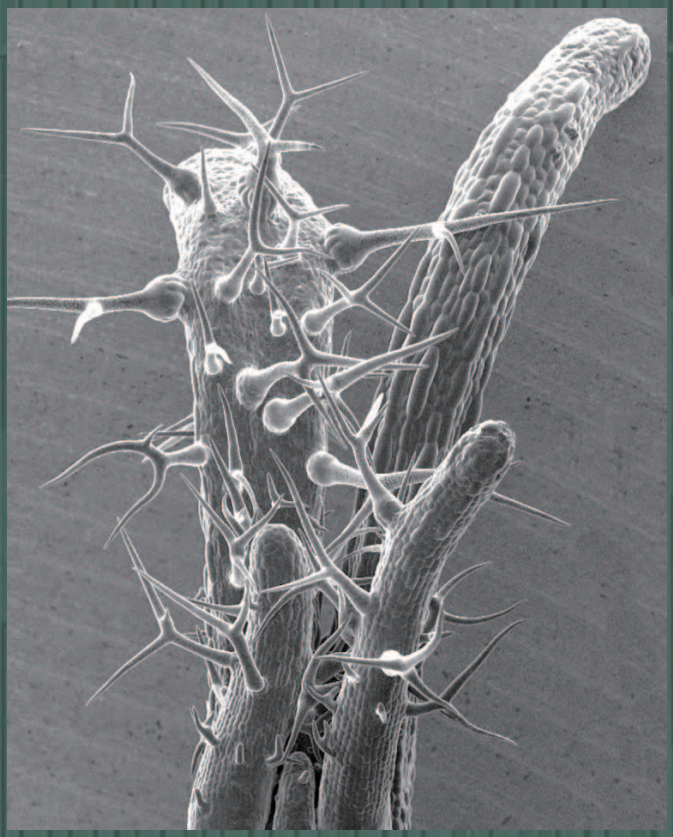
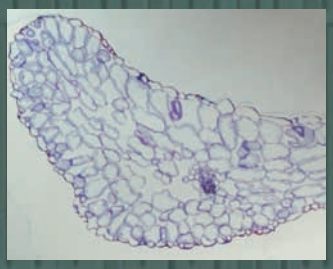
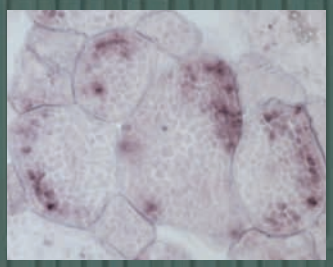



National Institute for Basic Biology

2012 ANNUAL REPORT

CONTENTS

The cover photographs are related to a study showing the role of the middle domain-specific *WOX* genes in leaf development of *Arabidopsis thaliana* (Nakata *et al*., Plant Cell, 2012). This study led a proposal that a new domain, named the middle domain, located between the adaxial and abaxial domains, has a distinct role from that of the previously identified two domains above: namely, the middle domain-specific *WOX* genes organize blade outgrowth together with adaxial and abaxial regulators and prevent the mixing of the adaxial and abaxial domains to maintain proper patterns during leaf development. See page 34 of this report for details.

INTRODUCTION

In order to understand the survival strategies of a variety of organisms NIBB uses model organisms in collaboration with Japanese and international researchers to uncover both the basic mechanisms that are common to all living things, as well as the varied and unique systems organisms have evolved to allow them to adapt to their environments. It is necessary to continue to promote top-level research in this field, therefore it is important that we boldly pioneer new areas of study, and exert our energies to foster the young researchers who will lead us in the future.

In addition, as an inter-university research institute it is essential that the National Institute for Basic Biology spares no effort in promoting cooperative use and collaborative research. In answer to the desires of the research community, NIBB has been strengthening our cooperative use and collaborative research structure by setting up the DSLM (Digital Scanned Light-sheet Microscope) cooperative research program, the next Generation DNA sequencer cooperative research program, and we have established the "NIBB BioResource Center" and the "NIBB Core Research Facilities". In addition, in order to protect biological resources that are indispensable to the advancement of the biological sciences we have begun operating as the core facility of the Interuniversity Bio-Backup Project (IBBP), together with implementing plans in this fiscal year to begin joint-use research aimed at the development of new cryopreservation technology of biological resources. With the current climate of emphasis being placed on the practical application of research NIBB is expected to play a central role in promotion of the importance of the study of basic biology. To this end I believe it is necessary for NIBB to come together and strive to propel research, as well as expanding cooperative use and collaborative research with universities and institutes throughout the world.

In 2012 we welcomed several new colleagues, including 4 assistant professors and 5 NIBB research fellows, while 2 colleagues transferred to other institutes.

Finally I would like to congratulate Prof. Yoshinori Ohsumi for winning the Kyoto Prize, which is widely regarded as one of the most prestigious international awards for lifetime achievement in the arts and sciences, and Assistant Prof. Shinichi Miyagawa for winning the NINS young Researcher Award. I would also like to congratulate Dr. Kazuya Kuboyama, Dr. Michitaro Shibata, and Dr. Kenji Fukushima for receiving recognition for their research achievements as detailed on page 7.

We always welcome any questions, comments and suggestions concerning research activities and administration of NIBB.

Mikio Nishimura, D. Agric.
Deputy Director General
June 11, 2013

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).

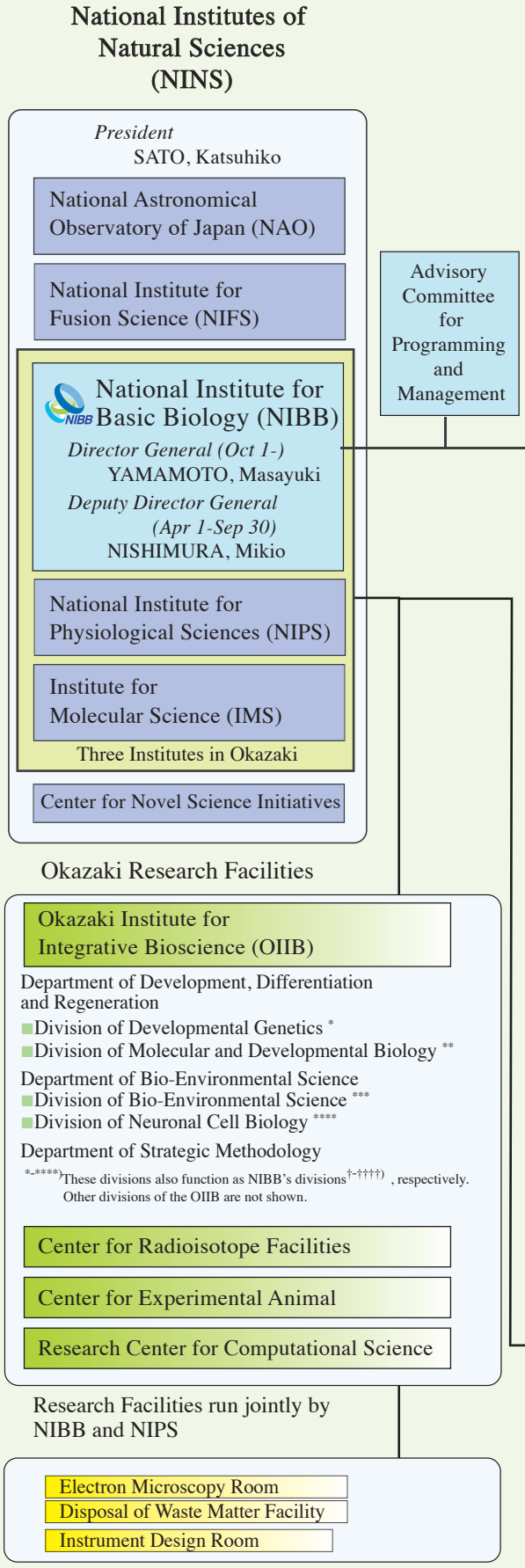
Policy, Decision Making, and Administration

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.

Organization



National Institute for Basic Biology (NIBB)

As of April 1, 2013

Research Units

- **Cell Biology**
  - Division of Cell Mechanisms
  - Division of Intercellular Signaling Biology
  - Laboratory of Neuronal Cell Biology††††
  - Laboratory of Cell Sociology
- **Developmental Biology**
  - Division of Morphogenesis
  - Division of Developmental Genetics †
  - Division of Molecular and Developmental Biology ††
  - Division of Embryology
  - Division of Germ Cell Biology
  - Laboratory of Molecular Genetics for Reproduction
- **Neurobiology**
  - Division of Molecular Neurobiology
  - Division of Brain Biology
  - Division of Brain Circuits
  - Laboratory of Neurophysiology
- **Evolutionary Biology and Biodiversity**
  - Division of Evolutionary Biology
  - Division of Symbiotic Systems
  - Laboratory of Morphodiversity
  - Laboratory of Bioresources
  - Laboratory of Biological Diversity
- **Environmental Biology**
  - Division of Molecular Environmental Endocrinology †††
  - Division of Environmental Photobiology
  - Division of Seasonal Biology (Adjunct)
- **Theoretical Biology**
  - Laboratory of Genome Informatics
- **Imaging Science**
  - Laboratory for Spatiotemporal Regulations

Strategic Planning Department

Research Support Units

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

Other Research Support Sections

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

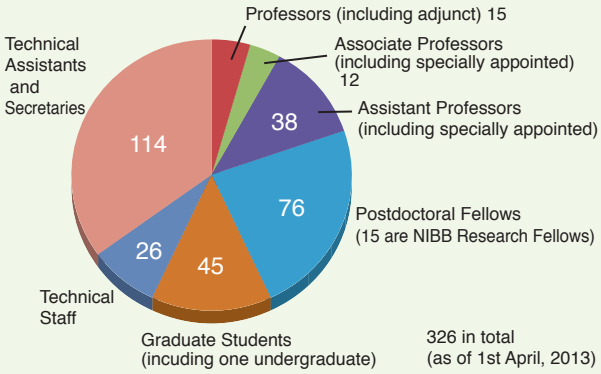
Okazaki Administration Office

Research and Research Support

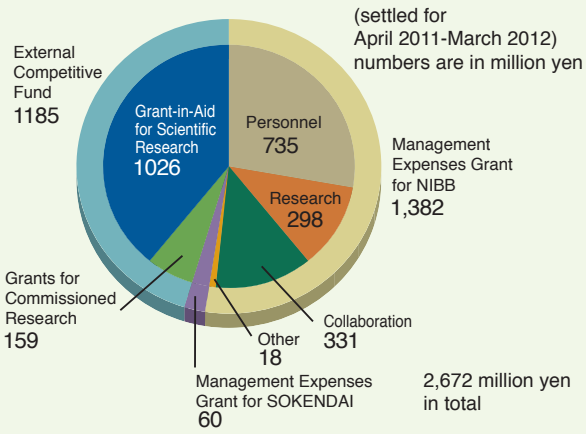
NIBB's research programs are conducted in research units and research support units. Each research unit is called either a division, led by a professor, or a laboratory, led by an associate professor or an assistant professor. Each research unit forms an independent project team. The NIBB Core Research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

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

Members in NIBB



Financial Configuration of NIBB



Members of the Advisory Committee for Programming and Management# (as of April, 2013)

Non-NIBB members	HAKOSHIMA, Toshio*	Professor, Nara Institute of Science and Technology
	HIGASHIYAMA, Tetsuya	Professor, Nagoya University
	KONDO, Takao	Professor, Nagoya University
	KURUMIZAKA, Hitoshi*	Professor, Waseda University
	MIZUSHIMA, Noboru	Professor, The University of Tokyo
	MORI, Ikue	Professor, Nagoya University
	OHTA, Kunihiro*	Professor, The University of Tokyo
	TAKABAYASHI, Junji	Professor, Kyoto University
	TANAKA, Ayumi	Professor, Hokkaido University
	TSUKITA, Sachiko*	Professor, Osaka University
NIBB members	FUJIMORI, Toshihiko	Professor, National Institute for Basic Biology
	HASEBE, Mitsuyasu	Professor, National Institute for Basic Biology
	IGUCHI, Taisen	Professor, Okazaki Institute for Integrative Bioscience
	KAWAGUCHI, Masayoshi	Professor, National Institute for Basic Biology
	KOBAYASHI, Satoru	Professor, Okazaki Institute for Integrative Bioscience
	NISHIMURA, Mikio	Professor, National Institute for Basic Biology
	NODA, Masaharu	Professor, National Institute for Basic Biology
	TAKADA, Shinji	Professor, Okazaki Institute for Integrative Bioscience
	UENO, Naoto	Professor, National Institute for Basic Biology
	YAMAMORI, Tetsuo	Professor, National Institute for Basic Biology
	YOSHIDA, Shosei	Professor, National Institute for Basic Biology

* new member from April 2013
# Chairperson and vice-chair will be nominated at meeting scheduled for the end of June, 2013.

GOALS OF THE NATIONAL INSTITUTE FOR BASIC BIOLOGY

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

Promotion of Collaborative Research

Collaborative Research Support

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

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

NIBB Core Research Facilities

year	2009	2010	2011	2012
Priority collaborative research projects	1	4	6	5
Collaborative research projects for model organisms/ technology development	3	2	2	3
Individual collaborative research projects	54	68	88	89
NIBB workshops	3	3	6	6
Collaborative experiments using the large spectrograph	10	8	9	14
Collaborative experiments using the DSLM	–	7	8	5
Collaborative experiments using the next generation DNA sequencer	–	11	45	47
Facility Use (Training Course Facility)	–	1	0	2
total total	71	94	164	171

Collaborative Research Projects by Year

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 (p. 68).

The Functional Genomics Facility maintains a wide array of core research equipment, including cutting edge tools such as next generation DNA sequencers. The facility is dedicated to fostering NIBB's collaborative research by providing these tools as well as expertise. The current focus is supporting functional genomics works that utilize mass spectrometers and DNA sequencers, holding training courses as one of these undertakings (p. 86). 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.

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

The 2011 Tohoku Earthquake and Tsunami in March, 2011, caused massive damage to important biological resources. To prevent such loss, NIBB established the IBBP Center in 2012 in collaboration with seven national universities for multiplicate preservation of genetic libraries and other invaluable bioresources (p.76).

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. The 10th NIBB-EMBL symposium "Quantitative Bioimaging" was held in March, 2013, and will appear in the 2013 Annual Report.

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

Collaborative programs have also been started with the Temasek Life Sciences Laboratory (TLL), of Singapore and Princeton University. The 4th NIBB-MPIPZ-TLL Joint Symposium "Arabidopsis and Emerging Model Systems" was held in November, 2012 at NIBB (p. 85).

NIBB Conference

The NIBB Conferences are international conferences on hot topics in biology organized by NIBB's professors. Since the first conference in 1977 (the year of NIBB's foundation), NIBB Conferences have provided researchers in basic biology with valuable opportunities for international exchange. The 58th/60th conference "Germline -Speciation, Sex, and Stem Cells-" was held

in July, 2012 (p.84) and the 59th conference "Neocortical Organization" was held in March, 2012 (p. 83).

International Practical Course

With the cooperation of researchers from Japan and abroad the NIBB international practical course is given at a laboratory specifically prepared for its use. The 7th course "Genetics, Genomics and Imaging in Medaka and Zebrafish" was held jointly with TLL and the National University of Singapore (NUS) in July, 2012 at NUS and TLL (p. 86). Graduate students and young researchers from various areas including Germany, China, India, and Italy, were provided with training in state-of-the-art research techniques. International conferences and courses are managed by the Office of International Cooperation (p. 79).

Bio-Resources

The National BioResource Project (NBRP) is a national project for the systematic accumulation, storage, and supply of nationally recognized bio-resources (experimental animals and plants, cells, DNA, and other genetic resources), which are widely used as materials in life science research. To promote this national project, NIBB has been appointed as a center for research on Medaka (*Oryzia latipes*) whose usefulness as a vertebrate model was first shown by Japanese researchers. The usability of Medaka as a research material in biology has drawn increasing attention since its full genome sequence recently became available. NIBB is also a sub-center for the NBRP's work with Japanese morning glory. The NIBB BioResource Center has equipment, facilities, and staff to maintain Medaka and Japanese morning glory safely, efficiently, and appropriately. The center also maintains other model organisms, such as mice, zebrafish, *Arabidopsis*, *Lotus japonicus*, and *Physcomitrella patens*, and provides technical support and advice for the appropriate use of these organisms (p. 73).

Strains of Japanese morning glory maintained in the center

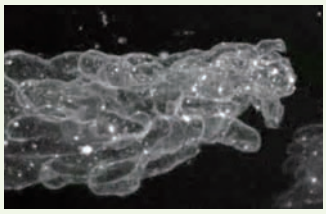
Outreach

NIBB's outreach activities aim to present cutting edge research results to the public via mass media through press releases or directly through the internet. We made a major revision of our English web page and opened a video gallery containing an English language introduction of NIBB in 2011. NIBB also cooperates in the education of undergraduate and younger students through lectures and workshops. Outreach activities are mostly managed by the Office of Public Relations (p. 79).

Development of New Fields of Biology

Bioimaging

NIBB aims to maximize the application of modern light microscopes and biophotonic probes for real time visualization of biological phenomena and to develop new imaging techniques. As part of our collaborative work with EMBL, NIBB introduced a DSLM, which is effective for the three-dimensional observation of living samples, and has developed an improved model (p. 67). The Advisory Committee on Bioimaging, comprised of leading researchers in the bioimaging field in Japan, is organized to formulate advice on NIBB's imaging research. The Bioimaging Forum provides an opportunity for researchers and company engineers to frankly discuss practical difficulties and needs regarding imaging (p. 87).



A frame from a 3D movie of living *Amoeba proteus* first visualized by DSLM owing to its excellent time resolution.

Okazaki Biology Conferences

NIBB holds Okazaki Biology Conferences (OBC) that, with the endorsement of the Union of Japanese Societies for Biological Science, aim to explore new research fields in biology and support the formation of international communities in these fields. Dozens of top-level researchers from Japan and abroad spend nearly one week together for intensive discussions seeking strategies for addressing future critical issues in biology. Past Conferences have promoted the formation of international researcher communities. The OBC8 "Speciation and Adaptation II: Environment and Epigenetics" was held in March, 2012 (p. 81), and the OBC9 "Marine Biology II" was held in October, 2012 (p. 82).

Cultivation of Future Researchers

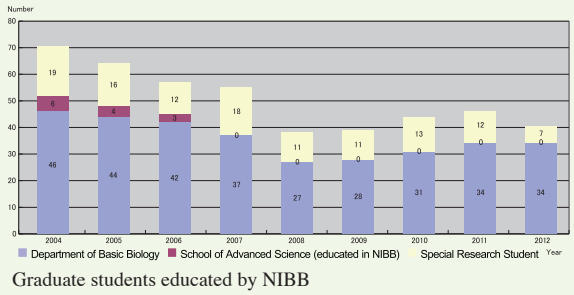
NIBB constitutes the Department of Basic Biology in the School of Life Science of the Graduate University for Advanced Studies (SOKENDAI). The department provides a five-year course for university graduates and a three-year doctoral course for graduate students with a master's degree.

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

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

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

Students from Japan and abroad can also come to NIBB through our Internship Program. Internships give students an excellent way to build international connections while experiencing hands on research in a world class research institute (p. 87).



Graduate students educated by NIBB

Personnel changes in 2012*

Newly assigned in NIBB

Name	Position	Research Unit	Date
MAEDA, Taro	NIBB Research Fellow	Functional Genomics Facility	March 16
MII, Yusuke	Assistant Professor	Division of Molecular and Developmental Biology	April 1
ISHIKAWA, Masaki	Assistant Professor	Division of Evolutionary Biology	April 1
WAKE, Hiroaki	Assistant Professor	Division of Brain Circuits	April 1
GOYO-YAMADA, Shino	NIBB Research Fellow	Division of Cell Mechanisms	April 1
MIYAKAWA, Hitoshi	NIBB Research Fellow	Division of Molecular Environmental Endocrinology	April 1
TANAKA, Yasuhiro	NIBB Research Fellow	Division of Brain Circuits	April 1
SOYANO, Takashi	NIBB Research Fellow	Division of Symbiotic Systems	July 1
TAKEHANA, Yusuke	Assistant Professor	Laboratory of Bioresources	November 1

Newly affiliated in other universities and institutes

Name	New Affiliation	Position	Date
HIWATASHI, Yuji	Aberystwyth University	Marie Curie Fellow	April 1
WATANABE, Takaaki	Lerner Research Institute, Cleveland Clinic	Postdoctoral Research Fellow	April 1

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

Awardees in 2012

Name	Position	Award
KUBOYAMA, Kazuya	NIBB Research Fellow	Young Investigator Award of Japanese Association for Protein phosphatase Research
FUKUSHIMA, Kenji	Graduate Student	Japanese Society for Plant Systematics Meeting Presentation Award (Oral Presentation Section)
SHIBATA, Michitaro	Graduate Student	Sokendai President's Award
MIYAGAWA, Shinichi	Assistant Professor	NINS Young Researcher Award
OHSUMI, Yoshinori	Professor Emeritus	The Kyoto Prize

Note: On the unit member lists from P. 8 all members who belonged to the unit during 2012 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 2012. The former title is indicated by an asterisk (*).

DIVISION OF CELL MECHANISMS

Professor
NISHIMURA, Mikio

Associate Professor
HAYASHI, Makoto

Assistant Professor:	*MANO, Shoji*
	YAMADA, Kenji
Technical Staff:	*KONDO, Maki*
NIBB Research Fellow:	*GOTO-YAMADA, Shino*
Postdoctoral Fellow:	*OIKAWA, Kazusato*
	KANAI, Masatake
	WATANABE, Estuko
	TANAKA, Mina
	KAMIGAKI, Akane
	NITO, Kazumasa
	*GOTO-YAMADA, Shino**
Graduate Student:	*NAKAI, Atsushi**
	*CUI, Songkui**
	SHIBATA, Michitaro
Technical Assistant:	*SAITO, Miyuki*
	NAKAYAMA, Tomomi
	HIKINO, Kazumi
	YOSHINORI, Yumi
	YAMAGUCHI, Chinami
	NAKAI, Atsushi
	CUI, Songkui
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 stored in oil body (Figure 1) via β-oxidation and the glyoxylate cycle, are transformed into leaf peroxisomes that function in several crucial steps of photorespiration. After the functional transition of glyoxysomes to leaf peroxisomes during the greening of pumpkin cotyledons, the reverse transition of leaf peroxisomes to glyoxysomes occurs during senescence. Gene expression, alternative splicing, protein translocation and protein degradation control the functional transformation between glyoxysomes and leaf peroxisomes.

II. Transcriptomics, proteomics and phenomics of plant peroxisomes

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

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

Bioinformatic analysis of the *Arabidopsis* genome predicted the presence of 15 kinds of genes, called *PEX* genes, for peroxisomal biogenesis factors. We demonstrated that *PEX5* and *PEX7* form a cytosolic receptor complex and recognize PTS1- and PTS2-containing proteins, respectively. *PEX14* is a peroxisomal membrane docking protein that captures the

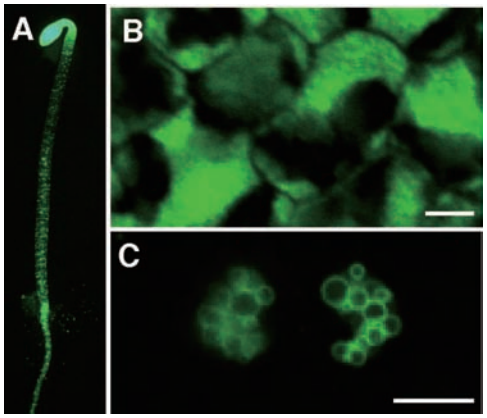


Figure 1. Oil body visualized by oleosin-GFP. (A) Five-day-old dark grown seedlings expressing oleosin-GFP. (B) Numerous oil bodies in the cell of dry seed. (C) High-resolution image of aggregated oil bodies. Bar = 5 μm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

receptor-cargo complex. We also comprehensively investigated whether or not these predicted *PEX* genes function in peroxisome biogenesis by generating knock-down mutants that suppress *PEX* gene expression by RNA-interference. Phenotypes of these mutants allowed us to identify the functional *PEX* genes, which can be classified into two groups: *PEX* genes regulating for peroxisomal protein import and *PEX* genes regulating for peroxisomal morphology. We continue to investigate the detailed molecular functions of other *PEX* genes. Of these, we recently proposed that function of PEX7 is maintained by a quality control mechanism involving RabE1c.

