB, MPIPZ and TLL, pledged to keep supporting this symposium as a center for collaboration between plant scientists from Japan, Germany and Singapore.

Speakers

Bucher, Marcel (Univ. Cologne), Coupland, George (MPIPZ), Davis, Seth Jon (MPIPZ), Frederic, Berger (TLL), Hao, Yu (TLL), He, Yuehui (TLL), Ito, Toshiro (TLL), Jose, Jimenez-Gomez (MPIPZ), Koornneef, Maarten (MPIPZ), Nakabayashi, Kazumi (MPIPZ), Renier van der, Hoorn (MPIPZ), Saijo, Yusuke (MPIPZ), Schulze-Lefert, Paul (MPIPZ), Soppe, Wim (MPIPZ), Torti, Stefano (MPIPZ), Turck, Franziska (MPIPZ), Zhongchao, Yin (TLL)

Araki, Tadashi (Kyoto Univ.), Fukuda, Hiroo (Univ. of Tokyo), Hasebe, Mitsuyasu (NIBB), Kakimoto, Tatsuo (Osaka Univ.), Kawaguchi, Masayoshi (NIBB), Machida, Yasunori (Nagoya Univ.), Mano, Shoji (NIBB), Matsubayashi, Yoshikatsu (Nagoya Univ.), Minagawa, Jun (Hokkaido Univ.), Murata, Tadashi (NIBB), Okada, Kiyotaka (NIBB), Shimamoto, Ko (NAIST), Takeda, Naoya (NIBB), Toyokura, Koichi (NIBB), Ueda, Tadashi (Univ. Tokyo), Yamada, Kenji (NIBB)

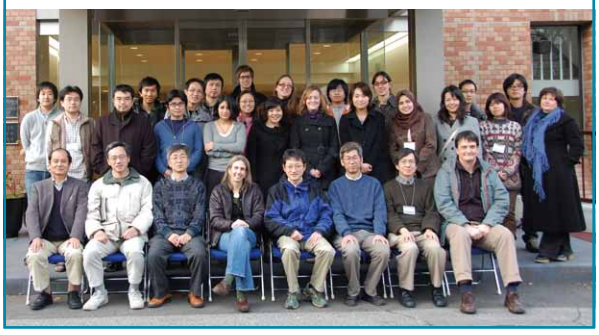
The NIBB International Practical Course

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 course replaced our Bioscience Training Course, which had been held for twenty years and which had, for the most part, accepted participants from universities and institutes in Japan. The Fourth International Practical Courses was held in 2009 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. We hope that the techniques and knowledge provided in these courses will prove useful in the future work of all of the participants.

The Fifth NIBB International Practical Course: "Developmental Genetics of Zebrafish and Medaka III"

- Period: January 26 (Tue)– February 2 (Tue), 2010
- Participants: 15 (three from Taiwan, three from Germany, two from Japan, one each from China, Singapore, Israel, UK, Canada, Norway, and Italy)
- Lecturers:
  Dr. Katie Peichel (Fred Hunchinson Cancer Research Center, USA)
  Dr. Kunio Inoue (Kobe University, Japan)
  Dr. Shin-ichi Higashijima (National Insitute for Physiological Science, Japan)
  Dr. Christoph Winkler (National University of Singapore, Singapore)
  Dr. Kohei Hatta (University of Hyogo, Japan)
- Course Staff from NIBB:
  Minoru Tanaka, Kinoshi Naruse, Kayo Kobayashi, Shuhei Nakamura, Takao Sasado

Contents of the course: This course focused on skills and technology on medaka. Participants tried learning modification of BAC using homologous recombination, cryopresevation and artificial inseminination and heat induction of gene. Participants also practiced retrieving and extracting informations using medaka genome blowser.



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

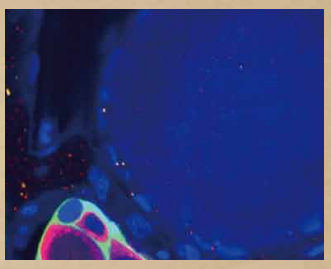
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

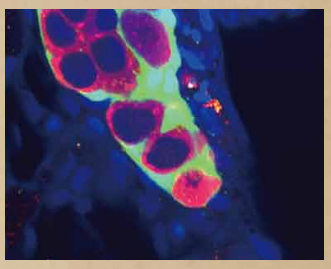
From Higashi Okazaki Station to Each Area

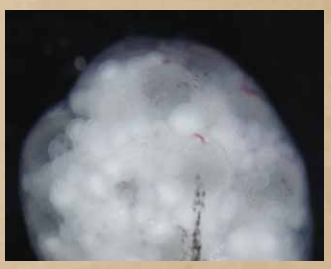
Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

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).







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