III. Identification of novel components essential for peroxisome biogenesis

To better understand peroxisome biogenesis and functions, we isolated a number of *Arabidopsis* mutants having aberrant peroxisome morphology (*apem* mutants) based on them having 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 *apem* mutants, *APEM1* gene (whose defect causes the elongation of peroxisomes and mitochondria) encodes dynamin-related protein 3A, which is involved in division of both organelles. *APEM2* and *APEM4* (whose defects cause a decrease in the efficiency of protein transport) were revealed to encode proteins homologous to PEROXIN 13 (PEX13) and PEX12, respectively, and both proteins are responsible for protein translocation on peroxisomal membranes. APEM9 is the plant-specific PEX that has a role in tethering the PEX1-PEX6 complex on peroxisomal membranes. In addition, we found that *APEM3* encodes Peroxisomal membrane protein 38, and that its defect causes enlargement of peroxisomes (Figure 2).

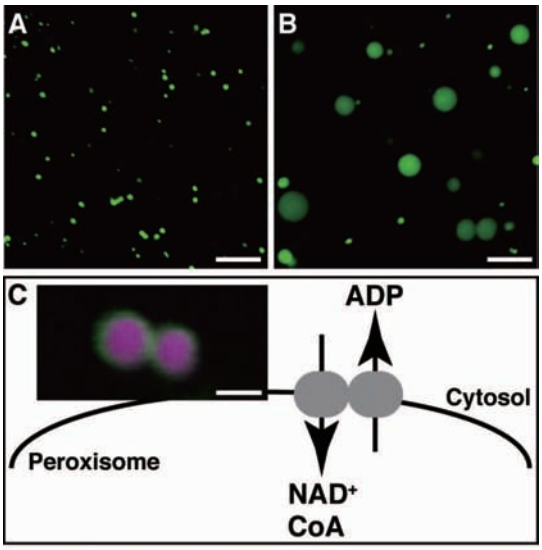


Figure 2. PMP38/APEM3 has a role as a transporter on the peroxisomal membrane. (A, B) Compared to the parent plant (A), peroxisomes are enlarged in the *apem3* mutant (B). (C) Subcellular localization and function of PMP38. *PMP38-GFP* (green) and *RFP-PTS1* (magenta) were transiently expressed in Arabidopsis leaf cells (Inset). PMP38 is present as a dimer, and has a role in transport of NAD^+, CoA and ADP. Scale bars, 10 μm (A, B) and (B) 1 μm (C).

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

IV. ER derived organelles for protein storing and defense strategy

Plant cells develop various types of endoplasmic reticulum (ER)-derived structures with specific functions. ER bodies are ER-derived compartments observed in *Arabidopsis*. They are rod-shaped structures surrounded by ribosomes, and are widely distributed in the epidermal cells of whole seedlings. Undamaged rosette leaves have no ER bodies, but accumulate ER bodies after wounding or jasmonic acid treatment. This suggests that ER bodies function in the defense against herbivores. ER bodies in seedlings include β-glucosidase PYK10. When plant cells are damaged, PYK10 forms large protein aggregates. The aggregate formation increases glucosidase activity, possibly producing toxic products. *Arabidopsis nai1* mutants have no ER bodies in the entire plant and do not accumulate PYK10. *NAI1* encodes a transcription factor and regulates the expression of *PYK10* and *NAI2*. The *Arabidopsis nai2* mutant has no ER bodies and reduced accumulation of PYK10. *NAI2* encodes a unique protein that localizes to the ER body. We found that the membrane protein of ER body 1 (MEB1) and MEB2 are integral membrane proteins of the ER body. MEB1 and MEB2 have homology to yeast iron/manganese transporter CCC1. Heterologous expression of MEB1 and MEB2 in yeast *ccc1* mutant suppresses the iron toxicity (Figure 3), indicating that MEB1 and MEB2 are iron/manganese transporters. These results suggest that the ER body has specific membrane proteins that are involved in defense against metal stress as well as pathogens and herbivores. NAI2 deficiency relocates MEB1 and MEB2 to the ER network. These findings indicate that NAI2 is a key factor that enables ER body formation. We are now investigating the function of NAI2 on ER body formation by heterologously expressing it in onion and tobacco cells.

V. Roles of molecular chaperones on cell differentiation

Molecular chaperones are cellular proteins that function in the folding and assembly of certain other polypeptides into oligomeric structures. We have found that HSP90 inhibitor induces genes with heat shock response element (HSE)

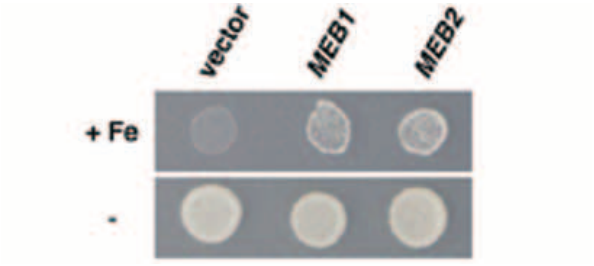


Figure 3. MEB1 and MEB2 suppress the toxicity of iron. Yeast iron sensitive *ccc1* mutants harboring empty vector, vector for *MEB1*, or *MEB2* were grown on synthetic minimal medium with (upper) or without (lower) 2 mM iron.

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. These data indicate that HSP90 regulates correct gene expression for heat acclimatization in plants. We also observed that HSP90 is involved in hormone responses in *Arabidopsis*. The evolutional and functional characterizations are now being investigated.

VI. Update of The Plant Organelles Database 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 five individual units: the perceptive organelles database, the organelles movie database, the organellome database, the functional analysis database, and external links. The perceptive organelles database, which was added as new content, shows organelles dynamics in response to environmental stimuli (Figure 4). The organelles movie database contains time-lapse images and 3D structure rotations. The organellome database is a compilation of static image data of various tissues of several plant species at different developmental stages. The functional analysis database is a collection of protocols for plant organelle research. The amount of included data is increasing day by day. We will add new content, which is dedicated to ultrastructures of organelles by electron microscopy, soon. It is expected that PODB2 will contribute to systems biology through the combination of the included data with other 'omics' data and computational analyses. In addition, we updated the 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.

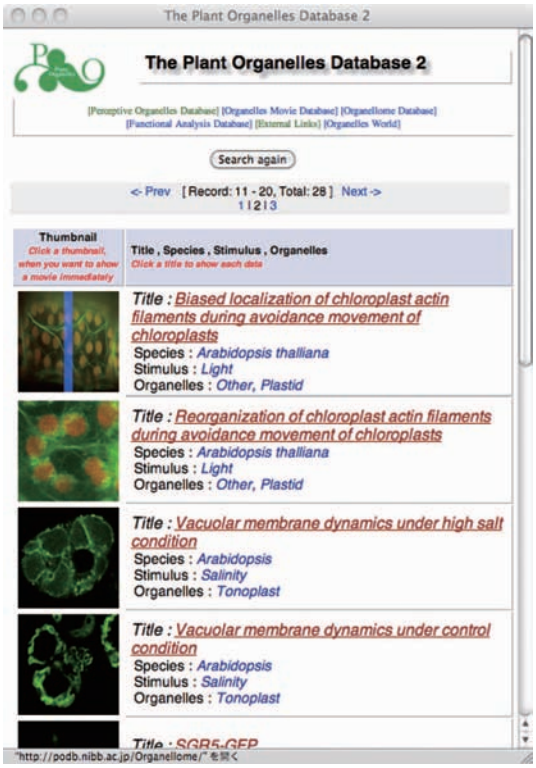


Figure 4. The graphical user interface of the perceptive organelles database in PODB2 (http://podb.nibb.ac.jp/Orgenellome).

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- Nakano, R.T., Matsushima, R., Nagano, A.J., Fukao, Y., Fujiwara, M., Kondo, M., Nishimura, M., and Hara-Nishimura, I. (2012). ERMO3/MVP1/GOLD36 is involved in a cell type-specific mechanism for maintaining ER morphology in Arabidopsis thaliana. PLoS ONE *7*, e49103.
- Nakayama, M., Kaneko, Y., Miyazawa, Y., Fujii, N., Higashitani, N., Wada, S., Ishida, H., Yoshimoto, K., Yamada, K., Nishimura, M., and Takahashi, H. (2012). A possible involvement of autophagy in amyloplast degradation in columella cells during hydrotropic response of Arabidopsis roots. Planta *236*, 999-1012.
- Negishi, T., Oshima, K., Hattori, M., Kanai, M., Mano, S., Nishimura, M., and Yoshida, K. (2012). Tonoplast- and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. PLoS ONE *7*, e43189.
- Toyokura, K., Hayashi, M., Nishimura, M., and Okada, K. (2012). Adaxial-abaxial patterning: A novel function of the GABA shunt. Plant Signal. Behav. *7*, 705-707.

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- Kobayashi, K., Narise, T., Sonoike, K., Hashimoto, H., Sato, N., Kondo, M., Nishimura, M., Sato, M., Toyooka, K., Sugimoto, K., Wada, H. Masuda, T., and Ohta, H. Role of galactolipid biosynthesis in coordinated development of photosynthetic complexes and thylakoid membranes during chloroplast biogenesis in Arabidopsis. Plant J. 2012 Nov. 26.
- Yamada, K., Nagano, A.J., Nishina, M., Hara-Nishimura, I., and Nishimura, M. Identification of two novel endoplasmic reticulum body-specific integral membrane proteins. Plant Physiol. 2012 Nov. 19.

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- Mano, S., Kimori, Y., Takeda, T., Miwa, T., Nishikawa, S., Mimura, T., Nagatani, A., and Nishimura, M. (2012). Using image-based resources: Databases for plant organelle dynamics and applications based on image information. In Introduction to Sequence and Genome Analysis. iConcept Press Ltd. ed. (Brisbane, iConcept Press Ltd.), chapter 16, pp. 1-25.
- Tanaka, Y., Kimura, T., Hikino, K., Goto, S., Nishimura, M., Mano, S., and Nakagawa, T. (2012). Gateway vectors for plant genetic engineering: Overview of plant vectors, application for bimolecular fluorescence complementation (BiFC) and multigene construction. In Genetic Engineering – Basics, New Applications and Responsiblities. Barrera-Saldaña, H. A ed. (Rijeka, INTECH), pp. 35-58.

DIVISION OF INTERCELLULAR SIGNALING BIOLOGY

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

I. Secreted peptide signals

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

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

1-1 Root meristem growth factor (RGF)

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

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

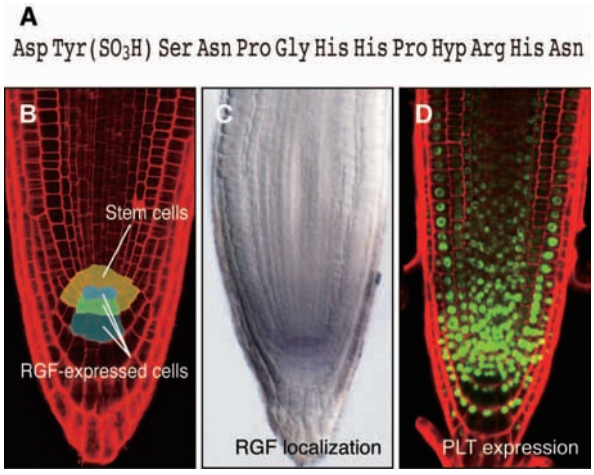

Figure 1. Mode of action of RGF peptide. (A) Structure of RGF1. (B) Expression domain of *RGF* genes. (C) Localization of RGF peptides visualized by anti-RGF1 antibody. (D) Expression pattern of PLETHORA (PLT2-GFP) transcription factor, a downstream target of RGF signaling.

1-2 Other novel peptide signal candidates

The common feature of known small post-translationally modified peptide signals is that they are encoded by multiple paralogous genes whose primary products are approximately 70- to 110-amino-acid cysteine-poor secreted polypeptides that share short conserved domains near the C-terminus. We have identified several novel polypeptide families that fulfill the above criteria by *in silico* screening and determined their mature structures by analyzing apoplastic peptide fractions by nano LC-MS/MS. Functional analysis of these peptides is now going on.

II. Post-translational modification mechanisms

Post-translational modifications are known to affect peptide conformation through steric interactions with the peptide backbone, thereby modulating the binding ability and specificity of peptides for target receptor proteins. To date,

the following types of post-translational modification have been identified in secreted peptide signals in plants: tyrosine sulfation and hydroxyproline arabinosylation (Figure 2).

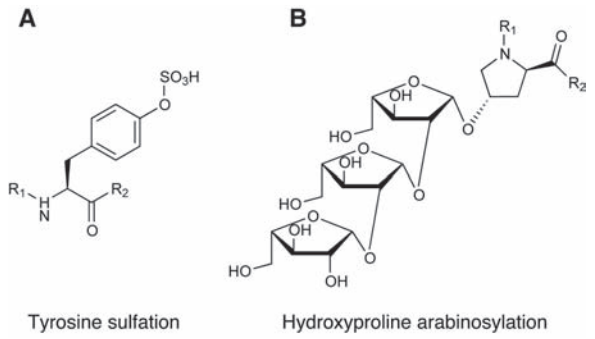

Figure 2. Post-translational modifications in secreted peptide signals in plants.

2-1 Tyrosine sulfation

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

2-2 Hydroxyproline arabinosylation

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

2-3 Chemical synthesis of arabinosylated peptides

Arabinosylation of hydroxyproline (Hyp) is a posttranslational modification often found in secreted peptide signals in plants. We have succeeded in the stereoselective total synthesis of β-1,2-linked tri-arabinosylated CLV3 peptide ([Ara_3]CLV3) (Figure 3 and 4). Comparison of mono-, di- and tri-arabinosylated CLV3 glycopeptides revealed that the biological activity increased progressively as arabinose chain length increased. Thus, arabinose chain length of CLV3 is important for its biological activity.

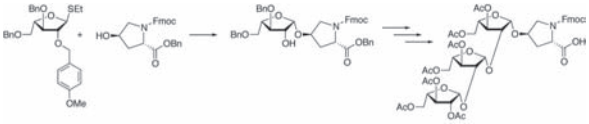

Figure 3. Synthesis of triarabinosylated Hyp building block.

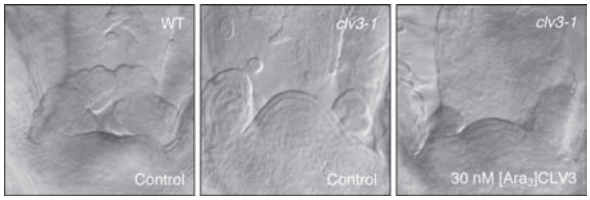

Figure 4. Shoot apical meristem of wild-type, *clv3-1* seedlings and *clv3-1* seedlings treated with chemically synthesized [Ara_3]CLV3 at 30 nM.

2-4 Conformation of arabinosylated peptides

NMR spectroscopy and NOE-based structure calculations revealed the structural impact of the arabinose chain on peptide conformation. The arabinose chain of [Ara_3]CLV3 extends toward the C-terminal end of the peptide, and its non-reducing end is positioned proximal to the peptide backbone (Figure 5). Consequently, the arabinose chain causes distinct distortion in the C-terminal half of the peptide in a highly directional manner. The established synthetic route of [Ara_3]CLV3 will greatly contribute to our understanding of the biology and biochemistry of arabinosylated peptide signals in plants.

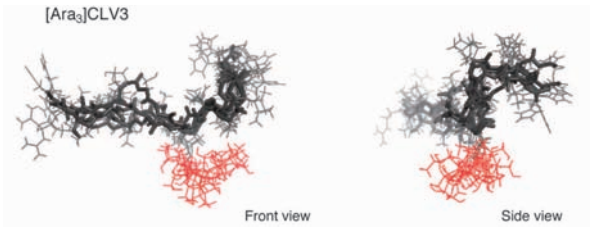

Figure 5. Energy-minimized structure of [Ara_3]CLV3 resulting from a simulated annealing protocol that incorporated NOE-derived distance restraints.

III. Receptors for secreted peptide signals

The receptors or putative receptors for peptide signals identified to date belong to the receptor kinase (RK) or

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

3- 1 Receptor expression library

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

3- 2 Structural basis for ligand recognition

Leucine-rich repeat receptor kinases (LRR-RKs) comprise the largest subfamily of the transmembrane receptor kinases in plants. In several LRR-RKs, a loop-out region called an "island domain" that intercepts the extracellular tandem LRRs at a position near the transmembrane domain constitutes the ligand-binding pocket, but the absence of the island domain in numerous LRR-RKs raises questions about which domain specifically recognizes the corresponding ligands in non-island domain-carrying LRR-RKs. We determined, by photoaffinity labeling followed by chemical and enzymatic digestion, that BAM1, a CLV1/BAM family LRR-RK whose extracellular domain is comprised of 22 consecutive LRRs, directly interacts with the small peptide ligand CLE9 at the LRR6-8 region that is relatively distal from the transmembrane domain (Figure 6). Multiple sequence alignment and homology modeling revealed that the inner concave side of LRR6-8 of the CLV1/BAM family LRR-RKs is slightly deviatory from the LRR consensus. Our results indicate that ligand recognition mechanisms of plant LRR-RKs are more complex and diversified than anticipated.

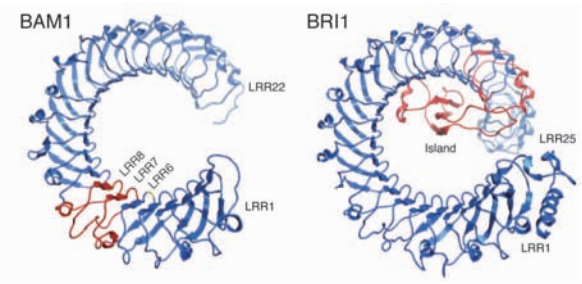

Figure 6. Deduced structure of BAM1 extracellular LRR domain by homology modeling and comparison with the BRI1 crystal structure. Ligand-binding domains are highlighted in red.

Publication List

〔Original paper〕

- Shinohara, H., Moriyama, Y., Ohyama, K., and Matsubayashi, Y. (2012). Biochemical mapping of a ligand-binding domain within *Arabidopsis* BAM1 reveals diversified ligand recognition mechanisms of plant LRR-RKs. Plant J. *70*, 845-854.

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

- Shinohara, H., and Matsubayashi, Y. Chemical synthesis of *Arabidopsis* CLV3 glycopeptide reveals the impact of Hyp arabinosylation on peptide conformation and activity. Plant Cell Physiol. 2012 Dec 19.

〔Review article〕

- Matsubayashi, Y. (2012). Recent progress in research on small post-translationally modified peptide signals in plants. Genes Cells. *17*, 1-10.

LABORATORY OF NEURONAL CELL BIOLOGY

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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.

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

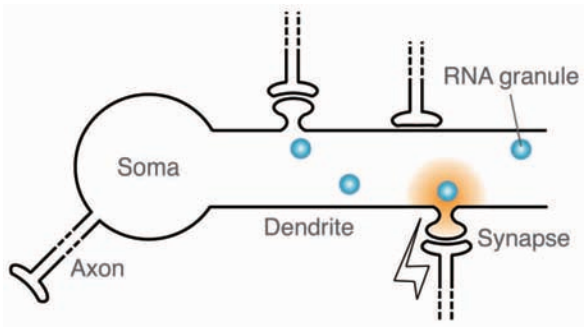

Figure 1. A model for local translation in neuronal dendrites. Specific mRNAs are recruited into RNA granules and transported to dendrites. Translation of the mRNAs is induced locally upon synaptic stimulation, which modifies local postsynapses to regulate synaptic connection and network formation.

I. Knockout mice for RNA granule proteins

We previously identified RNA granule protein 105 (RNG105), an RNA-binding protein, as a component of RNA granules. RNG105 is responsible for mRNA transport to dendrites, which is required for the encoded proteins to be translated and function in dendrites for proper networking of neurons (Shiina *et al*., *J. Neurosci*. **30,** 12816-12830, 2010).

RNG105 knockout mice exhibit reduced dendritic synapse formation and reduced dendritic arborization, which results in poor development of neuronal networks. The knockout neonates die soon after birth due to respiratory failure, which is associated with defects in fetal brainstem development. To investigate the role of RNG105 in higher brain functions, e.g., memory and learning, in adult mice, we generated RNG105 conditional knockout (cKO) mice using the Cre/loxP system. Alpha-CaMKII promoter was used to drive Cre recombinase since its promoter activity was low during embryonic stages but elevated after birth in the brain. We successfully obtained RNG105 cKO mice that grew into adults (Figure 2A). Expression of RNG105 was markedly reduced in the cerebrum, especially in the hippocampal pyramidal neurons of adult cKO mice (Figure 2B). Open field behavior testing revealed that exploratory activity of RNG105 cKO mice in a novel environment was not changed between the first and later trials, although exploratory activity of wild-type mice was reduced with increasing number of trials, suggesting that the knockout mice had some problems in being acclimated to a new environment. We are currently analyzing learning and memory in the cKO mice.

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

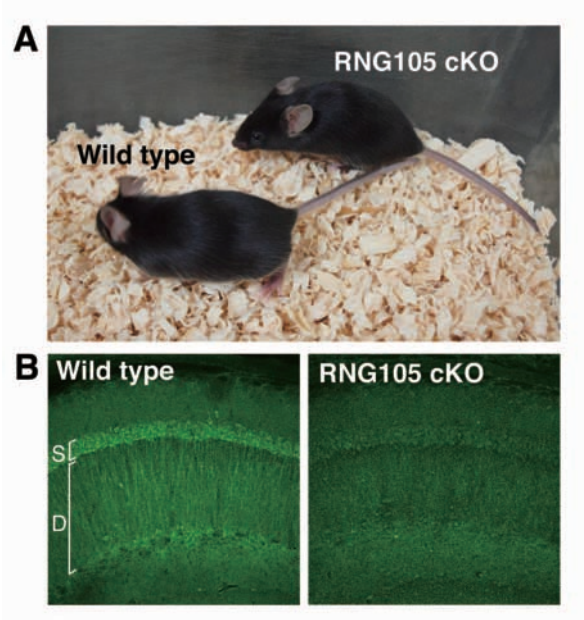

Figure 2. RNG105 conditional knockout (cKO) mice. A, 10-week-old adult wild-type and RNG105 cKO mice. B, Brain hippocampal slices from wild-type and RNG105 cKO mice were immunostained with an anti-RNG105 antibody. S, somatic layer; D, dendritic layer of hippocampal pyramidal neurons. RNG105 was reduced in pyramidal neurons of RNG105 cKO mice.

II. Molecular characterization of the RNA granule complex

RNA granules are abundantly formed in neurons, but not observed in many other types of cells. However, the formation of RNA granules is induced by stress such as oxidation and virus infection in the cells. They are called "stress granules" and have common features with neuronal RNA granules, e.g., they are macromolecular complexes containing ribosomes and mRNAs, and repress translation of cargo mRNAs. We previously found that RNG105 was a component of not only neuronal RNA granules but also stress granules, and further that overexpression of RNG105 in fibroblastic cells induced the formation of stress granules which contain mRNAs. Because knowledge about stress granules helps us in studying neuronal RNA granules, our research includes identification and characterization of molecular components of stress granules.

We have identified a novel component of stress granules, NFAR2. NFAR2 was recruited into RNG105-induced stress granules and enhanced the assembly of the stress granules (Figure 3). It is known that cellular stress activates master kinases including PKR, which induces translation repression and stress granule formation. We found that NFAR2 phosphorylation by PKR enhanced its localization to, and the formation of, stress granules. Furthermore, we found that NFAR2 was bound by its partner NF45 and antagonized its effect on stress granules in basal condition, but it overcame the antagonistic regulation by NF45 when phosphorylated by PKR. Because PKR is reportedly implicated in learning and memory, these results will provide insights into the regulatory mechanism of neuronal RNA granule formation in the brain.

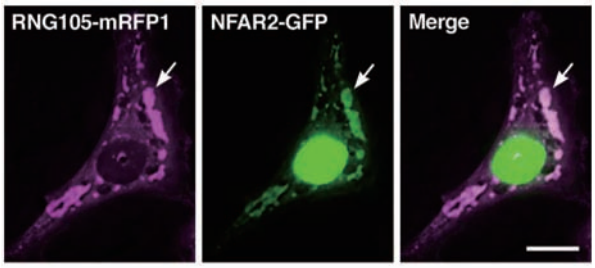

Figure 3. NFAR2 is a component of stress granules. A6 cells were co-transfected with RNG105-monomeric red fluorescent protein 1 (mRFP1) and NFAR2-green fluorescent protein (GFP). Expression of RNG105 induced the formation of cytoplasmic stress granules. NFAR2 was predominantly localized to the nucleus, and it was also recruited into and enlarged the stress granules (arrows). Scale bar, 10 μm.

LABORATORY OF CELL SOCIOLOGY

Assistant Professor
HAMADA, Yoshio

Technical Assistant: *GONDA, Naoko*

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

Primates and rodents have the ancestral type of placenta, classified as heomochorial, in which maternal blood is not contained within endothelial cell lined vessels but rather is in direct contact with epithelial cells of the fetal placenta that are derived from the trophoblast cell lineage. In mice, maternal blood coming out from the spiral arteries invaded by trophoblast cells that replace the endothelium passes across the fetal layers of the placenta and then enters into the labyrinth where fetal blood vessels form and feto-maternal exchange occurs. Maternal and fetal vascular circuits in the established mouse placenta are shown schematically in Figure 1.

Notch2 null mutation induces lethality around embryonic day 11 because of impaired maternal vasculature formation. However, both cellular and molecular events in the process of vasculature formation are little known, especially as to how the maternal blood bed expands among the tightly packed trophoblast mass. Our histological studies showed that a stream of maternal blood along the interface between labyrinthine and spongiotrophoblast was slightly opened in the mutant placenta. Expression of the Notch2 gene is observed in spongiotrophoblast and Giant cells, but in neither the labyrinthine trophoblast or multi-nuclear Syncytiotrophoblast that is derived by cell fusion of the mono-nuclear labyrinthine trophoblast (Figure 2). Thus, we conclude that cellular and molecular events to form the maternal blood bed occur in spongiotrophoblast and Giant cell layers.

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

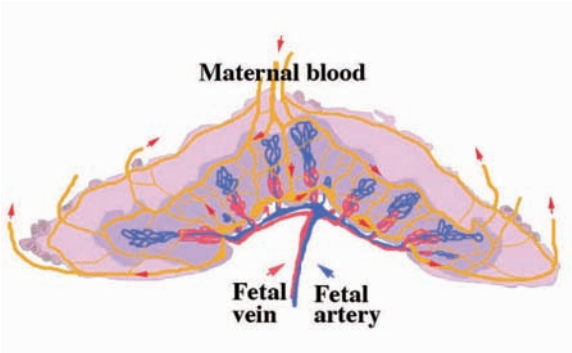

Figure 1. Schematic maternal and fetal vascular circuits in mouse placenta. Maternal blood flows in streams drawn in yellow in the direction shown by small red arrows. Fetal blood running in the artery (blue line) reach at the border spongio- and labyrinth layers and return to fetal vein (red line).

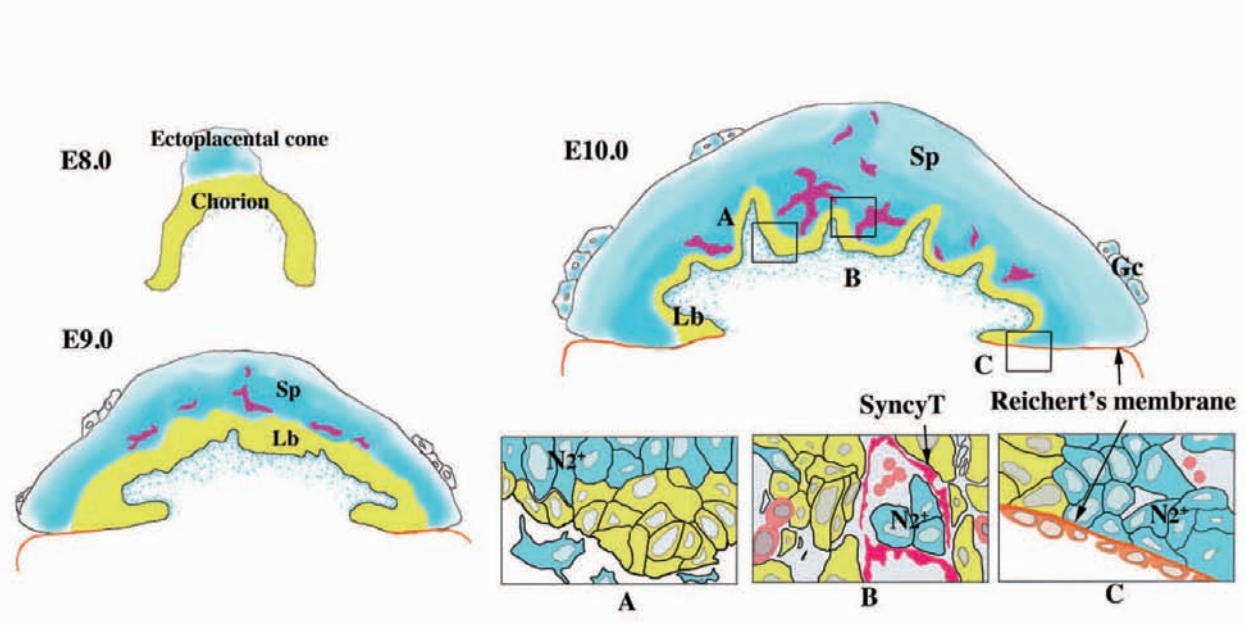

Figure 2. Expression of Notch2 gene in developing mouse placenta. Notch2 gene is expressed in the ectoplacental cone at E8.0, but not in the chorion. At E9.0, the ectoplacental cone and chorion differentiate into spongiotrophoblast (Sp) and labyrinthine trophoblast (Lb), respectively. The expression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

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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 force for morphogenesis

Physical forces are a non-negligible environmental factor that can guide the morphogenesis of organisms. Such forces are generated by tissue-tissue interactions during early development where drastic tissue remodeling occurs. One good example is neural tube formation. In vertebrates, the neural tube that is the primordial organ of the central nervous system and is formed by the bending of the neural plate that is a flat sheet of neuroepithelial cells. The tissue remodeling is driven by cellular morphogenesis in which selected cells in the neural plate change their shapes from cuboidal to an elongated wedge-like shape. Recent studies have revealed that this cell shape change is controlled by cytoskeletal dynamics, namely the remodeling of F-actin and microtubules (Suzuki, M. et al. Dev. Growth Differ., 2012) . In addition, we also found that non-neural deep layer cells that underlie the non-neural ectoderm generate force to bring the two neural folds to the dorsal midline to close the tube (Morita, H. et al. Development, 2012).

Another example is the axial mesoderm, which elongates along the anterior-posterior axis during gastrulation cell movements by which rearrangement of the three germ layers is driven. The axial mesoderm is led by the anteriorly precedent tissue Leading Edge Mesoderm (LEM). When surgically isolated the LEM migrates fairly rapidly toward the predetermined anterior side, while the following axial mesoderm shows little directed tissue migration. We hypothesized that the LEM generates traction force on the following axial mesoderm. To prove the biological significance of the force generated by the LEM, we have removed the LEM, cultured to the end of gastrulation, and found that the movement of the LEM is required for the proper narrowing and elongation of the axial mesoderm (Figure 1).

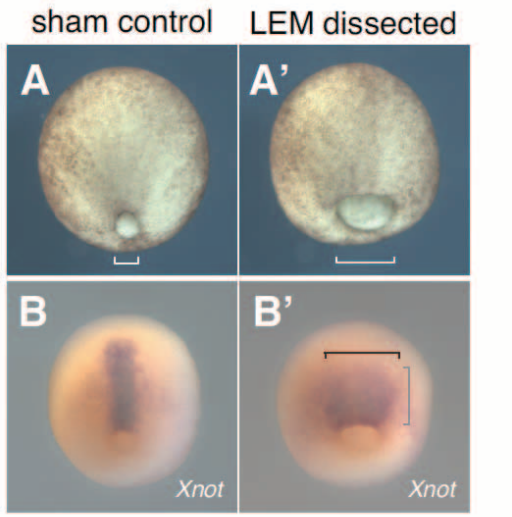

Figure 1. (A, A’) External appearance of early neurula embryos. Sham-operated embryo (A) in which the LEM was remained but not the LEM-removed (A’) embryo closed the blastopore and underwent convergent extension (B, B’, respectively)

II. The roles of PCP core components in mouse development

In epithelia, the roles of planar cell polarity (PCP) have been extensively studied, whereas in non-epithelia, they have yet to be fully understood. We are exploring the roles of PCP in the mesenchymal and neural tissues by using mouse genetics and *RNAi* knock-down technology.

Recently, we generated a hypomorphic allele of mouse *Prickle1*, one of the core PCP factors. As we have reported (Tao, H. et al. Proc. Natl. Acad. Sci., USA, 2009) *Prickle1* null/null mice die around E6.0 in gestation due to the failure of gastrulation. In contrast, *Prickle1* hypomorphic mutant mice survive to P0 in a Mendelian manner but eventually die a day after birth. Interestingly, the mutant mice also displayed abnormal morphology in some tissues where *Prickle1* is expressed. Now, we are investigating the cellular and molecular mechanisms underlying the abnormal morphology. We hope that in combination with comparative genomic analysis, these PCP mutant mice will serve as good models that can explain morphological variations of mammals.

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

During gastrulation, dorsal mesoderm cells migrate toward the midline and align along the anteroposterior axis to form the notochord. In this process, cells change their shape and migrate in a highly coordinated manner. To achieve this systematic cell movement, cell-to-cell interaction must be tightly regulated. The cell adhesion molecule cadherin plays a crucial role in this regulatory system. It is known that of the cadherin family members, paraxial protocadherin (PAPC) is an important molecule that regulates cell adhesion in mesoderm cells. We have found that the localization and the stability of PAPC protein is regulated by the ubiquitin system. The inhibition of E3 ubiquitin ligase SCF/β-TrCP blocks its plasma membrane localization. The inhibition of PAPC ubiquitination by the dominant-negative ubiquitin protein also impaired the plasma membrane localization and weakened cell adhesion of mesoderm cells (Figure 2). Furthermore, this ubiquitin system regulates the localization of another cell adhesion molecule, C-cadherin. Our findings uncovered a novel mechanism of regulation of cell adhesion proteins by the ubiquitin system, which plays a crucial role in the actively-migrating mesoderm cells.

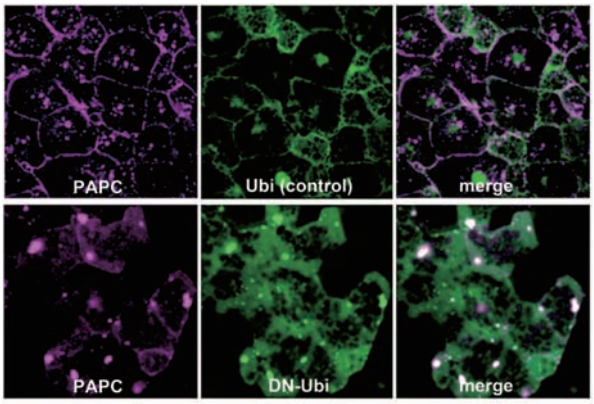

Figure 2. The ubiquitin system regulates PAPC localization and cell adhesion. In the normal mesoderm cells, PAPC localizes at the plasma membrane and cytoplasmic vesicles. Dominant-negative ubiquitin (DN-Ubi), which inhibits polyubiquitination, impairs PAPC localization to the plasma membrane and weakens cell adhesion.

IV. Cellular morphogenesis during neural tube closure

For the morphogenesis of organs, cellular morphogenesis as well as cellular behaviors plays critical roles. During early development of the central nervous system (CNS) in vertebrates, the neuroepithelial cells undergo a typical shape change, called apical constriction (AC), the cumulative action of which cause the neural plate to bend to form the neural tube. In AC, cell apices are contracted and stabilized, causing cells to adopt wedge-like shapes from columnar ones. Recent studies have revealed that AC is controlled by cytoskeletal dynamics, namely the remodeling of F-actin, and non-muscle myosin II activity, yet how AC is dynamically controlled in time and space is not fully understood.

Calcium ions act as second messengers, triggered by both extra- and intracellular cues. The level of cytoplasmic calcium is increased by its influx from either extracellular space or intracellular storage areas such as the endoplasmic reticulum (ER). We found that inhibition of calcium influx delayed neural tube closure (NTC), suggesting that calcium signaling plays an important role(s) in AC. Long-term time-lapse imaging with calcium indicators revealed the dynamic calcium transients throughout NTC. Occurrence of the transients correlated with AC at the cellular level and with the speed of NTC at tissue level. Furthermore, the forced increase of cytoplasmic calcium by caged-compounds caused cell shape change similar to AC. These suggest that calcium is a positive regulator of AC and accelerates NTC to achieve the correct formation of the hollow structure of the primitive CNS.

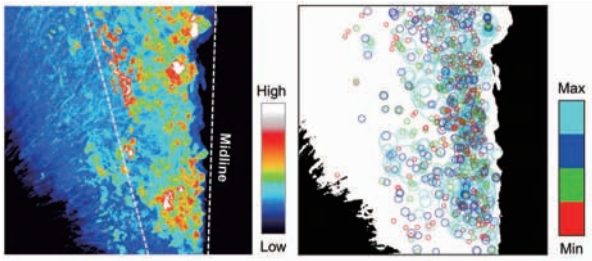

Figure 3. By injecting in vitro transcribed mRNA, we observed the intracellular calcium patterns during *Xenopus* neural tube closure. During neural cells undergoing apical constriction, calcium transients at single cell level dynamically occurred, sometimes resulting in multicellular propagation. (Left) Frequency of calcium transients visualized by pseudocolor, (right) Time-projection of calcium patterns by colored circle representing area of the transients.

V. Notochord and evolution of chordates

Gastrulation is a morphogenetic movement that is essential for the formation of two- or three-germ-layered embryos. *Brachyury* is transiently expressed in the blastopore region, where it confers on cells the ability to undergo invagination. This process is involved in the formation of the archenteron in all metazoans. This is a “primary” function of *Brachyury*. During the evolution of chordates, they gained an additional expression domain at the dorsal midline region of the blastopore. In the new expression domain, *Brachyury* served its “secondary” function, recruiting another set of target genes to form a dorsal axial organ, the notochord (Figure 4). In order to better understand the molecular mechanisms underlying the origin of the notochord during chordate evolution, we are currently investigating to compare the *Brachyury* gene regulatory networks of hemichordates (a nonchordate deuterostome closest to chordates) and cephalochordates (the most basal chordate).

VI. Epigenetic modification in the invertebrate genome

Epigenetic modifications, such as DNA methylation and histone modification, alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. These processes are crucial to normal development and

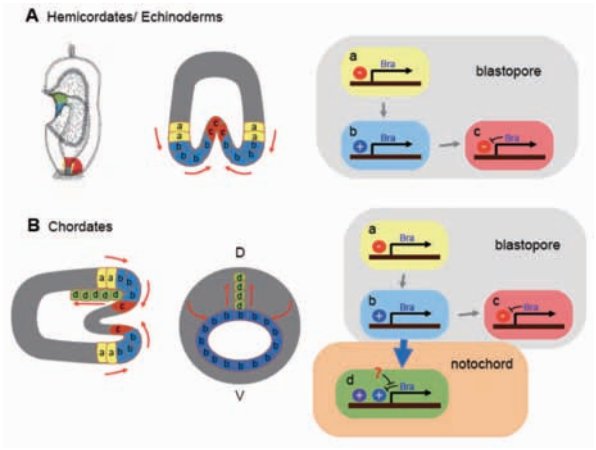

Figure 4. A schematic representation of the innovation of secondary *Brachyury* function that leads the notochord formation during chordate evolution. (A) *Brachyury* appears to be expressed continuously in the blastopore, but engaged cells never continue expressing the gene. (B) The expression of *Brachyury* in the cells fated to form the notochord is not transient, but instead continues till the process of notochord differentiation progresses to some extent.

differentiation of distinct cell lineages in mammals. In invertebrates, gene coding regions are the primary targets of DNA methylation, but the role of DNA methylation in actively transcribed genes is unknown. We investigated the tissue variability of both the global levels and distribution of 5mC in the seasquirt *Ciona intestinalis*. We found that global 5mC content of early developmental embryos is high, but is strikingly reduced in body wall tissues. We chose sperm and adult muscle cells, with high and reduced levels of global 5mC respectively, for genome-wide analysis of 5mC targets. By means of CXXC-affinity purification followed by deep sequencing (CAP-seq), and genome-wide bisulfite sequencing (BS-seq), we designated body-methylated and unmethylated genes in each tissue. Surprisingly, body-methylated and unmethylated gene groups are identical in the sperm and muscle cells. We conclude that gene body methylation is not a direct regulator of tissue specific gene expression in *C. intestinalis*. Instead, methylated genes are often stably or maternally expressed. Moreover, we demonstrate that transgenes can be modified by gene body methylation, when their expression is driven by promoters of endogenously body-methylated genes. Our findings reveal constant targeting of gene body methylation irrespective of cell types, and they emphasize a correlation between gene body methylation and ubiquitously expressed genes.

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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 function in *Drosophila*.

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

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

II. Mechanism regulating sex determination of PGCs

It is widely accepted in mammals and *Drosophila* that male sexual development is imposed in PGCs by the sex of the gonadal soma, and that PGCs assume a female fate in the absence of a masculinizing environment. How PGCs initiate female development, however, is a long-standing question in reproductive and developmental biology.

We have reported that *Sex lethal* (*Sxl*) was expressed in XX female, but not XY male PGCs, during their migration to the gonads. To determine whether *Sxl* induces female development in XY PGCs, we induced *Sxl* expression in XY PGCs using *nanos-Gal4* and *UAS-Sxl*, and transplanted these PGCs into XX females. We found that these XY PGCs entered the oogenic pathway and produced mature oocytes in XX females. These oocytes contributed to progeny production. In contrast, XY PGCs did not enter the oogenic pathway. These observations demonstrate that *Sxl* expression in XY PGCs during embryogenesis induces functional egg differentiation in the female soma.

Our findings provide powerful evidence for *Sxl* as a master gene that directs a female germline fate. Experiments for identifying the downstream genes regulated by *Sxl* and the upstream genes inducing Sxl only in XX female PGCs are in progress.

III. The role of heparan sulfate proteoglycans in JAK/ STAT signaling and distribution of its ligand, Unpaired.

The JAK/STAT pathway plays vital roles in development and homeostasis in animals. *Drosophila*, with its complete set of JAK/STAT components, provides a powerful genetic system to analyze the molecular functions of this essential pleiotropic pathway. In addition to its functions in embryonic, larval, and imaginal development, JAK/STAT signaling plays a critical role during several steps of *Drosophila* oogenesis. First, JAK activity is necessary in the somatic cells of the GSC niche to regulate production of the BMP signal that maintains GSCs. Furthermore, in the germarium, JAK signaling is required for maintenance and function of the supporting somatic escort cells. As cysts exit the germarium, JAK activity regulates the formation of stalk cells that separate developing egg chambers. Later in oogenesis, constant JAK activity is necessary for proper migration of the border cell cluster towards the posterior of the egg chamber. In ovaries with reduced JAK activity, there is a reduction in the number of border cells and stretched cells (terminal fates), and a concomitant increase in the number of main body cells. Furthermore, increased JAK activity from overexpression of the Janus kinase, Hop, or the Upd ligand causes cells to adopt terminal fates within the follicular epithelium. These results are consistent with the observed graded activation of JAK in the follicular epithelium and suggest that Upd acts as a morphogen during oogenesis. However, it has not been shown whether this

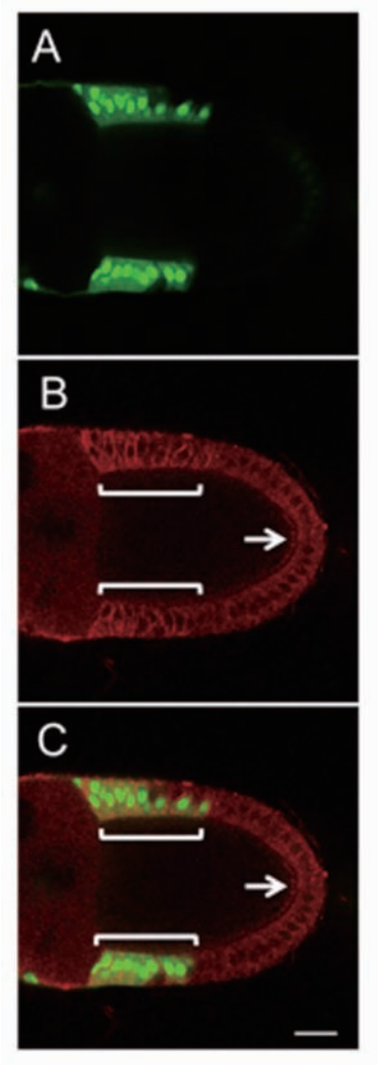

Figure 1. Dally regulates distribution of Upd.
(A-C) Distribution of Upd protein in *dally*-over expressed ovary. (A) *dally*-overexpressed clone (Green). (B) Upd protein distribution (Red). Upd is normally distributed at the apical surface of posterior follicle cells (Arrow). Upd accumulate on the cell surface of dally-over expressed clone (Brackets). (C) Merged image of A and B.

putative morphogen forms a concentration gradient that reflects the pattern of JAK activation.

Morphogens are important molecules in development and are defined by their ability to direct different cell fates over a distance in a concentration-dependent manner. It has been well established that extracellular signaling molecules of the Wingless/Wnt, Hedgehog (Hh), and Bone Morphogenetic Protein (BMP) families act as morphogens during *Drosophila* development. Despite extensive studies on the activities of these morphogens, it is not fully understood how these molecules generate and maintain their gradients in a tissue. A class of molecules that affect the gradient formation of all these morphogens is heparan sulfate proteoglycans (HSPGs). HSPGs are a family of carbohydrate-modified proteins abundantly found in the extracellular matrix and on the cell surface. Three families of HSPGs are widely conserved during animal evolution: syndecans, glypicans, and perlecans. In particular, two *Drosophila* HSPGs of the glypican family, *dally* and *dally-like protein* (*dlp*), have been shown to control BMP, Wnt, and Hh signaling. A previous study has shown that Upd protein expressed in cultured cells is tightly associated with the extracellular matrix and the addition of free heparin releases Upd into the medium. These observations suggested that Upd normally associates with HSPGs and thus may be regulated in a mechanism analogous to other morphogens.

We demonstrated that Upd indeed forms an extracellular gradient that activates JAK in a concentration dependent manner. As is the case for other secreted morphogens, Upd signaling was regulated by glypicans. Mutations in *dally* and *dlp* or in the HS biosynthetic enzymes, *sulfateless* (*sfl*) and *HS 2-O sulfotransferase* (*Hs2st*) led to aberrant JAK/STAT pathway activation and disruption of stalk cell specification. These alterations in JAK/STAT signaling and cell differentiation can be attributed to effects on the normal extracellular gradient of Upd by loss or changes in modification of the glypicans. Biochemical and histochemical studies showed that Dally and Upd physically bind to each other and co-localize on the surface of *Drosophila* S2 cells. In vivo, Upd accumulation on cells lacking glypicans was dramatically reduced, and reciprocally was enhanced upon ectopic expression of Dally (Figure 1). These results suggest that *Drosophila* glypicans serve to stabilize a novel morphogen, Upd, at the cell surface during oogenesis.

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

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

We identified *dally* and *dlp* as important components of the GSC niche in both sexes. In the female GSC niche, *dally* was expressed in niche cells. On the other hand, both of the glypicans were expressed in male GSC niche cells. Mutations for these glypicans caused a significant reduction in GSC number in both ovary and testis. In the *dally* mutant ovary, GSC lost appropriate activation of the signaling pathway by Dpp (a BMP homologue acting as a niche signal in female GSC niche). Conversely, ectopic expression of *dally* in female gonads caused an increase in GSC number with ectopic activation of the Dpp signaling pathway. These

results strongly suggest that *dally* defines the female GSC niche by regulating distribution of Dpp.

To address this, we have been trying hard to visualize Dpp distribution in the female GSC niche. By modifying protocols for antibody staining and generating new antibodies, we succeeded in visualization of Dpp distribution. We found that Dpp distribution was significantly expanded when *dally* was ectopically expressed in female gonads, while Dpp-producing cells were unaffected (Figure 2). The above results support our model that glypicans define the GSC niche by regulating extracellular distribution of niche signals.

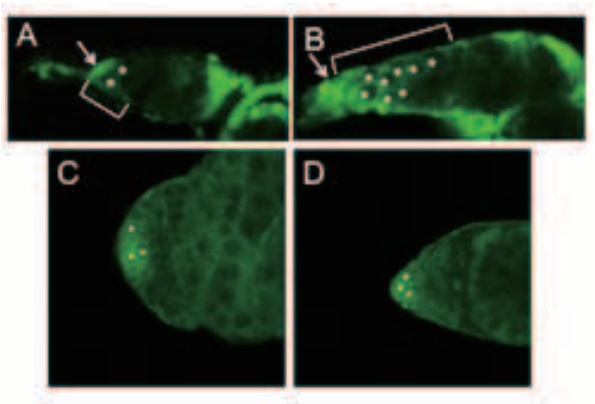

Figure 2. Distribution of GSC niche signals.
(A,B) Dpp distribution in distal tip region (germarium) of normal ovary (A) and of ovary expressing *dally* in the somatic cells throughout germarium (B). Green signal indicates Dpp protein (Brackets). Dpp distribution is expanded in *dally*-expressing ovary, compared to that observed in normal ovary. Arrows show niche cells, which are the source of Dpp. Asterisks indicate GSCs. (C, D) Distribution of male GSC niche signals, Upd (Green, C) and Gbb (Green, D). Distribution of both niche signal was limited within male GSC niche. Asterisks indicate GSCs.

We also investigated molecular function of glypicans in the male GSC niche. We found that *dlp* is required for signaling pathway by Gbb (the other BMP homologue acting as a niche signal in male GSC niche). Upd is also known as male GSC niche signal. Since our data showed that *dally* regulates the morphogen gradient of Upd (see section III), we speculate that *dally* and *dlp* separately regulate Upd and Gbb distribution in the male GSC niche, respectively. For the first step to address this possibility, we tried to visualize the Upd and Gbb distribution in the male GSC niche. We found that Upd and Gbb were both enriched in the male GSC niche (Figure 2). By utilizing this system, we are now investigating whether these glypicans define the male GSC niche via regulating distribution of niche signals.

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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." Our laboratories aim to understand the molecular mechanism underlying pattern formation by several different approaches.

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

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

I. Lipid modification and extracellular trafficking of Wnt proteins

The Wnt family of secreted signal proteins plays a key role in numerous aspects of embryogenesis. Most Wnt proteins transmit their signals locally in most of the cases where they function, presumably since their secretion and transport are under tight control. One important step regulating the extracellular transport of various secreted signal proteins involves post-translational modification with lipid moieties. We found that murine Wnt-3a is modified with a mono-unsaturated fatty acid, palmitoleic acid, at a conserved Ser residue. 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). Thus, the Wnt protein appears to require 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.

The palmitoleoylation requires an enzyme, Porcupine (Porcn), a member of the family of membrane-bound O-acyltransferases (MBOAT). The *Porcn* gene was first identified as a segment polarity gene, like *wingless*, in *Drosophila* and is evolutionally conserved from worms to mammals. Porcn proteins localize to the endoplasmic reticulum (ER) and are required for Wnt trafficking from the ER in mammalian cells and probably in *Drosophila* embryos. Porcn overexpression promotes lipid-modification of Wnt1 and Wnt3a and causes a steepened Wnt gradient in the chick neural tube, suggesting its important function in vertebrate embryogenesis.

To reveal the biological significance of palmitoleoylation by Porcn, we used zebrafish as a model system and examined the effects of defects in Porcn function on Wnt signals mainly in early embryonic stages because the roles of Wnt signals have been precisely characterized in early zebrafish embryos. We identified two zebrafish homologs of *porcupine, porcn* and *porcupine-like (porcn-l)*. Zebrafish *porcn*, but not *porcn-l*, restores secretion of Wnt proteins in

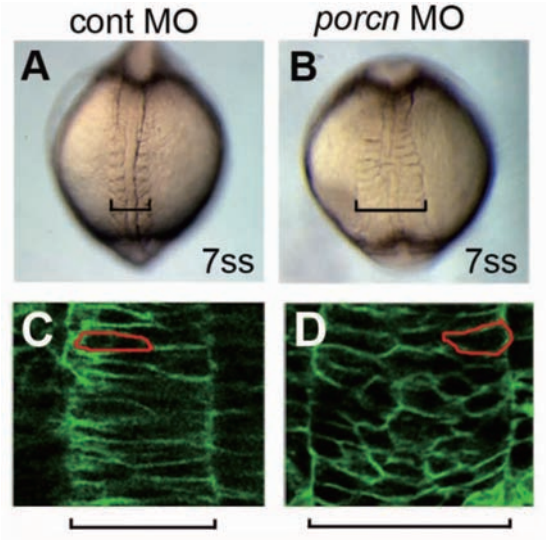

Figure 1. Porcn deficiency causes abnormalities in the convergence and extension. Zebrafish embryos injected with control (A, C) and Porcn-specific (B, D) MOs were shown, respectively. The notochord was expanded and shortened due to defect in the convergence and extension movement during gastrulation. The brackets indicate the notochords.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

porcn-deficient mouse L cells. Morpholino-mediated knockdown of *porcn* in zebrafish embryos impairs convergence and extension (CE) during gastrulation without changing embryonic patterning (Figure 1). Moreover, *porcn* interacts genetically with *wnt5b* and *wnt11* in regulating CE. In contrast, *porcn*-deficient embryos do not exhibit phenotypes caused by failure in canonical Wnt signaling, which is activated by several Wnt ligands, including Wnt3a. Furthermore, expression of genes regulated by the canonical Wnt signaling pathway is not perturbed in knockdown embryos relative to that in the controls. While the trafficking and lipidation of ectopically expressed zebrafish Wnt5b and mouse Wnt5a are impaired in *porcn*-deficient embryos, those of ectopically expressed Wnt3a are less or not affected (Figure 2). In addition, the secretion of Wnt5a is inhibited by less porcn inhibitor than that of Wnt3a in 293 cells. Thus, decrease of Porcn activity does not equivalently affect trafficking and lipidation of different Wnt proteins in zebrafish embryos and in mammalian culture cells. These results suggest that the mechanism of trafficking and modification of Wnt proteins appear to be inconsistent between different types of Wnts. We are currently examining the molecular mechanism underlying the variability of Wnt modification.

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

II. Molecular mechanism of somite development.

The molecular mechanism underlying the periodical formation of somites is coupled to an internal oscillator, referred to as the "segmentation clock," which has been evidenced by the cyclic expression of genes in the presomitic mesoderm (PSM). For example, *hairy/Enhancer of split* (*Espl*)-related bHLH genes, including *her1* and *her7* in zebrafish, are expressed in a dynamic pattern of stripes across the PSM in a posterior to anterior direction. The oscillating and anteriorly propagating wave of gene expression, which is maintained in the posterior PSM, becomes fixed to cause segmentation in the anterior PSM. Prior to morphological segmentation, a segmental 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 two Ripply genes, Ripply1 and Ripply2 play essential roles in this patterning. To gain insight into the mechanism of somite segmentation, we are examining the segmental patterning in zebrafish embryos by focusing on Ripply and related molecules.

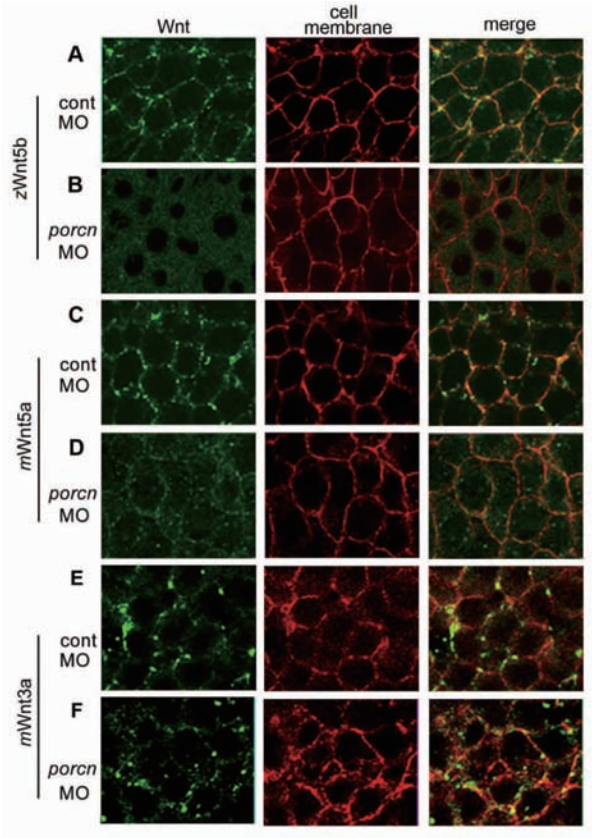

Figure 2. Secretion of Wnt proteins in zebrafish embryos. Confocal images of localization of EGFP-tagged zebrafish Wnt5b (A, B), mouse Wnt5a (C, D), and mouse Wnt3a (E, F) with membrane markers, in epiblast cells of embryos injected with 5mis control MO (A, C, E,) or *porcn* MO (B, D, F) are shown. Merged images of Wnts and membrane markers are also presented. Secretion of Wnt5a, but not Wnt3a, is defective in Porcn-deficient embryos.

III. Molecular mechanism of mesoderm development from stem cell-like progenitors.

The posterior body of the vertebrate is generated from progenitor cells residing in the tailbud. The mesodermal progenitor cells (MPCs), which appear to have stem cell-like characteristics, continuously produce the presomitic mesoderm (PSM) cells, which further differentiate into somites. Accumulating evidence has revealed the molecular mechanism underlying the maintenance of the undifferentiated state of the MPCs. For instance, in zebrafish embryos, *wnt* and zebrafish orthologues of brachyury, *no tail (ntl)* and *bra*, mutually activate their expressions in the MPCs and this autoregulatory loop is essential for maintenance of the undifferentiated state of the MPC.

On the other hand, the molecular mechanism promoting MPC differentiation into PSM cells should also be elucidated for a better understanding of the MPC-based development of the paraxial mesoderm. Of note, genetic studies with the zebrafish have indicated that the molecular mechanisms underlying the development of somites are not the same between trunk and tail. For instance, *spadetail (spt)/tbx16*

mutant embryos are impaired in the development of their trunk somites, but generate relatively normal tail ones. However, in contrast to this phenotype restricted to the trunk somites, other evidence suggests that *spt* is required for PSM differentiation in both trunk and tail somites and that some additional factors compensate the loss of Spt function during tail development. Therefore, for understanding the molecular mechanism that controls the maintenance and subsequent differentiation of the MPCs, it is important to reveal the function of Spt and these additional factors during the development of tail somites, especially in terms of their interaction with the Wnt/Brachyury autoregulatory loop.

One candidate as an additional factor seems to be Mesogenin1 (Msgn1), which is a bHLH transcription factor expressed in the PSM. Interestingly, mouse embryos deficient in functional Msgn1 have impaired development of their posterior somites, in spite of having normal formation of the first 7 somites, as well as show an abnormal accumulation of an undifferentiated cell mass at the tip of their tail. Thus, Msgn1 seems to be involved in PSM differentiation during the development of posterior, or tail, somites. However, it is still uncertain as to how the differentiation from the MPCs to PSM cells is controlled by *msgn1* during somite development. Furthermore, it has remained to be elucidated whether zebrafish *msgn1* interacts with *spt* during PSM differentiation during tail development.

We assessed the functions of *msgn1* in zebrafish development by injecting *msgn1* specific MO into wild-type and *spt* mutant eggs. Zebrafish embryos defective in *msgn1* and *spt* failed to differentiate into PSM cells in tail development and show increased expression of *wnt8* and *ntl* (Figure 3). Msgn1 acted in a cell-autonomous manner and as a transcriptional activator in PSM differentiation. The expression of *msgn1* initially overlapped with that of *ntl* in the ventral tail bud, as previously reported; and its mis-expression caused ectopic expression of *tbx24,* a PSM marker gene, only in the tail bud and posterior notochord, both of which expressed *ntl* in zebrafish embryos. Furthermore, the PSM-inducing activity of misexpressed *msgn1* was enhanced by co-expression with *ntl*. Thus, Msgn1 exercised its PSM-inducing activity in cells expressing *ntl*. Based on these results, we speculate that *msgn1* expression in association with that of *ntl* may allow the differentiation of progenitor cells to proceed during development of somites in the tail.

Publication List

〔Original papers〕

- Chen, Q., Takada, R., and Takada, S. (2012). Loss of Porcupine impairs convergent extension during gastrulation in zebrafish. J. Cell Sci. *125*, 2224-2234.
- Chiu, C.H., Chou, C.W., Takada, S., and Liu, Y.W. (2012). Development and fibronectin signaling requirements of the zebrafish interrenal vessel. PLoS ONE *7*, e43040.
- Yabe, T., and Takada, S. (2012). Mesogenin causes embryonic mesoderm progenitors to differentiate during development of zebrafish tail somites. Dev. Biol. *370*, 213-222.

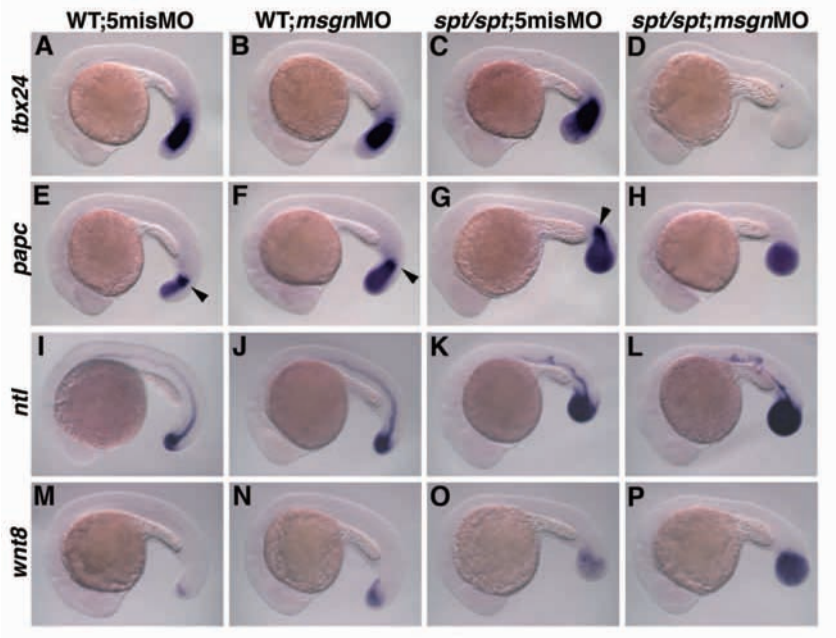

Figure 3. *msgn1* and *spt* are required for the differentiation of posterior mesoderm cells in zebrafish. Wild-type embryos injected with control (5mis) MO (A, E, I, and M) or *msgn1* MO (B, F, J, and N) and *spt* homozygous embryos injected with 5mis MO (C, G, K, and O) or *msgn1* MO (D, H, L, and P) at the 24-somite stage were hybridized with *tbx24* (A-D), *papc* (E-H), *ntl* (I-L) or *wnt8* (M-P) probes. The expression of paraxial mesoderm markers, *tbx24* and *papc* in the PSM is severely reduced in the *msgn1* MO-injected *spt* homozygous embryo (D, H). Injection of *msgn1* MO into wild-type embryos increased the expression area of *ntl* and *wnt8,* both of which are expressed in the mesoderm progenitor cells in the tailbud, in the tailbud (J, N) compared with that for the control (5mis) MO (I, M). In addition, *ntl* and *wnt8*-expressing cells are also increased by *msgn1* MO in *spt* mutant embryos (K, L, O, and P).

DIVISION OF EMBRYOLOGY

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

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

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

To understand the mechanisms underlying embryonic development and morphogenesis, it has become common to observe the behavior of cells and gene expression in living embryos in a variety of animal species. Progress in GFP technologies, genetic engineering and embryonic manipulation concomitantly with the progress in optics and microscope technologies, including development of highly sensitive photo-detectors, has allowed us to observe developing embryos live, even in mammals. It is useful to visualize nuclei, cell shapes, cytoskeleton or other organelles to observe cells and cell behaviors in living mouse embryos. We have established a series of transgenic mouse lines for live imaging, which is a part of a collaborative project taking place in the Laboratory for Animal Resources and Genetic Engineering, Riken CDB. In each mouse, cDNA encoding fusion protein with fluorescent protein and a localization sequence were inserted into Rosa26 locus. The sequence for the fusion protein was following stop sequences that are surrounded by loxP sites on both sides. These loxP sites can be recognized by an enzyme, Cre recombinase, that catalyzes recombination between two loxP sites to remove the stop sequences. Thus, when Cre recombinase is activated in a spatial-temporally specific manner, the following reporter fluorescent proteins are expressed in a specific way. And once this irreversible reaction is induced in the germ line, the derived offspring possess the transgene without the stop sequence and express the reporter fusion protein ubiquitously.

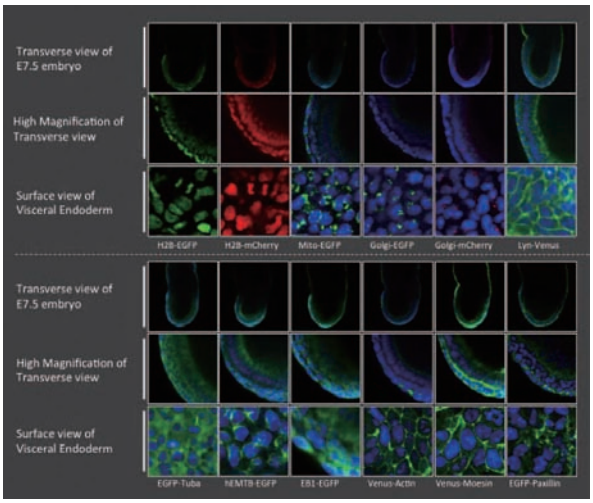

Figure 1. Examples of 7.5 day embryos expressing reporter fluorescent fusion proteins ubiquitously. These embryos express fusion proteins shown on the bottom.

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

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

We are trying to observe and reveal aspects of cell shape, morphogenesis, cell lineage, gene expression and cell differentiation by combining these techniques.

II. Histological observation of mouse embryos developing in the uterus

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

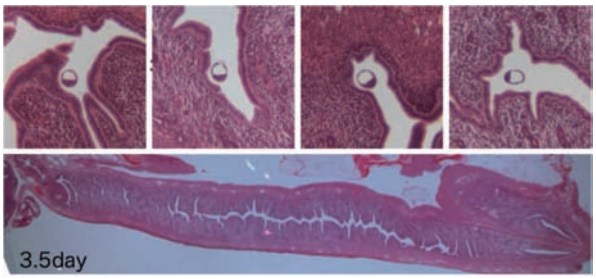

Figure 2. A pregnant uterus 3.5 days after fertilization. Developing embryos and the shape of uterus epithelium can be observed.

III. Studying early development of rabbits as a new model of mammalian embryogenesis

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

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

The oviducts (fallopian tubes) are tubes connecting the periovarian spaces and the uterine horns. The ova released from the ovary are transported through the oviduct, where fertilization occurs with the spermatozoon moving from the uterus. We are focusing on the region of the oviduct close to the opening to the ovary. In this region many multi-ciliated cells exist. These cilia move in one direction along the ovary-uterus axis. This directional movement of multi-cilia might play a major role in the transportation of ovum from periovarian space, although muscle contractions also play roles in the region close to the uterus. This suggested that the ovum was transported by the directionally beating cilia. This directional beating of cilia was based on the polarity of the microtubule assembly in the cilia. Skeletal microtubules in the cilia are arranged in a “9 +2”array, and the central two bundles are aligned facing the same direction in each cell. This suggests that oviduct epithelial cells possess polarity along the cell surface parallel to the longitudinal axis of the oviduct; this type of cellular polarity is called “Planner Cell Polarity (PCP)”. We have been studying how PCP is established during development, and how this polarity is maintained over a long period in later stages.

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

IV. Analysis of mechanical properties of cells during embryonic development

Mechanics is one of the essential components for biological processes including cell shape transformation, tissue morphogenesis etc. However, how mechanical properties such as force and material stiffness regulate these processes is poorly understood. To approach this problem, measuring cellular geometric information and mechanical properties are necessary. We developed an image processing based technique to measure cellular geometric information from fluorescent microscopic images and a framework to theoretically estimate the mechanical properties. The image processing technique enabled us to robustly detect cell contour from images with high noise and non-uniform illumination, although the algorithm of the technique is quite simple. By employing the image processing technique, we successfully extracted geometric information of early embryonic cells during cytokinesis in *C. elegans* and of mouse cells in cell sheets. In the framework for estimating mechanical properties, geometric information was combined with a mechanical simulation, which was technically based on the data assimilation (Figure 3). We spatio-temporally estimated cell surface stiffness during cytokinesis by systematically fitting the *in vivo* cell shape to the mechanical simulation. We found that cell polar and equatorial regions were stiffer and softer, respectively. Further theoretical

modeling showed that the relative difference of stiffness between the two regions could be a primary determinant for cleavage furrow ingression during cytokinesis. We speculated that the relative contributions of cell surface stiffness and the contractile ring could explain the contractile ring dependent and independent cytokinesis.

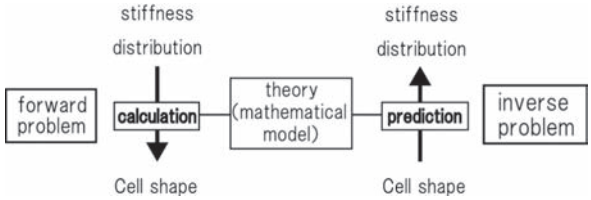

Figure 3. Theoretical estimation of cell surface stiffness. Schematic illustration of estimation.

Publication List

〔Original papers〕

- Bashar, K., Komatsu, K., Fujimori, T., and Kobayashi, T.J. (2012). Automatic extraction of nuclei centroids of mouse embryonic cells from fluorescence microscopy images. PLoS ONE, 7, e35550.
- Cao, L., Kobayakawa, S., Yoshiki, A., and Abe, K. (2012). High resolution intravital imaging of subcellular structures of mouse abdominal organs using a microstage device. PLoS ONE, 7, e33876.
- Koyama, H., Umeda, T., Nakamura, K., Higuchi,T., and Kimura, A. (2012). A high-resolution shape fitting and simulation demonstrated equatorial cell surface softening during cytokinesis and its promotive role in cytokinesis. PLoS ONE, 7, e31607.

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Mammalian testes produce numerous sperm for a long period. This property that is fundamental for continuity of life across generations is supported by the robust stem cell system, which is defined by the ability of proliferation that is associated with self-renewal (maintenance of their own undifferentiated population) and differentiation (production of different cell type(s)),

The Division of Germ Cell Biology, which was launched in 2008, aims to fully understand the mouse sperm stem cell system *in vivo* under the context of tissue architecture of testis. So far, we have revealed some characteristics of this potent and interesting stem cell system. First, differentiating germ cells that had been believed to be irreversibly committed to differentiation still retain the self-renewing potential and can contribute to stem cell pool maintenance ("potential stem cells"). Secondly, "reversion" from potential stem cells occurs at a higher frequency when testicular tissue is damaged and regeneration is induced. Thirdly, the undifferentiated spermatogonia population that includes both "actual" and "potential" stem cells localized to the vasculature (vascular- associated niche). We also discovered that stem cells turn over in an unexpectedly frequent and mathematically stochastic manner under steady-state situations that continuously produce sperm. (Nakagawa et al., Dev. Cell 2007, Yoshida et al., Science 2007; Nakagawa et al., Science 2010; Klein et al., Cell Stem Cell 2010).

In 2012, we have extended these investigations for the deeper understanding of this stem cell system. Firstly, we have investigated the nature and the behavior of the stem cells at a single cell resolution. Secondly, we have challenged the cellular and molecular nature of the vascular-associated niche. Thirdly, a mechanism underlying the coordinated spatio-temporal regulation of stem cells has been proposed.

I. Stem cell behavior at a single cell resolution in undisturbed testicular tissue

For the purpose of function-based investigation of stem cells, it is essential to analyze the behavior of cells over time. We have previously established experimental systems to study stem cell behavior in mouse spermatogenesis over time, without affecting the tissue architecture, namely live-imaging and pulse-labeling experiments. For live-imaging, taking advantage of the fluorescent protein-labeling, we have achieved continual filming of particular subsets of spermatogonia in the testes (Figure 1: Yoshida et al., Science 2007). For pulse-labeling, we have also established irreversible labeling of a particular spermatogonial population using tamoxifen-dependent activation of cre recombinase fused with a domain of the mutated human estrogen receptor (Figure 2: Yoshdia et al., Development 2006, Nakagawa et al., Dev. Cell 2007, Nakagawa Science 2010).

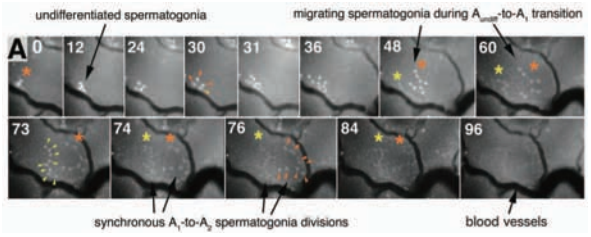

Figure 1. Live-imaging of fluorescence-labeled cells. An example of live-imaging observation of spermatogonia in a living testis. Spermatogonia labeled with GFP under Ngn3 promoter (white) were continuously filmed, shown cell division (30h and 76h) and active migration. A part of selected frames are shown. Numerals indicate the elapsed time in hours. (From Yoshida et al. Science 2007)

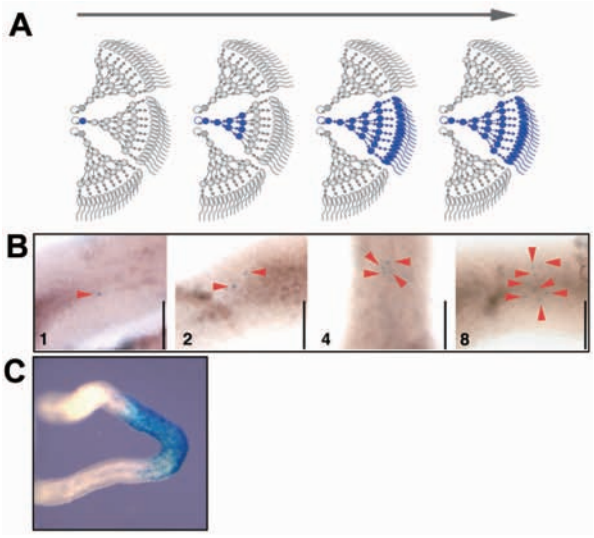

Figure 2. Pulse-labeling of subset spermatogonia with marker expression. (A) Principle of pulse-label of a stem cell. When a self-renewing stem cell is pulse-labeled, its descendants (shown in blue here) include both stem and differentiating cells. Then, the clone will persist in the tissue, as shown in the actual experiments in the following panels. (B) Spermatogonia labeled with LacZ expression (colored in blue) observed 2 days after induction of labeling with Tamoxifen administration. A_{single} (1), A_{pair} (2), $A_{aligned\text{-}4}$ (4), $A_{aligned\text{-}8}$ (8) are shown. (C) Three months after the pulse, labeled cells that are descendant of the induced spermatogonia form patches occupying a continual segment of the tubules including stem and differentiating cells. This reflects the stem cell function of the originally labeled cell. (From Yoshida Reproduction 2012 and Nakagawa et al., Dev. Cell 2007)

In addition, our results strongly suggest that within the undifferentiated group of spermatogonia consisting of A_{single}, A_{paired} and $A_{aligned}$ cells, a small subset expressing GFRα1 (glial cell line-derived neurotropic factor receptor alpha 1) is responsible for the stem cell functions, while those expressing Ngn3 (neurogenin 3, a bHLH transcription factor) are destined for differentiation while maintaining their potential to get back into being GFRα1-positive and self-renew (Nakagawa et al., 2010).

In addition, in collaboration with Professor Benjamin Simons, a theoretical physicist of Cambridge University, we have shown that the spermatogenic stem cells show a stochastic and continuous turnover between neighbors (Nakagawa et al., Dev. Cell 2007, Klein et al., Cell Stem Cell 2010): Instead of the 'text book' type of asymmetric divisions that always give rise to one self-renewing and one differentiating daughter, fate selection for stem cells either to remain undifferentiated or to differentiate appears to be stochastic. As a result, fates of stem cell-derived cohorts are highly variable and show neutral competition between stem cell clones.

Based on these preceding findings, we began investigating the behavior of GFRα1+ spermatogonia in undisturbed testis using live-imaging and pulse-labeling at a single cell resolution. These have allowed us to directly demonstrate for the first time that this tiny subset of spermatogonia indeed acts as the stem cell pool supporting the steady-state turnover of spermatogenic cells. We are now intensively challenging the problem of how to link the actual single-cell-level behavior (such as cell division, fragmentation of spermatogonial syncytia, fate selection, whether to differentiate or remain undifferentiated, and death) of the GFRα1+ spermatogonia and the overall robustness of the stem cell system at a level of population, with the aid of mathematical modeling.

II. Nature of the stem cell niche microenvironment in the mouse testis

It is generally believed that some specialized microenvironment within tissue is involved in the stem cell system as an indispensable element that regulates the stem cell behavior, designated as the "stem cell niche". In contrast to well-defined examples of the stem cell systems accompanying anatomically defined niches where stem cells always settle and differentiation accompanies their exit from the niche region, such as *Drosophila* gonads, mammalian testis do not exhibit such a defined substructure. Therefore it has long been a mystery how stem cell behavior is regulated in the context of this tissue, namely the seminiferous tubules (Figure 3). Regarding this issue, it has been shown that, within the 'basal compartment' of seminiferous tubules, the undifferentiated spermatogonia show a preferential localization to the area adjacent to blood vessels that make a network running through the interstitial space between the seminiferous tubules (Chiarini-Garcia, Biology of Reproduction 2001, Yoshida et al., Science 2007). However, again, lacking a discrete niche structure, this example of a stem cell niche has been considered to stand on a facultative nature.

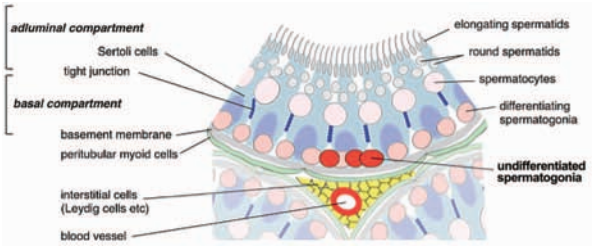

Figure 3. Architecture of seminiferous tubules, the spermatogenic center in the testis. Schema of seminiferous tubules. In basal compartment between the basement membrane and tight junction between Sertoli cells, undifferentiated spermatogonia (the population responsible for the stem cell pool) show a preferential localization to blood vessels running between the tubules. The differentiating progeny of the stem cells stratify into four layers, each of which is comprised of mitotic spermatogonia, meiotic spermatocytes, and haploid round and elongating spermatids. (Modified from Spradling et al, 2011)

Based on these preceding observations of our own and others, we hypothesize that the blood vessel-adjacent area is specialized and optimal for harboring and regulating the stem spermatogonia, and that this feature is reflected by the specialized gene expression in this area. We therefore have been challenging this issue by searching for genes showing localized expression to such a presumptive niche area. We first collected the area of interest using microdissection techniques on sectioned testis specimens, followed by cDNA microarray and *in situ* hybridization analyses to search for genes showing preferential expression. We have been studying these genes with regard to the spatial relationship between the sites of expression of these genes with undifferentiated (and stem) spermatogonia, as well as the possible function of these genes in stem cell regulation. These findings will provide basic and fundamental information on stem cell regulation in tissues harboring facultative niche.

III. Spatio-temporal stem cell regulation by retinoic acid metabolism

In seminiferous tubules, differentiation of Ngn3-positive undifferentiated spermatogonia into Kit-positive stage occurs periodically with an interval of 8.6 days, which is followed by programmed differentiation process toward spermatozoa that takes 35 days. This temporal regulation is essential for the integrity of the tissue and continuity of spermatogenesis in that this process supports the regular stratification of germ cells in the wall of seminiferous tubules from stem cells to mature testis (Figure 3).

Based on the classic finding that retinoids (whose prominent active form is retinoic acid) are essential for the timed differentiation of undifferentiated spermatogonia, we investigated how the differentiation of spermatogonia is regulated by retinoic acid signaling, taking advantage of experimental modulation of retinoid supply and examination of retinoid metabolism-related enzyme expression patterns (Sugimoto et al., Mech. Dev. 2012). It has been demonstrated that a series of enzymes involved in retinoic acid metabolism at different steps are expressed separately but in a beautifully

orchestrated manner among multiple differentiating germ cell types (i.e., meiotic and haploid cells) and somatic Sertoli cells (which nourish all the steps of germ cells in seminiferous tubules), suggesting strongly that RA concentration changes in a 8.6 day cyclic manner, and that it increases at the same timing with that of differentiation of spermatogonia into Kit-positive. In addition, expression of RA metabolism-related genes in Sertoli cells are under regulation of RA signaling, forming positive and negative regulatory loops, leading us to propose that it is RA itself that coordinates the orchestration of metabolism between neighboring cell types that constitute the seminiferous tubules (Figure 4).

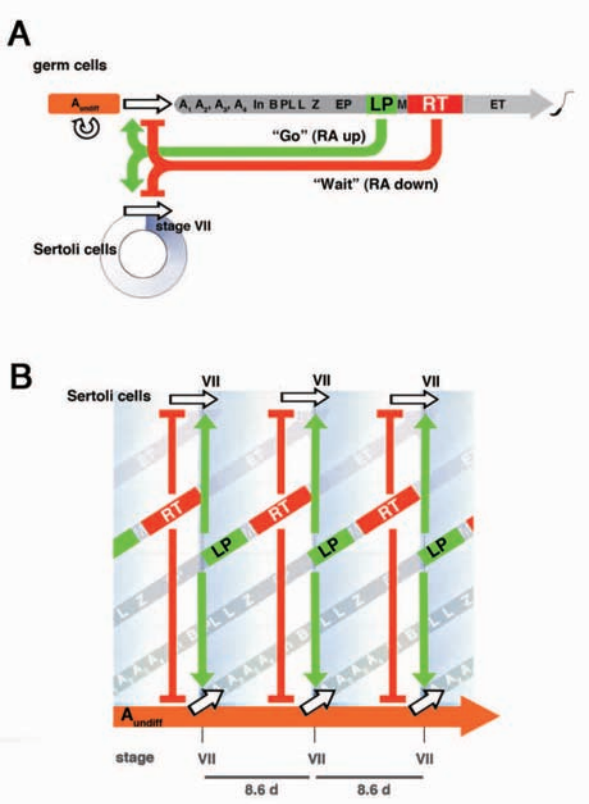

Figure 4. Proposed model for coordination of periodic differentiation of germ cells linked by retinoid metabolism. (A) Particular stages of meiotic cells (LP) and haploid cells (RT) send the *'go'* and *'wait'* signals to A_{undff} and Sertoli cells, by regulating the local RA metabolism to increase and decrease the RA concentration, respectively. (B) These signals will occur reciprocally and periodically to maintain the seminiferous epithelial cycle. (From Sugimoto et al., Mech. Dev. 2012)

IV. Perspectives

As described above, we have been tackling the robust stem cell system that supports mouse spermatogenesis from different aspects and scales. Once this research is achieved it will be quite intriguing to ask how these events are linked with each other and integrated into the robustness of the whole stem cell system. For that purpose, it is clear that we still need to know about molecules (and genes that encode these factors) that play essential roles but have not been identified. As well, mathematical analyses and modeling will be quite powerful to link the observations obtained in different scales.

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〔Original papers〕

- Koyanagi, S., Hamasaki, H., Sekiguchi, S., Hara, K., Ishii, Y., Kyuwa, S., and Yoshikawa, Y. (2012). Effects of ubiquitin C-terminal hydrolase L1 deficiency on mouse ova. Reproduction *143*, 271-279.
- Nakamura, Y., Usui, F., Miyahara, D., Mori, T., Ono, T., Kagami, H., Takeda, K., Nirasawa, K., and Tagami, T. (2012). X-irradiation removes endogenous primordial germ cells (PGCs) and increases germline transmission of donor PGCs in chimeric chickens. J. Reprod. Dev. *58*, 432-437.
- Sato, T., Yokonishi, T., Komeya, M., Katagiri, K., Kubota, Y., Matoba, S., Ogonuki, N., Ogura, A., Yoshida, S., and Ogawa, T. (2012). Testis tissue explantation cures spermatogenic failure in c-Kit ligand mutant mice. Proc. Natl. Acad. Sci. USA *109*, 16934-16938.
- Sugimoto, R., Nabeshima, Y., and Yoshida, S. (2012). Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium. Mech. Dev. *128*, 610-624.

〔Review article〕

- Yoshida, S. (2012). Elucidating the identity and behavior of spermatogenic stem cells in the mouse testis. Reproduction *144*, 293-302.

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.

I. Cellular biphasic process critical for manifestation of the sex

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

As the results of our previous studies, we have revealed that germ cells are critical for the biphasic process. In the absence of germ cells, we found that medaka exhibit complete male secondary characteristics at both endocrine and gene levels (Kurokawa et al., 2007 PNAS). This indicates that germ cells are essential for formation of ovaries. In addition, in the absence of germ cells, somatic cells are predisposed to male development.

This view was also supported by our recent mutant analysis (Nakamura et al., 2012 PLoS ONE). Medaka with a mutation in the *sox9b* gene show a female to male sex reversal. In the mutant, an initial pathway to determine the sex functions and the gonad normally is formed. But we found that *sox9b* mutant does not maintain the germ cells. The extracellular matrix produced by supporting cells surrounding germ cells is largely disorganized in the mutant (Figure 1). Therefore it was expected that loss of germ cells but not impairment of the initial male pathway might cause a female to male sex reversal. In order to prove this hypothesis, we tried recovering the number of germ cells using *sox9b* and *amhrII* heterozygous compound mutant. In the compound mutant, germ cells come back to the normal number and no sex reversal was observed, supporting our idea that germ cells are important for the proper manifestation of sex

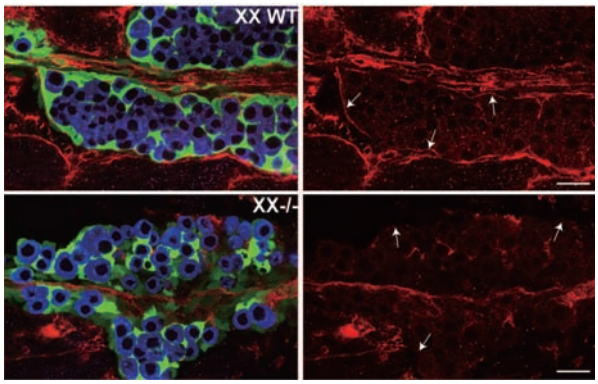

Figure 1. Extracellular matrix (laminin: red) in the *sox9b* mutant gonad (bottom panels) and in the wild-type gonad (upper panels). Distribution is impaired in the mutant. Green; *sox9b*-expressing supporting cells. Blue; germ cells (from PLoS ONE 2012).

II. How the number of germ cells are regulated during the course of early sex determination process

As mentioned above we can say that regulation of germ cell number is an important component of proper sex differentiation. We have previously identified the gene responsible for this regulation, *amhrII* (anti-Mullerian hormone receptor). The ligand for this receptor is AMH (anti-Müllerian hormone), which is known to be secreted from male supporting cells (Sertoli cells) during mammalian sex differentiation, and is critical for Müllerian duct (female reproductive organ) regression in males. But in teleosts, there are no organs found that are equivalent to the Müllerian duct. In addition, AMH belongs to a phylogenetically old and conserved type of TGFβ superfamily. These collectively suggest some conserved function other than Müllerian duct regression.

The mutant, called *hotei*, has a mutation in *amhrII* and exhibits a hypertrophic phenotype of germ cells and a male to female sex reversal. This suggested that an AMH system regulates germ cell number and sex.

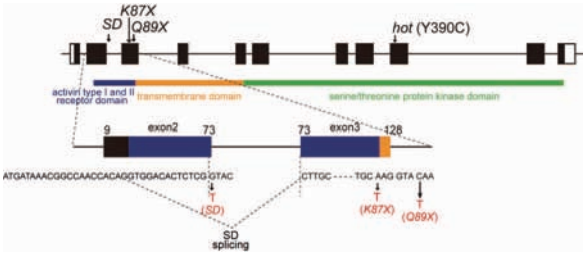

Figure 2. Hetrozygous *sox9b*-mutant medaka have a normal appearance but some exhibit female to male sex reversal.

First we isolated other alleles of *amhrII*, which causes a stop codon at the very last N terminal region and lacks important domains. Therefore it is very likely that these alleles are a functional null. Consistent with this, the mutated AMHRIIs fail to transmit an intercellular signal in the cultured cells. These allelic mutants displayed the same phenotype as the *hotei* mutant, excessive number of germ cells and sex reversal, indicating that a *amhrII* mutation in *hotei* also causes a functional null phenotype.

Next we investigated the expression of both ligand and receptor. As expected from the expression in mammals, medaka *amh* is expressed in supporting cells that directly surround germ cells. Interestingly *amhrII* is also detected in supporting cells. This suggests that the AMH system functions in supporting cells in an autocrine manner. To further confirm this, chimeric gonads were generated with somatic cells from the *hotei* mutant and wild-type germ cells. These chimeric gonads showed an increasing number of germ cells. On the other hand, gonads with normal somatic cells, and germ cells from the *hotei* mutant did not exhibit any *hotei* phenotype. The result of chimeric analyses supports the expression analysis, whereby hyperactive germ cell proliferation is a result of loss of the AMH signal in supporting cells but is not due to a direct effect of AMH on germ cells.

III. The AMH system regulates mitotically active type I germ cells.

During sex differentiation in medaka gonads, there are found two different types of germ cells. In males, germ cells divide intermittently and the daughter germ cells are readily enclosed with surrounded supporting cells (type I division). In females, however, there is an additional type of germ cell, dividing synchronously and successively to form germ cell cysts (type II division). Therefore females have more germ cells than males. Our previous results using the *zenzai* mutant indicated that type I is a self-renewal type of division while type II division occurs in germ cells that are committed to gametogenesis (Saito et al., 2008 Dev. Biol.). In addition, we proved the presence of germline stem cells in mature medaka ovaries (Nakamura et al., 2010 Science).

A BrdU incorporation experiment revealed the presence of mitotically quiescent germ cells, a good indication of the stem character, in the developing gonads (Figure 3). In the *hotei* mutants, the number of the quiescent type I germ cells did not change, compared to that in wild-type. But the mutant has more type I germ cells totally than the wild type. This suggests that the AMH system does not regulate transition from a quiescent type of type I germ cells to mitotically active type I germ cells but promotes mitosis of typeI germ cells that had already been committed to the mitotic process (Nakamura et al., 2012 Development).

Collectively these analyses demonstrate that the AMH system acts in supporting cells autonomously and that this activity consequently regulates mitotically active type I germ cells. In the mutant, loss of AMH activity results in more germ cells, which then causes a male to female sex reversal.

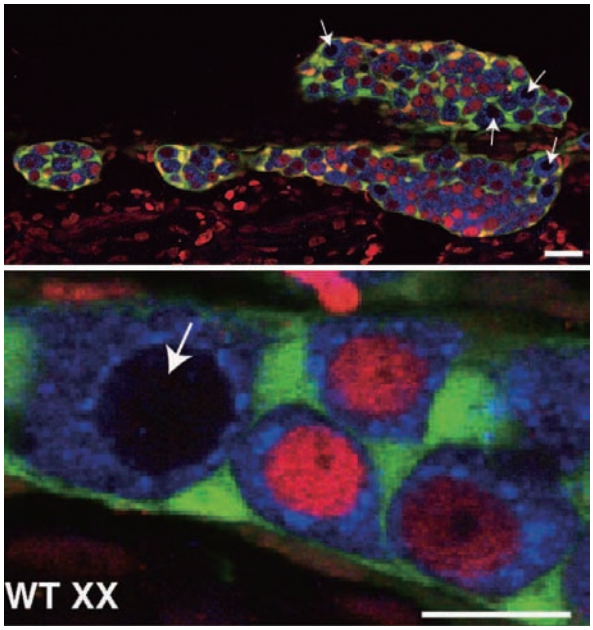

Figure 3. Presence of mitotically quiescent type I germ cells (arrows). Blue; germ cells, Red; BrdU, Green; supporting cells..

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〔Original papers〕

- Ichimura, K., Bubenshchikova, E., Powell, R., Fukuyo, Y., Nakamura, T., Tran, U., Oda, S., Tanaka, M., Wessely, O., Kurihara, H., Sakai, T., and Obara, T. (2012). A comparative analysis of glomerulus development in the pronephros of medaka and zebrafish. PLoS ONE *7*, e45286.
- Nakamura, S., Watanabe, I., Nishimura, T., Picard, J-Y., Toyoda, A., Taniguchi, Y., di Clemente, N., and Tanaka, M. (2012). Hyperproliferation of mitotically active germ cells dues to defective anti-Müllerian hormone signaling mediates sex reversal in medaka. Development *139*, 2283-2287.
- Nakamura, S., Watanabe, I., Nishimura, T., Toyoda, A., Taniguchi, Y., and Tanaka, M. (2012). Analysis of medaka *sox9* orthologue reveals a conserved role in germ cell maintenance. PLoS ONE *7*, e29982.

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

- Kobayashi, K., Kamei, K., Kinoshita, M., Czerny, T., and Tanaka, M. A heat-inducible cre/loxP gene induction system in medaka. Genesis 2012 Nov 3.

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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, and biochemical approaches.

I. Genetic approach

Recent studies of *Arabidopsis* mutants show several genes are involved in the axes-dependent control of lateral organ development. The adaxial- and abaxial-specific tissue differentiation in the leaf primordium is determined by the precise expression of the adaxial marker genes, *HD-Zip III* including *PHABULOSA* (*PHB*), and the abaxial marker genes, *FILAMENTOUS FLOWER* (*FIL*) and *YABBY*. Using the reporter gene-system, we visualized the function domain of microRNA165/166 (miR165/166), which targeted the *HD-Zip III* messenger RNA, and showed that miR165/166 act in the epidermal and mesophyll cells locating in the abaxial side and determine the adaxial-specific expression of PHB. One of the *MIR165/166* genes, *MIR165A*, is expressed in the abaxial epidermal cells. We revealed that *MIR165A* is enough to repress the PHB expression in the cells located in the abaxial side, indicating that miR165 is likely to move to cells at the adaxial side. When miR165 was produced by another primary miRNA transcript backbone, pri-miR319a, in the abaxial epidermal cells, the activity of miR165 was observed in the whole region of leaf primordia, showing that sequence and/or structure of *MIR165A* primary transcript is necessary to restrict the miR165-active region to the abaxial-side cells. Thus we concluded that the number of cells in which miR165 can move is determined depending on the *MIR165A* primary transcript (Tatematsu et al., in preparation).

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*). We revealed that one of them, *enf1*, has a mutation in a gene that encodes SUCCINIC SEMALDEHYDE DEHYDROGENASE, which catalyzes the conversion of succinic semialdehyde (SSA) to succinate, and that SSA and/or its derivatives affect the axis-dependent cell fate in leaf primordia (Toyokura et al., 2011, 2012). We also isolated some suppressor mutants of *enf1*, which show normal leaf shapes and expression domain of *FIL* promoter:*GFP*. We determined the genes, which have the mutation for the suppression of *enf1* phenotype, using next-generation sequence methods, and revealed that one of them has the mutation in a gene which encodes a transaminase enzyme.

Detailed analysis of temporal and spatial expression pattern of *FIL* in a developing leaf primordium using reporter gene combined with the Cre-LoxP recombinant system showed dynamic shift of the *FIL*-expression domain (Figure 1). This

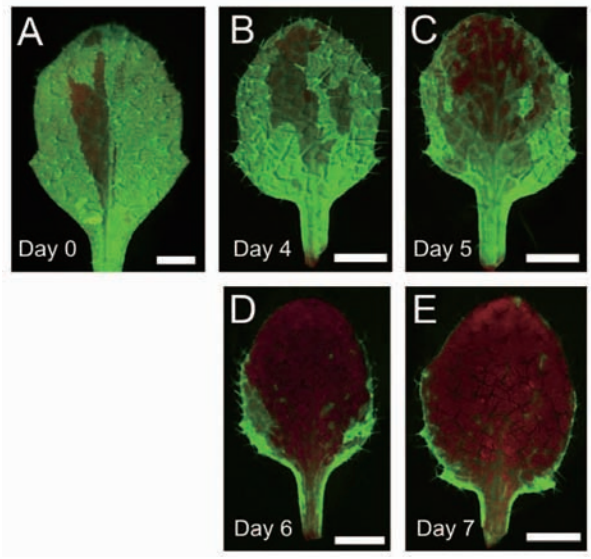

Figure 1. Cell lineages of *FIL*-expressing cells were visualized using a transgenic line having *FIL* promoter:*Cre-GR* and *35S:LoxP-ter-LoxP-YFP*. After dexamethasone (DEX) treatment, YFP is expressed constitutively in the *FIL*-expressing cells by Cre-LoxP recombinant system. We applied DEX at various timing after seed germination and checked YFP signal in the third leaves of 12-day-old seedlings. Photo was taken from the adaxial side. Green and red colors indicate YFP and autofluorescence of chlorophyll, respectively. Strong green colors indicate YFP expressed in the adaxial epidermal- and adaxial mesophyll cells, and weak colors indicate YFP expressed in the adaxial mesophyll cells, but not in the adaxial epidermis. The day of DEX treatment after seed germination is indicated at the bottom left of each panel. Bars represent 1 mm.

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

†: This laboratory was closed on 31 March, 2013.

result indicates that the boundary between the expression domain of *FIL* and that of *PHB* moves to the abaxial side during leaf development. Another enlarged-*FIL*-expression domain mutant, *enf2*, which has abaxialized leaves, showed slower moving of the boundary than that in wild type. We also indicated that the *ENF2* gene encoded a plastid-localized unknown protein, and that the state of expression of chloroplast genome-encoding genes might be a factor determining the rate of the boundary shifting between the *PHB*- and the *FIL*-expression domains. Using a simple mathematical model, we also predicted that general cellular functions, such as production and/or degradation of the determining genes, regulate the rate of the boundary shifting during leaf development (Tameshige et al., in press).

We examined the role of homeobox-related gene, *PRESSED FLOWER* (*PRS*) and its homolog, *WOX1*, and showed that they are required for forming the margin-specific oblong cells. Genetic analyses showed that *PRS* and *WOX1* control blade outgrowth and determine the expression domains of the adaxial- and the abaxial-determining genes in the marginal region of leaf primordia. Based on the observations we proposed the middle domain model (Nakata et al., 2012; Nakata and Okada 2012). To identify the genes acting in the downstream of *WOX1*, we carried out gene expression profiling using Arabidopsis genome array in the *WOX1* overexpressing transgenic plants. This analysis indicates that *WOX1* directly regulates the expression level of several adaxial and abaxial determining genes, suggesting that three domains, the adaxial-, the abaxial-, and the middle-domain, interact mutually during leaf developments. We also found that the expression of some auxin-inducible genes was repressed in the *WOX1* overexpression plants. To reveal the relationships between *WOX1* and auxin, we are analyzing the auxin responsibility and expression of auxin-related genes in the shoot apex of wild type and *prs wox1* seedlings (Nakata et al., in preparation).

To reveal how floral organs fix their forms through development processes, we analyzed a series of mutants named *folded petals* (*fop*) with stacked and folded petals when flowers open. We found that epidermal cells of the mutant petal show traces of stacking with the epidermal cells of sepals. FOP proteins are related to wax/cutin synthesis or transport. Thus, we proposed that wax/cutin components secreted by FOP proteins on the surface of the petal epidermis might act as a lubricant between the floral organs in a developing bud (Takeda et al., 2013).

II. Biochemical approach

We are taking a biochemical approach to study of the intercellular signaling system by analyzing small peptides, which are present in the apoplastic region of the SAM. We purified fractions containing small peptides from apoplast fractions of the curds of cauliflower (*Brassica oleracea* L. var. *botrytis*), and found a putative lipid transfer protein (LTP) has a role in increasing the number of SAMs in Arabidopsis seedling when applied exogenously. The cauliflower *LTP* gene was highly expressed in the curd, and the expression of the Arabidopsis ortholog was observed in the L1 cell layers of the SAM (Figure 2A, B). When *LTP* expressed under constitutive active promoter in Arabidopsis, the transgenic plants showed increased number of SAMs near the shoot apex (Figure 2C). Moreover, to analyze the role of *LTP* gene *in planta*, we made RNAi knockdown lines of Arabidopsis *LTP*. In some seedlings of the knockdown lines, multiple cotyledon and/or filamentous leaves were observed. Thus, we concluded that LTP proteins in apoplasts are responsible for SAM maintenance and formation.

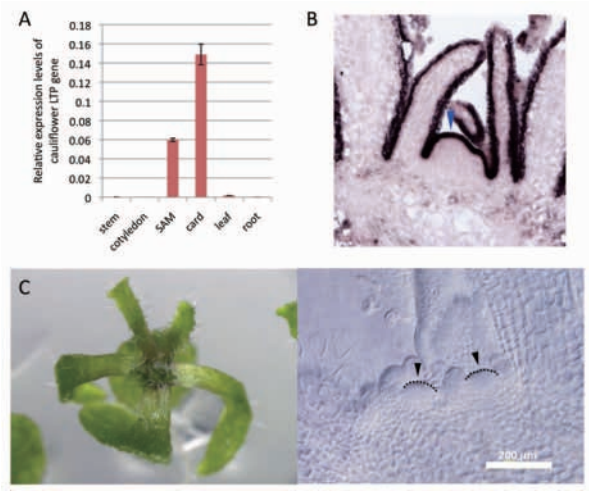

Figure 2. (A) Expression levels of cauliflower *LTP* genes in cauliflower organs analyzed by qRT-PCR methods. (B) Pattern of *in situ* hybridization of Arabidopsis *LTP* gene showing expression in epidermis of a wild type seedling. A blue arrowhead indicates the SAM. (C) (Left) 10-day-old seedling of Arabidopsis *LTP* overexpression plants. (Right) A longitudinal section of the seedling. Black arrowheads indicate the SAMs, and black dashed lines indicate shapes of the SAMs.

Publication List

〔Original papers〕

- Endo, A.*, Tatematsu, K.*, Hanada, K.*, Duermeyer, L., Okamoto, M., Yonekura-Sakakibara, K., Saito, K., Toyoda, T., Kawakami, N., Kamiya, Y., Seki, M., and Nambara, E. (2012). Tissue-specific transcriptome analysis reveals cell wall metabolism, flavonol biosynthesis, and defense responses are activated in the endosperm of germinating Arabidopsis thaliana seeds. Plant Cell Physiol. *53*, 16-27. (*: Equally contributed)
- Nakata, M., Matsumoto, N., Tsugeki, R., Rikirsch, E. Laux, T., and Okada, K. (2012). Roles of the middle domain-specific *WUSCHEL-RELATED-HOMEOBOX* genes in early development of leaves in Arabidopsis. Plant Cell *24*, 519-535.
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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. We are now focusing our attention on the molecular mechanisms underlying axon branching and arborization for synapse formation, along with elimination of mistargeted axons and branches. Among the region-specific molecules in the developing retina, we have already found several molecules that induce abnormal branching or arborization when their expression was experimentally manipulated *in vivo*.

One is adenomatous polyposis coli 2 (APC2), which is preferentially expressed in the nervous system from early developmental stages through to adulthood. APC2 is distributed along microtubules in growth cones as well as axon shafts of retinal axons. The knockdown of *Apc2* in chick retinas reduced the stability of microtubules in retinal axons and yielded abnormal behaviors of growth cones including a reduced response to ephrin-A2 and misprojection in the tectum without making clear target zones.

Recently, we generated *Apc2*-deficient mice by a gene-targeting technique. In the homozygous mutant mice, robust defects in neuronal lamination were observed in the cortex (Figure 1A), hippocampus, cerebellum (Figure 1B), and olfactory bulb. *In vivo* BrdU labeling and immunohistochemical analyses with specific markers revealed that the laminary abnormalities are a result of dysregulated neuronal migration by a cell-autonomous mechanism. Dissociated *Apc2*-deficient cerebellar granule cells showed no obvious alterations in migration under non-stimulated conditions, however, Brain-derived neurotrophic factor (BDNF)-stimulated directional migration was perturbed.

Total internal reflection fluorescence (TIRF) microscopy revealed that APC2 is distributed along actin fibers as well as microtubules. BDNF-stimulated F-actin formation at the leading edge was impaired in migrating *Apc2*-deficient neurons, along with dysregulation of Rho GTPase activity. Thus APC2 is an essential mediator of the cytoskeletal regulation at leading edges in response to extracellular signals. The phenotypes observed in *Apc2*-deficient mice suggest that mutations in *APC2* in humans may cause a neurodevelopmental disorder.

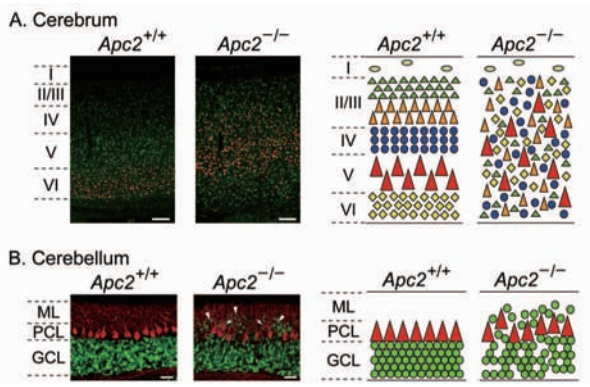

Figure 1. Laminary defects in the brain of *Apc2*-deficient mice. **A**, Sagital sections of P30 cerebral cortex stained with anti-NeuN (green, a neuron-specific marker) and anti-FoxP2 (red, a polymorphous cell-specific marker). The cerebral cortex of the wild-type (*Apc2*$^{+/+}$) mouse is organized into six layers and FoxP2-positive cells are mainly distributed in layer VI. In the *Apc2*-deficient mouse (*Apc2*$^{-/-}$), however, they are observed broadly in layers II to V. Right panels are schematic drawings of distribution patterns of neuronal subtypes in the wild-type and *Apc2*-deficient mouse. In the *Apc2*-deficient mouse, cortical layers were poorly organized and layer boundaries are blurred and indistinct. Scale bars, 50 μm. **B**, Sagital sections of P20 cerebellum stained with anti-NeuN (green, a granule cell-specific marker) and anti-calbindin D-28K (red, a Purkinje cell-specific marker). Arrows and arrowheads indicate ectopically distributed Purkinje and granule cells, respectively. Right panels are schematic drawings of distribution patterns of neuronal subtypes in the wild-type and *Apc2*-deficient mouse. ML, molecular layer; PCL, Purkinje cell layer; GCL, granule cell layer. Scale bars, 50 μm.

II. Regeneration of retinal axons

CNS neurons in fish can regrow their axons after nerve transection, while CNS neurons in mammals lose their capacity to regenerate. Thus, we have been investigating genes involved in optic nerve regeneration using fish visual systems. We found that expression of coagulation factor XIII

A subunit (FXIII-A), a protein cross-linking enzyme, is upregulated in the goldfish optic nerve and retina during regeneration. The cells producing FXIII-A were astrocytes/microglial cells and retinal ganglion cells (RGCs) in the optic nerve and retina, respectively. Overexpression of FXIII-A in RGCs and addition of extracts of optic nerves with injury to retinal explants induced significant neurite outgrowth from the retina. From these observations, we concluded that the increase of FXIII in RGCs promotes neurite sprouting from injured RGCs, whereas the increase of FXIII in optic nerves facilitates elongation of regrowing axons.

III.Physiological roles of protein tyrosine phosphatase receptor type Z

Protein-tyrosine phosphatase receptor type Z (Ptprz, also known as PTPζ/RPTPβ) is a member of the R5 receptor-like protein tyrosine phosphatase (RPTP) subfamily. Ptprz is predominantly expressed in glial and neuronal cells in the central nervous system (CNS) and its physiological importance has been demonstrated through studies with *Ptprz*-deficient mice. Ptprz modulates hippocampal synaptic plasticity: adult *Ptprz*-deficient mice display impairments in spatial and contextual learning. Ptprz is expressed also in the stomach, where it is used as a receptor for VacA, a cytotoxin secreted by *Helicobacter pylori*: *Ptprz*-deficient mice are resistant to gastric ulcer induction by VacA.

This year, we revealed that Ptprz plays a negative role in oligodendrocyte differentiation in early CNS development and remyelination in demyelinating CNS diseases, through the dephosphorylation of its substrates such as p190RhoGAP. We first found an early onset of the expression of myelin basic protein (MBP), a major protein of the myelin sheath, and early initiation of myelination *in vivo* during development of the *Ptprz*-deficient mouse, as compared with the wild-type (Figure 2A). In addition, oligodendrocytes appeared earlier in primary cultures from *Ptprz*-deficient mice than wild-type mice.

We subsequently found that adult *Ptprz*-deficient mice are less susceptible to experimental autoimmune encephalomyelitis (EAE) induced by active immunization with myelin/oligodendrocyte glycoprotein (MOG) peptide than were wild-type mice. However, the number of T-cells and macrophages/microglia infiltrating into the spinal cord were not decreased in *Ptprz*-deficient mice after MOG immunization, suggesting that the reduced tissue damage is not attributable to an inhibition of infiltration by inflammatory cells.

It is known that Fyn tyrosine kinase-mediated down-regulation of Rho activity through activation of p190RhoGAP is crucial for oligodendrocyte differentiation and myelination. Here, p190RhoGAP is one of the physiological Ptprz substrates. After EAE induction, the tyrosine phosphorylation of p190RhoGAP increased significantly, and the EAE-induced loss of MBP was markedly suppressed in the white matter of the spinal cord in *Ptprz*-deficient mice (Figure 2B).

Thus, selective inhibition of Ptprz signaling could be an effective and plausible therapeutic strategy for treating demyelinating diseases.

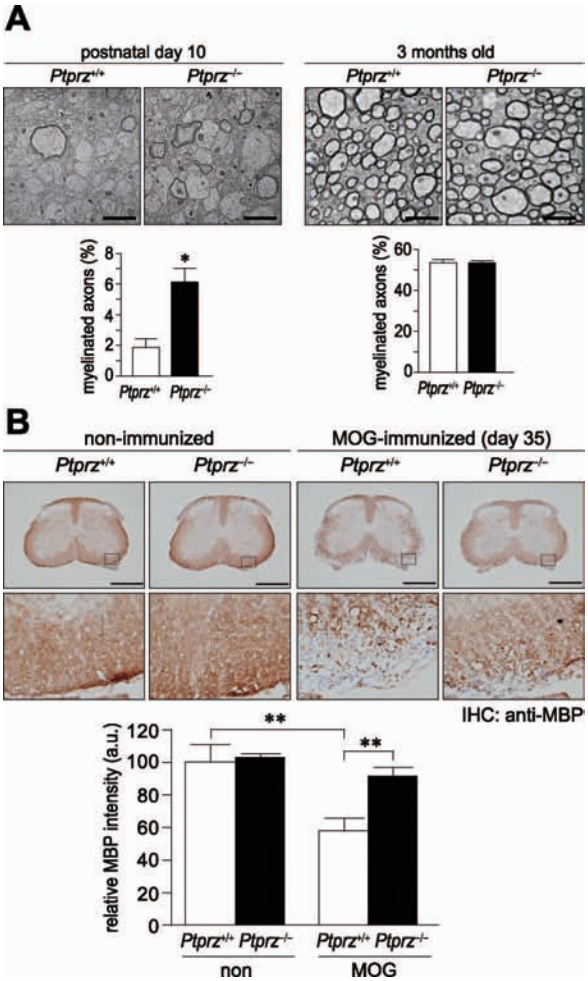

Figure 2. Ptprz plays a negative role in oligodendrocyte differentiation in early CNS development and remyelination in demyelinating CNS diseases. **A**, Early myelination in *Ptprz*-deficient mice. Electron micrographs of transverse sections at the corpus callosum from mice at postnatal day 10 (left), and 3 months old (right). Scale bars, 2 μm. Percentages of myelinated axons in total axons are shown at the lower position of each panel. Data are the mean ± SEM $*p < 0.05$. **B**, Reduced MBP loss in *Ptprz*-deficient mice after EAE induction. Anti-MBP staining of the spinal cord sections from wild-type and *Ptprz*-deficient mice 35 days after MOG immunization, or non-immunized control mice. The lower images are enlargements of the areas enclosed by squares in the upper images. Scale bars, 500 μm. MBP signals are expressed as the relative change compared with the non-immunized wild-type mice, and shown at the bottom. Data are the mean ± SEM. $**p < 0.01$.

IV. Brain systems for body-fluid homeostasis

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

In the brain, Na_x channels are specifically expressed in

astrocytes and ependymal cells in the sensory circumventricular organs, such as the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT), where Na_x-positive glial cells are involved in sensing an increase in $[Na^+]$ in body fluids. *Na_x*-deficient mice do not stop ingesting salt even when dehydrated, while wild-type mice avoid salt. This behavioral defect of *Na_x*-deficient mice is recovered by a site-directed transfer of the *Na_x* gene with an adenoviral vector into the SFO. Na_x thus functions as the brain's Na^+-level sensor for the homeostatic control of $[Na^+]$ in body fluids.

Na_x has a putative PSD-95/Disc-large/ZO-1 (PDZ)-binding motif at the carboxyl-terminus. We thus hypothesized that the Na_x channel may be regulated by PDZ-scaffold proteins. Very recently, we found that several PDZ proteins bind to Na_x by PDZ-array overlay assay. Among them, synapse-associated protein 97 (SAP97/DLG1) was coexpressed with Na_x in the SFO. In C6 glioblastoma cells, destruction of the PDZ-binding motif of Na_x, Na_x(T1679A), resulted in a decrease in cell-surface Na_x, which was attenuated with inhibitors of endocytosis (Figure 3A). Depletion of SAP97 also led to the reduction in the surface expression of wild-type Na_x (Figure 3B). Next, functional relevance of the binding of Na_x with SAP97 was confirmed by Na^+-imaging studies (Figure 3C). When the extracellular Na^+ concentration, $[Na^+]_o$ was increased from 145 mM to 170 mM, both C6M16 cells expressing wild-type Na_x and C6M(TA)8 cells expressing the Na_x(T1679A) mutant showed increases in the intracellular Na^+ concentration, $[Na^+]_i$, and the level eventually reached the same equilibrium point between Na^+ influx by Na_x and Na^+ export by Na^+/K^+-ATPase. However, importantly, C6M(TA)8 cells took longer to reach this equilibrium level than control C6M16 cells. This is probably because the reduction in the number of surface Na_x channels diminished the Na^+ influx. Thus, SAP97 appears to play an important role in sensing body-fluid $[Na^+]$ in the SFO through regulation of the surface expression of the sensor channel.

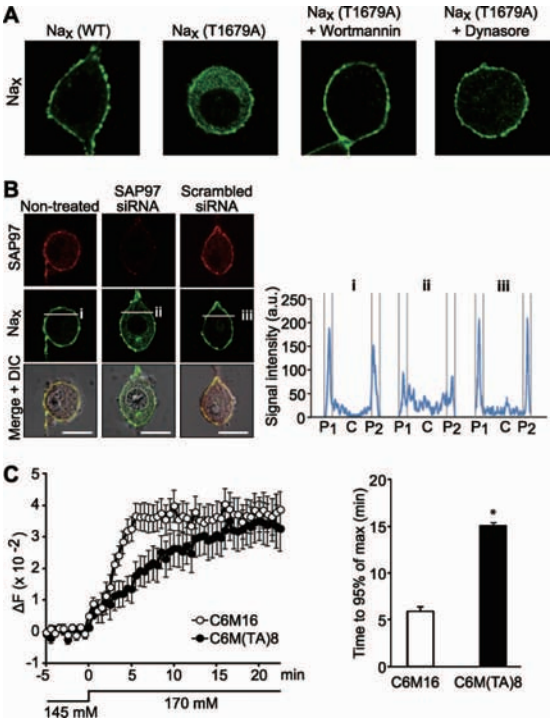

Figure 3. SAP97 promotes the stability of Na_x channels at the plasma membrane. **A**, Decrease in cell-surface expression of the Na_x mutant with Thr-1679 changed to Ala (mutation in the PDZ-binding motif) in C6 cells and its improvement by treatment with endocytosis inhibitors, wortmannin and dynasore. After the induction of the expression of Na_x(T1679A) channels, cells were treated with 100 nM wortmannin or 200 μM dynasore for 6 h. Then the cells were fixed, permeabilized, and stained with anti-Na_x. **B**, Reduction in the cell-surface expression of Na_x with depletion of SAP97. Non-treated C6M16 cells, or C6M16 cells transfected with siRNA for SAP97 or scrambled siRNA were immunostained with anti-SAP97 and anti-Na_x. The fluorescence intensity profiles along the white lines are shown in the right panel. Scale bars, 10 μm. a.u., arbitrary unit. **C**, Reduced sodium influx in C6 cells in the absence of any association between Na_x and SAP97. Left: Na^+ imaging of C6M16 (wild-type Na_x) and C6M(TA)8 (T1679A mutant Na_x) cells upon elevation of the extracellular Na^+ concentration from 145 mM to 170 mM. The coordinate gives the fluorescence ratio (ΔF340/380 nm) in Na^+ imaging with sodium-binding benzofuran isophthalate acetoxymethyl ester (SBFI/AM), representing the intracellular Na^+ concentration. The physiological 145 mM Na^+ solution was changed to a 170 mM solution at 0 min. Right: Summary of the time to reach 95% of the maximum fluorescence ratio. Data represent the mean ± SE (n = 40 for each); $*P < 0.01$, two-tailed *t* test.

Publication List

〔Original papers〕

- Kuboyama, K., Fujikawa, A., Masumura, M., Suzuki, R., Matsumoto, M., and Noda, M. (2012). Protein tyrosine phosphatase receptor type Z negatively regulates oligodendrocyte differentiation and myelination. PLoS ONE *7*, e48797.
- Matsumoto, M., Fujikawa, A., Suzuki, R., Shimizu, H., Kuboyama, K., Hiyama, T.Y., Hall, R.A., and Noda, M. (2012). SAP97 promotes the stability of Na_x channels at the plasma membrane. FEBS Lett. *586*, 3805-3812.
- Shintani, T., Takeuchi, Y., Fujikawa, A., and Noda, M. (2012). Directional neuronal migration is impaired in mice lacking adenomatous polyposis coli 2. J. Neurosci. *32*, 6468-6484.
- Sugitani, K., Ogai, K., Hitomi, K., Nakamura-Yonehara, K., Shintani, T., Noda, M., Koriyama, Y., Tanii, H., Matsukawa, T., and Kato, S. (2012). A distinct effect of transient and sustained upregulation of cellular factor XIII in the goldfish retina and optic nerve on optic nerve regeneration. Neurochem. Int. *61*, 423-432.

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

We continue to study genes that are expressed in specific areas of the neocortex in order to understand the principles governing the formation of the primate brain. In addition, this year we have reported a novel genetic manipulation method to visualize cortical projection neurons with retrograde TET-off lentiviral vector, area-specific substratification of deep layer neurons in the rat cortex, and the behavioral analysis of zfhx2-deficient mice as follows.

I. Area-specific substratification of deep layer neurons in the rat cortex

To identify cell types for fine mapping of neuronal circuits, we studied area-specific sublamina structures of the rat cerebral cortex using cholecystokinin (cck) and purkinje cell protein4 (pcp4) mRNAs as the markers for the subtypes of excitatory neurons in layers 5 and 6. We found a segregated expression, especially pronounced in layer 6, where corticothalamic and corticocortical projecting neurons reside (Figure 2). To examine the relationship between gene expression and projection target, we injected retrograde tracers into several thalamic subnuclei, ventral posterior (VP), posterior (PO), mediodorsal (MD), medial and lateral geniculate nuclei (MGN and LGN); as well as into two cortical areas (M1 and V1). This combination of tracer-in situ hybridization (ISH) experiments revealed that corticocortical neurons predominantly express cck and corticothalamic neurons predominantly express pcp4 mRNAs in all areas tested. In general, cck(+) and pcp4(+) cells occupied the upper and lower compartment of layer 6a, respectively. However, the sublaminar distribution and the

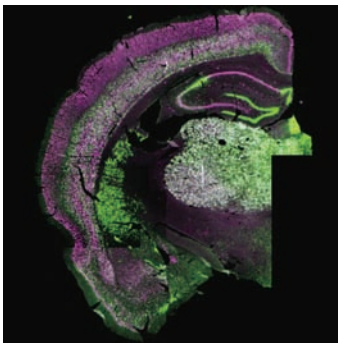

Figure 1. Cover letter for J. Comp. Neurology, Volume 520, Issue 16.

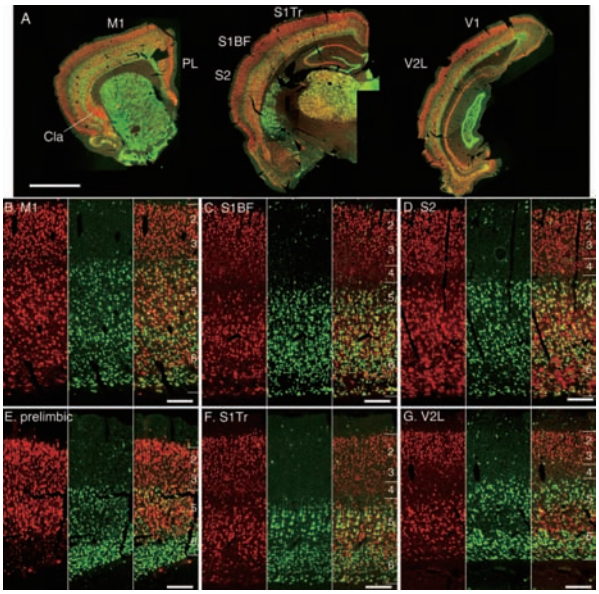

Figure 2. Expression patterns of cck and pcp4 mRNAs in various cortical areas. A: Low magnified views of the double-ISH of cck (red) and pcp4 (green) mRNAs in the rat brain. PL, prelimbic cortex; Cla, claustrum; M1, primary motor cortex; S1Tr, primary somatosensory cortex, trunk region; S1BF, primary somatosensory cortex, barrel field; S2, secondary somatosensory cortex; V1, primary visual cortex; V2L, secondary visual cortex lateral area. B–G: The magnified cortical areas labeled in panel A. Scale bars = 1 mm in A; 200 μm in B–G. (Watakabe et al., J Comp Neurol. 520, 3553-3573, 2012.)

relative abundance of cck(+) and pcp4(+) cells were quite distinctive across areas. For example, layer 6 of the prelimbic cortex was almost devoid of cck(+) neurons, and was occupied instead by corticothalamic pcp4(+) neurons. In the lateral areas, such as S2, there was an additional layer of cck(+) cells positioned below the pcp4(+) compartment. The claustrum, which has a tight relationship with the cortex, mostly consisted of cck(+)/pcp4(−) cells. The combination of gene markers and retrograde tracers revealed a distinct sublaminar organization, with conspicuous cross-area variation in the arrangement and relative density of corticothalamic connections. (Watakabe et al., J Comp Neurol. 520, 3553-3573, 2012.)

II. Visualization of cortical projection neurons with retrograde TET-off lentiviral vector.

We are interested in identifying and characterizing various projection neurons that constitute the neocortical circuit. For this purpose, we developed a novel lentiviral vector that carries the tetracycline transactivator (tTA) and the

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

transgene under the TET Responsive Element promoter (TRE) on a single backbone. By pseudotyping such a vector with modified rabies G-protein, we were able to express palmitoylated-GFP (palGFP) or turboFP635 (RFP) in the corticothalamic, corticocortical, and corticopontine neurons of mice. The high-level expression of the transgene achieved by the TET-Off system enabled us to observe characteristic elaboration of neuronal processes for each cell type (Figure 3). At higher magnification, we were able to observe fine structures such as boutons and spines as well. We also injected our retrograde TET-Off vector to the marmoset cortex, and therefore proved that this method can be used to label the long- long-distance cortical connectivity of the millimeter scale. In conclusion, our novel retrograde tracer provides an attractive option to investigate the morphologies of identified cortical projection neurons of various species (e.g., Figure 4) (Watakabe et al., PLoS One. 7, e46157, 2012).

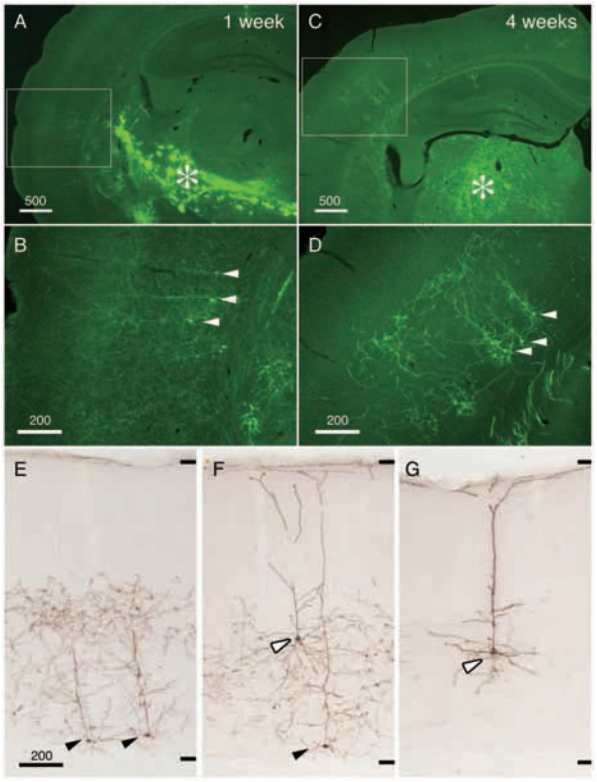

Figure 3. Retrograde infection of the corticothalamic cells by StTTrG/FuG-B vector. StTTrG/FuG-B vector was injected into the mouse thalamus. (A–D) After one week (for panels A and B) or four weeks (for panels C–G), the mouse was perfusion fixed and sliced at 40 μm thickness. The boxed areas in panels A and C were magnified in panels B and D, to show infection of corticothalamic neurons in layer 6 (indicated by arrowheads). The injection site is indicated by the asterisk (*). These images show the original green fluorescence of the infected cells. (E–G) The immunoperoxidase detection of the GFP-expressing cells. Bar: 500 μm (A and C) , 200 μm (for others). Watakabe et al., PLoS One. 7, e46157. The caudal block of the marmoset brain was parasagittally sectioned to visualize the reciprocal connectivity between V1 and V2.

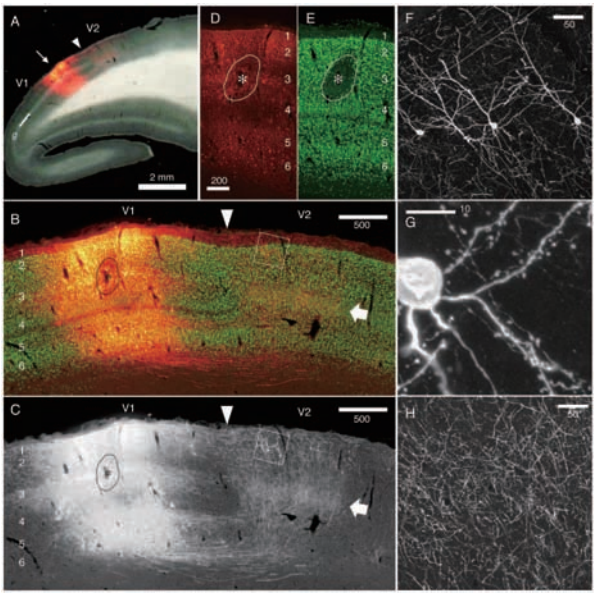

Figure 4. Reciprocal connectivity of marmoset V1 and V2 visualized by StTTrR/FuG-B vector.
(A) Dark field image overlaid with RFP fluorescence indicates the injection site. The V1/V2 border (shown by the arrowhead) was clearly delineated by the presence of the striate of Gennari (g). The arrow indicates the injection site. (B and C) The RFP signals around the injection site (red) are shown with NeuN counterstaining (green). The arrow indicates the plexus of V1 terminals in layer 4. The boxed region contains the retrogradely labeled V2 neurons with feedback projection to V1, which is magnified in panel F. The asterisk indicates the core of injection, where NeuN expression is lost by local damage. Only the RFP signals are shown in panel C. (D and E) RFP signals (red) and NeuN stains (green) in the injection center. The contrast for the RFP signals is adjusted so that the cell bodies of the infected neurons can be delineated. Note that the lamina positions of the infected cells are restricted mostly to layers 2, 3 and 6. (F–H) Confocal images of RFP in V2. Panel F shows the cell bodies and dendrites of V2 neurons that project back to V1. (G) The spines of the basal dendrites originating from one of these cells. (H) The plexus of axon terminals concentrated in layer 4 of V2 (shown by the arrow). Bar: 2 mm for A; 500 μm for B and C; 200 μm for D and E; 50 μm for F and H; 10 μm for G.
Figures 3 and 4 from Watakabe et al., PLoS One. 7, e46157, 2012.

III. Behavioral analysis of zfhx2-deficient mice.

Zfhx2 (also known as zfh-5) encodes a transcription factor containing three homeobox domains and 18 Zn-finger motifs. We have reported that Zfhx2 mRNA is expressed mainly in differentiating neurons in the mouse brain and its expression level is negatively regulated by the antisense transcripts of Zfhx2 (Figure 5). Although the expression profile of Zfhx2 suggests that ZFHX2 might have a role in a particular step of neuronal differentiation, the specific function of the gene has not been determined. We therefore generated a Zfhx2-deficient mouse line and performed a comprehensive battery of behavioral tests to elucidate the function of ZFHX2. Homozygous Zfhx2-deficient mice showed several behavioral abnormalities, namely, hyperactivity, enhanced depression-like behaviors, and an aberrantly altered anxiety-like phenotype (e.g., Figure 6). These behavioral phenotypes suggest that ZFHX2 might play roles in controlling emotional aspects through the function of monoaminergic neurons where ZFHX2 is expressed.

Moreover, considering their phenotypes, the Zfhx2-deficient mice may provide a novel model of human psychiatric disorders. (Komine et al., PLoS One. 7, e53114, 2012)

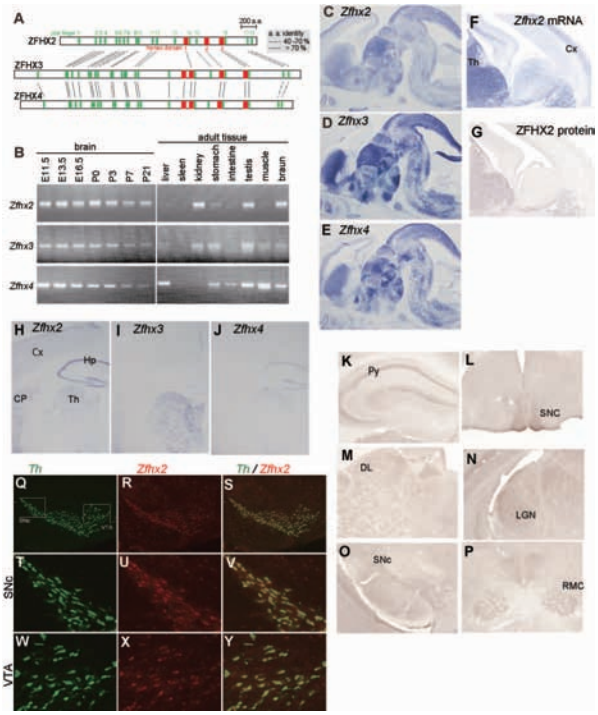

Figure 5. Structure and expression of mouse Zfhx2.(A) Structure of ZFHX2 and related proteins. ZFHX2 is a protein of 2562 amino acids containing 18 zinc fingers (green ovals) and three homeodomains (red squares). (B) Zfhx2, Zfhx3, and Zfhx4 mRNA detected by semi-quantitative RT-PCR in various RNA sources. Note that cDNAs were amplified for 30 cycles for brains of different developmental stages, whereas cDNAs were amplified for 35 cycles for various adult tissues. (C–E) Expression of Zfhx2 (C), Zfhx3 (D), and Zfhx4 (E) mRNA in the parasagittal sections of an E15.5 mouse brain. These three genes were expressed in substantially similar patterns with the highest expression level of Zfhx3. (F, G) Expression of Zfhx2 mRNA (F) and the ZFHX2 protein (G) was compared on adjacent coronal sections of an E15.5 brain. mRNA expressed in the thalamic region (Th) was translated, whereas mRNA expressed in the cerebral cortex (Cx) was not translated: this situation made the expression patterns of ZFHX2 and ZFHX3 more alike in protein level than in mRNA level. (H–J) Expression of Zfhx2 (H), Zfhx3 (I), and Zfhx4 (J) mRNA in the coronal sections of an adult brain. Cerebral cortex (Cx), hippocampus (Hp), thalamus (Th), caudate putamen (CP). Expression levels of all three genes were decreased compared with those in the embryonic brain, but Zfhx2 maintained a higher level of expression than the others. (K–P) Expression of ZFHX2 protein in the adult brain. The pyramidal layer of the hippocampus (K, Py), the suprachiasmatic nucleus (L, SCN), laterodorsal thalamic nucleus (M, LD), lateral geniculate nucleus (N, LGN), substantia nigra pars compacta (O, SNc), and magnocellular part of the red nucleus suprachiasmatic (P, RMC). (Q–Y) Double-color in situ hybridization with Zfhx2(red) and tyrosine hydroxylase (Th) probes (green).

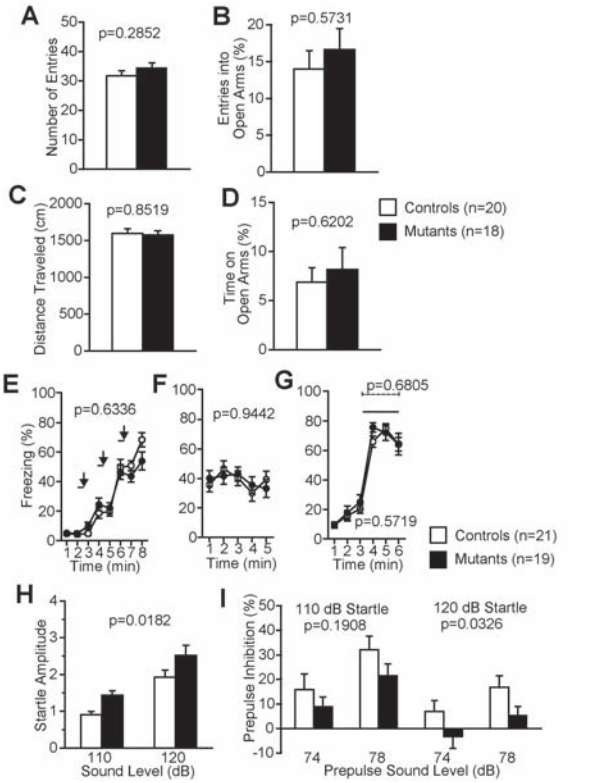

Figure 6. Locomotor activity measured in open field test. Distance traveled (A, B),stereotypic behavior (C), vertical activity (D), and time spent in the center area (E). Measurements are blocked in 5 min (A, C, D, and E) or in 1 min (B, shown only for the first 10 min). (Komine et al., PLoS One. 7, e53114, 2012)

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- Kinoshita, M., Matsui, R., Kato, S., Hasegawa, T., Kasahara, H., Isa, K., Watakabe, A., Yamamori, T., Nishimura, Y., Alstermark, B., Watanabe, D., Kobayashi, K., and Isa, T. (2012). Genetic dissection of the circuit for hand dexterity in primates. Nature *487*, 235-238.
- Komine, Y., Takao, K., Miyakawa, T., and Yamamori, T. (2012). Behavioral abnormalities observed in zfhx2-deficient mice. PLoS ONE *7*, e53114.
- Takahata, T., Shukla, R., Yamamori, T., and Kaas, J.H. (2012). Differential expression patterns of striate cortex-enriched genes among Old World, New World, and prosimian primates. Cereb. Cortex *22*, 2313-2321.
- Watakabe, A., Hirokawa, J., Ichinohe, N., Ohsawa, S., Kaneko, T., Rockland, K.S., and Yamamori, T. (2012). Area-specific substratification of deep layer neurons in the rat cortex. J. Comp. Neurol. *520*, 3553-3573.
- Watakabe, A., Kato, S., Kobayashi, K., Takaji, M., Nakagami, Y., Sadakane, O., Ohtsuka, M., Hioki, H., Kaneko, T., Okuno, H., Kawashima, T., Bito, H., Kitamura, Y., and Yamamori, T. (2012). Visualization of cortical projection neurons with retrograde TET-off lentiviral vector. PLoS ONE *7*, e46157.

DIVISION OF BRAIN CIRCUITS

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Various firing patterns of many cortical neurons represent information processing in the brain. The microarchitecture of synaptic connections control information processing in cortical circuits. The structure and location of synapses determine and modify the strength of this information processing. The aim of our laboratory is to reveal how information is formed, maintained, selected, and decoded in the brain at the levels of single cells and of single synapses. To do so, we mainly use two-photon microscopy that allows us to see fluorescence signals from deep within living tissue, developing novel photostimulation methods and animal behavioral tasks. The aim of our recent study is to reveal how voluntary movement is represented in cortical circuits. One of the most important problems in neuroscience is how a variety of spatio-temporally heterogeneous neural activity in the cortex emerges moment-by-moment at multiple stages of a movement.

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

To carry out two-photon calcium imaging while mice performed a self-initiated movement, we developed a head-restrained lever-pull task (Figure 1). Mice used their right forelimbs to pull the lever for a given amount of time and were subsequently rewarded with a drop of water from a spout near their mouth. With the reward at the cessation of the lever pull, a magnet-controlled solenoid pole would quickly push the lever back to the wait position. The mice then had to wait 3 s before they could receive another reward for a lever pull. During 8-9 days of the training sessions, the task difficulty was increased by gradually increasing the lever-pull time (100–1,000 ms) and mice were able to either increase or maintain the number of successful trials. Furthermore, the interval time between successful trials decreased, indicating that mice successfully learned how to pull the lever and also understood that they had to wait >3 s to pull the lever again and obtain another reward.

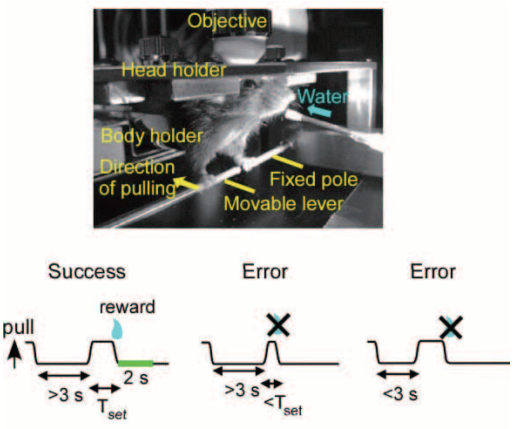

Figure 1. The lever-pull task in head-restrained mice. The head of the mouse was fixed under an objective, and the right forelimb was available to grasp and pull the movable lever. Before pulling the lever, mice had to wait for more than 3 s. After the lever pull was maintained for T_{set}, a drop of water was dispensed.

II. Identification of the mouse motor forelimb areas with photostimulation mapping

We used channelrhodopsin-2 (ChR2) mice to determine the forelimb motor areas over the broad neocortical surface by optogenetic stimulation mapping (OSM) using blue-laser scanning of the cortical surface. Laser illumination induced forelimb movements in two distinct areas: RFA (the rostral area) and CFA (the caudal area) (Figures 2). These motor forelimb areas corresponded to those reported by intracortical microstimulation in the rat and mouse cortex.

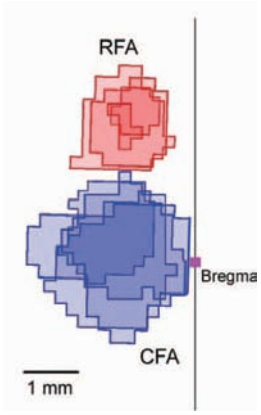

Figure 2. Dorsal view of left cortical hemisphere. Overlaid red and blue contours show the RFA and CFA, respectively, as revealed by OSM.

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

We performed two-photon calcium imaging in layer 2/3 of the left RFA or CFA while mice executed the task (Figure 3). Motion-corrected calcium transients reflected spiking activity, and particularly the burst firing of individual cells. We found two types of task-related cells in the mice: cells whose peak activities occurred during lever pulls (pull cells) and cells whose peak activities occurred after the end of lever pulls (post-pull cells). The activity of pull cells was strongly associated with lever-pull duration. In approximately 40% of imaged fields, functional clusterings were temporally detected during the lever pulls. Spatially, there were ~70 μm-scale clusters that consisted of more than four pull cells in approximately 50% of the fields. Ensemble and individual activities of pull cells within the cluster more accurately predicted lever movement trajectories than activities of pull cells outside the cluster. This was likely because clustered pull cells were more often active in the individual trials than pull cells outside the cluster. This

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

higher fidelity of activity was related to higher trial-to-trial correlations of activities of pairs within the cluster. We propose that strong recurrent network clusters may represent the execution of voluntary movements.

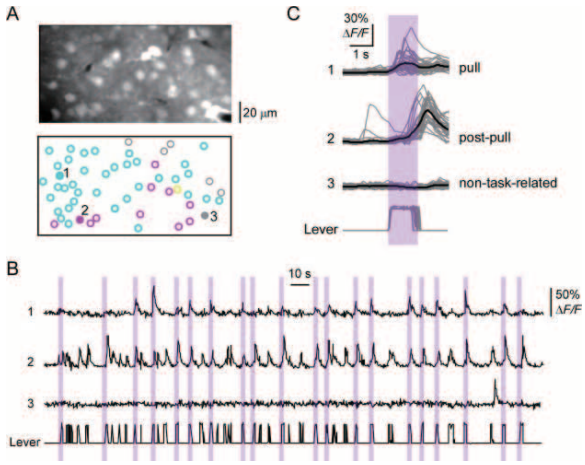

Figure 3. Activities of layer 2/3 motor cortex cells during task performance. A. A representative example of a two-photon imaged field in the RFA. Circles surround reconstructed neurons. Parts of fluorescent traces of numbered, closed circles are shown in (*B*). Cyan, magenta, yellow, and grey circles indicate pull cells, post-pull cells, other task-related cells, and non-task-related cells, respectively. B, 270-s traces of motion-corrected calcium transients of the numbered cells shown in (***A***). The trace indicated as Lever shows the lever trajectory. Shaded boxes indicate successful trials. C, The motion-corrected traces of cells 1, 2, and 3 are aligned with the start of all 30 successful lever pulls during the imaging session. Black thick lines indicate the mean traces. The lever trajectories in individual trials are overlaid in the bottom trace.

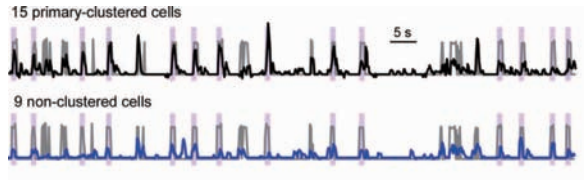

Figure 4. Prediction accuracy of the lever movement trajectory relative to neural ensemble activity of pull cells. The linear model prediction of the lever trajectory (grey) from 15 primary-clustered cells (black) and nine non-clustered cells (blue) in the imaged field shown in Figure 3. One of the test segments of the trajectory is shown. The correlation coefficients between the predicted and real trajectories for this segment were 0.55 and 0.40 in the 15 primary-clustered and nine non-clustered cells, respectively.

Publication List

〔Original paper〕

- Kimura R., Saiki A., Fujiwara-Tsukamoto Y., Ohkubo F., Kitamura K., Matsuzaki M., Sakai Y., and Isomura Y. (2012). Reinforcing operandum: rapid and reliable learning of skilled forelimb movements by head-fixed rodents. J. Neurophysiol. *108*, 1781-1792.

LABORATORY OF NEUROPHYSIOLOGY

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

"Why can we see?" This question is fundamental for a thorough understanding of vision-dependent animals, including human beings. In order to better understand the sensory systems of animals, we moved ahead from our previous research of the salt-sensing system to new research on the visual system.

I. Psychophysical study of Medaka fish

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

This year, we have made progress in studies of the prey-predator interaction using medaka and zooplankton. Visual motion cues are one of the most important factors for eliciting animal behavior, including predator-prey interactions in aquatic environments. To understand the elements of motion that cause such selective predation behavior, we used a virtual plankton system where the predation behavior in response to computer-generated prey was analyzed. First, we performed motion analysis of zooplankton (*Daphnia magna*) to extract mathematical functions for biologically relevant motions of prey. Next, virtual prey models were programmed on a computer and presented to medaka, which served as predatory fish. Medaka exhibited predation behavior against several characteristic virtual plankton movements, particularly against a swimming pattern that could be characterized as pink noise motion. Analyzing prey-predator interactions via pink noise motion will be an interesting research field in the future.

Furthermore, we have made progress in studies of the schooling behaviors of medaka. Many fish species are known to live in groups. Visual cues have been shown to play a crucial role in the formation of shoals (a shoal is defined as social group of fish). Using biological motion stimuli, depicting a moving creature by means of just a few isolated points, we examined for the first time whether physical motion information is involved in the induction of shoaling behavior. To generate biological motion stimuli, medaka were videotaped and then six points were placed along the body trunk using computer software (Figure 1). We found that the presentation of biological motion could prominently induce shoaling behavior. We are now analyzing what aspects of motion (such as movement speed and temporal order) are critical in the induction of shoaling behavior. Motion or behavioural characteristics can be valuable in recognizing animal species, sex, and group members. Studies using biological motion stimuli will enhance our understanding of how non-human animals extract and process the information which is vital for their survival.

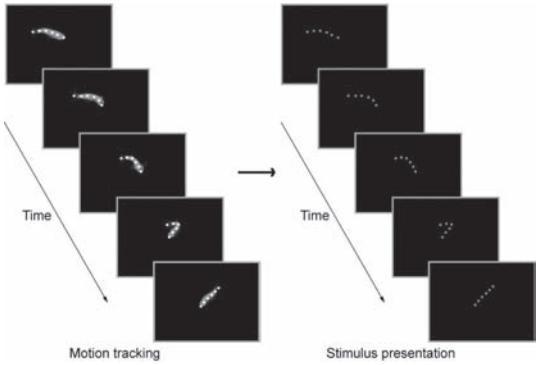

Figure 1. Animation sequence depicting biological motion of medaka. Six points were automatically placed along the body trunk (Motion tracking). Based on the tracking data, the movements of medaka were expressed as those of six gray dots (Stimulus presentation).

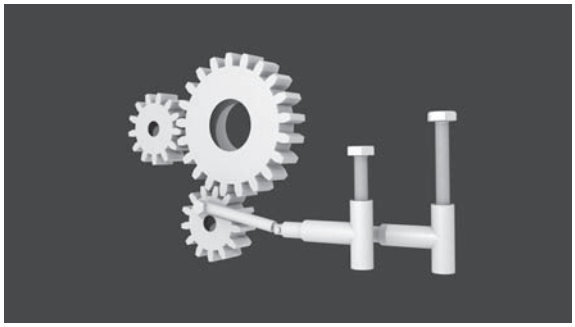

Figure 2. Sample video of Flash-lag effect. This year, we successfully produced a new version of the 3D Flash-lag effect using Blender 3D software. Please refer to YouTube (http://youtu.be/X8RiaNUFIaU).

II. Psychophysical study of Human vision

Another of our major subjects is the psychophysical and theoretical studies of the visual system of human beings (*Homo sapiens*). 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 (Figure 2). This effect has been utilized for understanding human motion perception. We developed a simple conceptual model explaining the flash-lag effect (Delta model, Watanabe *et al*., 2010).

Publication List

〔Original paper〕

● Matsunaga, W., and, Watanabe, E. (2012). Visual motion with pink noise induces predation behaviour, Scientific Reports, *2*, 219.

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I. Evolution of Complex Adaptive Characters

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

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

Different species have different morphology and also cellular characters vary between species. Stem cells self-renew and repeatedly produce differentiated cells during development. Conversely, differentiated cells can be converted into stem cells in some organisms. In plants, regeneration of a stem cell leads to a generation of a new individual, which is an effective strategy for propagation. The ability of reprogramming is different from species to species but the reason is unknown. The moss *Physcomitrella patens* has a rapid reprogramming ability (see http://www.nibb.ac.jp/evodevo/ERATO/movie/MacMovie.mp4) and is feasible for experiments. Cells in a dissected leaf are reprogrammed to become chloronema apical stem cells within 24 hours.

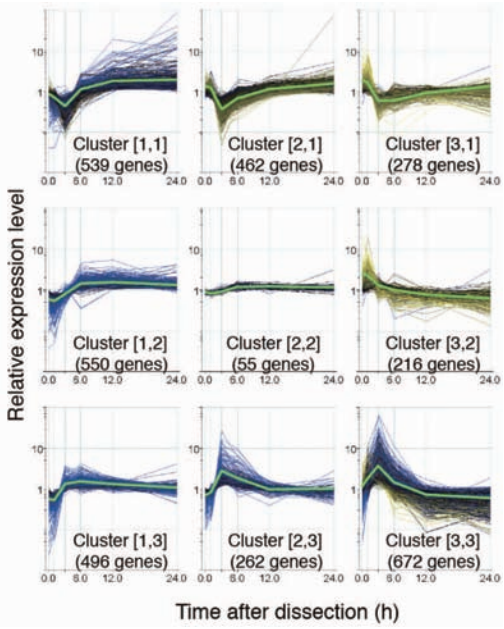

Figure 1. Expression changes of genes during reprogramming are classified into nine types. Reprogramming of leaf cells started at time 0.

To understand the underlying molecular mechanisms, a digital gene expression profiling method using mRNA 5'-end tags (5'-DGE) was established. The 5'-DGE method produced reproducible data with a dynamic range of four orders that correlated well with qRT-PCR measurements. After the excision of leaves, the expression levels of 10% of the transcripts changed significantly within 6 h. Genes involved in stress responses and proteolysis were induced and those involved in metabolism, including photosynthesis, were reduced. The later processes of reprogramming involved photosynthesis recovery and higher macromolecule biosynthesis, including of RNA and proteins. Comparison with stem cell formation via callus in flowering plants revealed that common phytohormone signaling pathways are activated during reprogramming, although no exogenous phytohormone is applied in the moss system, suggesting that an intrinsic phytohormone regulatory system may be used in the moss (Nishiyama et al. 2012).

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III. Evolution of Regeneration: Epigenetic regulation

In differentiated cells, active modifications such as trimethylation of histone H3 at lysine 4 (H3K4me3) are enriched at certain genes required for the cell function, and the repressive modifications including H3K27me3 are enriched at other unnecessary genes, to ensure an expression profile that fits the cell function. In contrast, in animal pluripotent stem cells, most genes with H3K27me3 also have H3K4me3, and this bivalent chromatin state is presumed to keep genes poised for transcription and to be required for the pluripotency. Thus, changes in the genome-wide chromatin modifications must be associated with and required for the process of reprogramming. We are currently attempting to analyze the dynamics of the chromatin modifications in the reprogramming process of *P. patens* with the combination of chromatin immunoprecipitation-sequencing (ChIP-Seq) using a next generation sequencer and live imaging of chromatin modifications. We have successfully established a 4D (3D + time) live-imaging method of a single *P. patens* nucleus, and also produced a H3K27me3 detector using fluorescent protein fused to *Drosophila melanogaster* Polycomb protein, which is known to bind to H3K27me3. We are now analyzing the ChIP-Seq data, performing the 4D single-nucleus live imaging for H3K27me3 during the reprogramming, and producing live-imaging detector for other chromatin modifications including H3K4me3. This study was mainly conducted by Yosuke Tamada.

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

Animal somatic cells can be reprogrammed to induce pluripotent stem (iPS) cells by introducing four transcription factors, while such factors have not been identified in plants. On the basis of the transcriptional profile during the moss reprogramming (Nishiyama et al., 2012), we selected genes, of which transcript levels are low in gametophores and up-regulated during reprogramming, of leaf cells after excision, and then overexpressed those genes in intact gametophores using the estrogen-inducible system. As a result, we identified a gene encoding a member of plant-specific transcription factor, STEM CELL-INDUCING FACTOR (STEMIN), that was able to induce direct reprogramming of differentiated leaf cells into chloronema apical stem cells without wounding signals. Using the 5'-DGE analysis, we found that many of the genes induced by the STEMIN-expression in intact gametophores overlapped with those upregulated during reprogramming in excised leaves. In addition, *STEMIN* promoter was activated at leaf cells that underwent reprogramming. Deletion of the *STEMIN* and its two paralogous genes delayed reprograming after leaf excision. Together, we suggest that STEMIN is a single master regulatory transcription factor governing *de novo* stem cell formation. Masaki Ishikawa was this study's main researcher.

V. Evolution of Elaborated Cell Division Machinery: Phragmoplast

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

VI. Evolution of Developmental Programs

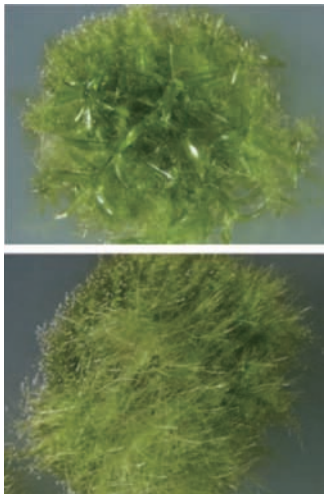

Figure 2. APB transcription factor is indispensable for a shoot body (gametophore) formation in the moss *Physcomiterlla patens*. Deletion of four APB genes caused no gametophores (lower panel) in comparison to wild type (upper panel).

The basal land plant mosses have two different developmental processes in the haploid generation, forming hypha-like protonemata and shoot-like gametophores. We found that four AP2-type transcription factors orthologous to Arabidopsis *thaliana AINTEGUMENTA*/*PLETHORA*/ *BABY BOOM* (*APB*) are indispensable for the formation of gametophore apical cells. Quadruple disruption of all *APB* genes blocked gametophore formation, even in the presence of cytokinin, which enhances gametophore apical cell formation in the wild type. Heat-shock induction of an *APB4*

transgene driven by a heat-shock promoter increased the number of gametophores. Expression of all APB genes was induced by auxin but not by cytokinin. Thus, the APB genes function synergistically with cytokinin signaling to determine the identity of the two types of stem cells (Aoyama et al. 2012).

V. Molecular mechanisms of mimicry

An excellent example of mimicry is the flower-mimicry of the orchid mantis *Hymenopus coronatus* with pink and white coloration and petal-like legs. HPLC and MS analyses indicated that xanthommatin, a common red pigment of the ommochrome family, almost solely contributes to the pink color of late-stage nymphs. On the other hand, 1st-instar nymph with yellowish red color contains a very labile pre-xanthommatin in addition to xanthommatin. These results suggest that the orchid mantis alters its body color by changing the composition of ommochrome pigments during post-hatching development. This work was mainly done by Hiroaki Mano.

VI. Molecular mechanisms of host shifting

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. A QTL analysis of a tiny moth, *Acrocercops transecta* revealed that only a restricted region of a single autosome was responsible for the larval performance, suggesting that a small number of genetic changes to larval performance allowed the successful host shifting. Identification of the responsive genes is in progress with Dr. Issei Ohshima in Kyoto Prefecture University.

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

The molecular mechanisms and evolutionary significance of plant movement, including seismonastic and nyctinastic movements, are enigmatic. To introduce the sensitive plant *Mimosa pudica* as a model, we established a method for transformation. We used a cotyledonary node as a target of *Agrobacterium*-mediated gene transfer because of its ability of shoot regeneration. We obtained a large number of transformed calluses (55-60%) and succeeded in regenerating transgenic plants with a transformation efficiency of >5%. This study was conducted mainly by Hiroaki Mano.

VIII. Evolution of pitcher leaves in carnivorous plants

Carnivorous plants form specialized leaves that are capable of attracting, trapping, and digesting prey and absorbing nutrients. The unusual plants evolved from non-carnivorous plants but their evolutionary process is mostly unknown. To understand the genomic changes associated with the evolution of carnivory, we sequenced 2-Gbp genome of the Australian pitcher plant *Cephalotus follicularis* in collaboration with Beijing Genomics Institute. Whole-genome shotgun data corresponding to 100-fold depth were produced by Illumina paired-end/mate-pair sequencing with 180-bp to 5-kb insert sizes. A *de novo* assembly yielded a total of 1.7 Gbp in 43,308 scaffolds with 15.6 kb of contig N50 and 83.3 kb of scaffold N50. We further produced 14 Gbp of PacBio reads with 2-kb mean max subread length for gap filling. Transcript-based gene prediction with RNA-seq reads found 45,469 gene models. Genomic data enable us to deduce the origin and evolution of carnivory-related genes, such as digestive enzyme genes. This study was conducted mainly by Kenji Fukushima and Tomoko Shibata.

Publication List

〔Original papers〕

- Aoyama, T., Hiwatashi, Y., Shigyo, M., Kofuji, R., Kubo, M., Ito, M., and Hasebe, M. (2012). AP2-type transcription factors determine stem cell identity in the moss *Physcomitrella patens*. Development *139*, 3120-3129.
- Nishiyama, T., Miyawaki, K., Ohshima, M., Thompson, K., Nagashima, A., Hasebe, M., and Kurata, T. (2012). Digital gene expression profiling by 5'-end sequencing of cDNAs during reprogramming in the moss *Physcomitrella patens*. PLoS ONE *7*, e36471.
- Nishimura, T., Matano, N., Morishima, T., Kakinuma, C., Hayashi, K.I., Komano, T., Kubo, M., Hasebe, M., Kasahara, H., Kamiya, Y., and Koshiba, T. (2012). Identification of indole-3-acetic acid transport inhibitors including compounds affecting cellular PIN trafficking by two chemical screening approaches using maize coleoptile systems. Plant Cell Physiol. *53*, 1671-1682.
- Suetsugu, N., Sato, Y., Tsuboi, H., Kasahara, M., Imaizumi, T., Kagawa, T., Hiwatashi, Y., Hasebe, M., and Wada, M. (2012). The KAC family of kinesin-like proteins is essential for the association of chloroplasts with the plasma membrane in land plants. Plant Cell Physiol. *53*, 1854-1865.
- Zhao, N., Ferrer, J.L., Moon, H.S., Kapteyn, J., Zhuang, X., Hasebe, M., Stewart, C.N., Jr., Gang, D.R., and Chen, F. (2012). A SABATH Methyltransferase from the moss *Physcomitrella patens* catalyzes S-methylation of thiols and has a role in detoxification. Phytochemistry *81*, 31-41.

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More than 80% of land plant families have symbiotic relationships with arbuscular mycorrhizal (AM) fungi. 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. Despite marked differences between the fungal and bacterial symbioses, common genes required for both interactions have been identified using model legumes. Our laboratory focuses on the early stages of the interaction between these microorganisms and *Lotus japonicus* in order to reveal the molecular mechanism and the origin of these symbiotic systems.

I. Nodulation

1-1 The site of auxin action during root nodule development and identification of *TRICOT* gene in *L. japonicus*

Nodulation is a form of *de novo* organogenesis that occurs mainly in legumes. During early nodule development, the host plant root is infected by rhizobia that induce dedifferentiation of some cortical cells; these cortical cells then proliferate to form the symbiotic root nodule primordium. We investigated the detailed patterns of auxin response during nodule development in *L. japonicus* (Figure 1). Our analyses showed that cytokinin signaling positively regulated this response. Additionally, we found that auxin response was inhibited by a systemic negative regulatory mechanism called autoregulation of nodulation (AON). Analysis of the constitutive activation of *LjCLE-RS* genes, which encode putative root-derived signals that function in AON, in combination with determination of auxin response patterns in proliferating cortical cells, indicated that activation of *LjCLE-RS* genes blocked the progress of further cortical cell division, probably through controlling auxin response. Our data provide evidence for the existence of a novel fine-tuning mechanism that controls nodule development in a cortical cell stage-dependent manner.

Recent studies on AON and hormonal controls of nodulation have identified key mechanisms and also indicated a possible link to other developmental processes, such as the formation of the shoot apical meristem (SAM). However, our understanding of this process is still limited by the low number of nodulation-related genes that have been identified. We found that the induced mutation *tricot* (*tco*) can suppress the activity of *spontaneous nodule formation 2*, a gain-of-function mutation of the cytokinin receptor in *L. japonicus*. Our analyses of *tco* mutant plants demonstrate that *TCO* positively regulates rhizobial infection and nodule organogenesis. In addition to its role in nodulation, *TCO* is involved in the maintenance of the SAM. The *TCO* gene was isolated by a map-based cloning approach and found to encode a putative glutamate carboxypeptidase with greatest similarity to *Arabidopsis* ALTERED MERISTEM PROGRAM 1, which is involved in cell proliferation in the SAM. Taken together, our analyses have not only identified a novel gene for regulation of nodule organogenesis but also provided significant additional evidence for a common genetic regulatory mechanism in nodulation and SAM formation. This new data will contribute further to our understanding of the evolution and genetic basis of nodulation.

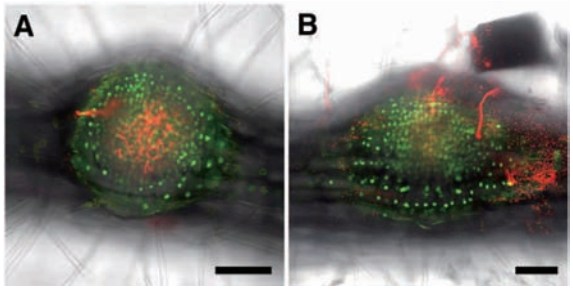

Figure 1. Auxin response patterns during nodule development in *L. japonicus*. (A, B) Auxin response patterns during nodule development are indirectly shown by *GFP* expression (green) in *DR5::GFP-NLS* plants. Fluorescence and nodule formation were observed from front (A) and side views (B) of infected roots. Red areas indicate the presence of rhizobia. Scale bars: 100 μm.

1-2 Isolation of a novel non-nodulation mutant, *daphne* that has a hyperinfection phenotype

The main events for making nodules are divided into "infection" and "organogenesis." For proper establishment of symbiosis, it is essential that the two phenomena proceed synchronously in different root tissues. Several non-nodulating mutants have been isolated using model legumes. However most of those have defects in both infection and organogenesis pathways. Therefore it has been difficult for understanding the molecular relationship between both pathways independently.

We isolated a novel non-nodulating mutant, *daphne* from C^{6+} beam mutagenized seeds of *L. japonicus*. Unlike

previously reported non-nodulation mutants, the *daphne* mutant has defects only in the organogenesis pathway. The *daphne* mutant has completely defective nodulation, but has increased number of infection threads. Map-based cloning identified the *daphne* mutation, reciprocal chromosomal translocation between chromosomal II and III which is located on the upstream of the *NODULE INCEPTION* (*NIN*) gene, whose expression is up-regulated dependent on rhizobial inoculation. In *daphne*, the expression level of *NIN* is lower than that in WT because of the mutation. Add to this, the result of the allelism test strongly suggest that *daphe* is a novel *nin* mutant allele, being different from the *nin* null mutant which cannot form any infection thread. The phenotype of the *nin* null mutant indicates that NIN, putative transcription factor, functions in both infection and organogenesis pathways. We speculate that daphne might have lost the specific expression of NIN that mediates the nodule organogenesis pathway.

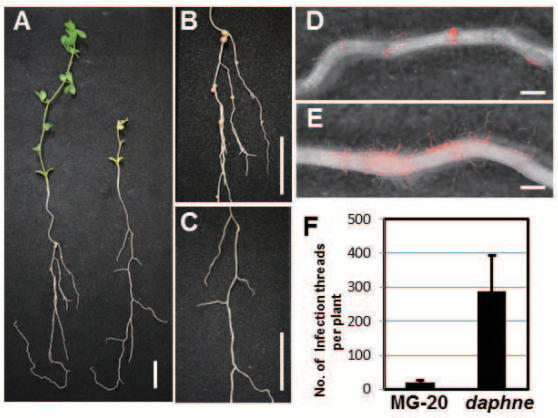

Figure 2. Isolation of *daphne* mutant. (A) The 4 week old seedlings of wild type (left) and *daphne* mutant (right) with rhizobial inoculation. (B, C) High-magnification images of wild type root (B) and *daphne* root (C). (D, E) The infection threads with red florescence signal and root images were merged. The number of infection threads is higher in *daphne* (E) than that in wild type (D). (F) The number of infection threads is about 20-fold greater in *daphne* than that in wild type. Scale bars: 2cm.

1-3 TOO MUCH LOVE, a novel F-box Kelch protein, functions in the long-distance control of the root-nodule symbiosis

In the legume-rhizobia symbiosis, the nodule number is tightly restricted by the host through a systemic suppression termed autoregulation of nodulation (AON). AON provides a long-distance control of nodulation via the shoot and is classified into 3 stages: (i) an early nodulation event induces the translocation of the root-derived signal to the shoot, (ii) the leucine-rich repeat receptor-like kinases (e.g., HAR1/SUNN/NARK or KLV) in the shoot are activated by the root-derived signal, and the shoot-derived inhibitor (SDI) is generated and translocated to the whole root system and (iii) an assumed root regulator activated by the SDI inhibits the subsequent nodule organogenesis. Earlier studies have suggested that CLE peptides are strong candidates for the root-derived signals, as they drastically suppresses the nodulation in a HAR1/SUNN/NARK- or KLV-dependent manner. However, a large part of the mechanism involved at stage (iii) of AON needs to be elucidated. Previously, we have shown that the *too much love* (*tml*) mutant in *L. japonicus* (a mutant that exhibits an excessive number of nodules) has a defect in the negative feedback regulation and that *TML* functions in the roots downstream of *HAR1* (Magori et al., 2009).

In this study, we found that *TML* acts downstream of *CLE-RS1/RS2* and *HAR1* and that *TML* suppresses the nodulation signaling downstream of the cytokinin receptor *LHK1/CRE1*. In addition, we identified the *TML* gene that encodes a Kelch repeat-containing F-box protein with two NLSs, and we show that *TML* is constitutively expressed in the root tips independent of rhizobial infection and is expressed in the root primordia upon rhizobial infection. With these results, we concluded that *TML* functions in the *CLE-RS1/RS2*- and *HAR1*-mediated long-distance control of nodule organogenesis. In addition, our finding that *TML* encodes the F-box protein strongly suggests that AON functions via proteasome-mediated target degradation. To our knowledge, this is the first report of ubiquitin-related component that functions during AON.

II. Arbuscular mycorrhiza symbiosis

Arbuscular mycorrhiza is mutualistic plant-fungal interaction which has several similar systems to root nodule symbiosis in host-symbiont recognition, infection process and nutrient material exchanges. Root nodule symbiosis (RNS) is thought to evolve by sharing AM factors, suggesting that the AM system contains a fundamental mechanism that also regulates root nodule symbiosis.

We isolated two novel common symbiosis mutants; *cerberus* and *nsp1* by screening of RNS mutants in *L. japonicus*. These mutant showed low symbiont infection phenotypes in AM and RNS (Figure 3). Common symbiosis genes isolated so far are supposed to act in the early signaling pathway for recognition of the symbionts and triggering symbiosis responses. However, analysis of the AM phenotypes revealed that CERBERUS and NSP1 quantitatively regulate AM fungal infection downstream or parallel to current models of symbiotic signaling, and extends the concept of commonality between RNS and AMS.

In order to obtain further insights about the function of these symbiotic factors, we performed transcriptome analysis in *nsp1* and *cerberus* mutant by RNAseq using next generation sequencing technology. The analysis will reveal effects of the mutation on the symbiotic signaling networks during AMS and RNS. In addition, we are also analyzing transcriptome profiles of the symbiotic partner AM fungi. AM fungi are an obligate symbiont which requires the host and symbiotic relationship to accomplish the life cycle, especially for the spore formation. The transcriptome analysis would indicate important clues about symbiotic and reproductive systems of AM fungi.

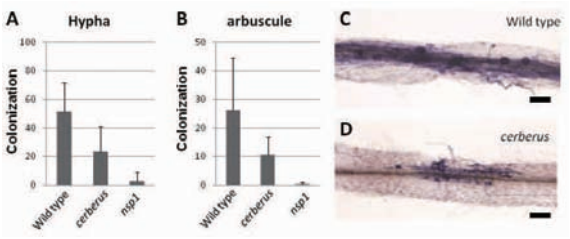

Figure 3. AM fungal colonization in RNS mutant cerberus and *nsp1*. Hyphae (A) and arbuscule (B) colonization in wild type, cerberus and nsp1 were examined at 4 weeks-after innoculation (wai) with AM fungi *Rhizophagus irregularis*. AM fungi colonized in the root of wild type (C) and *cerberus* (D) were stained with ink (purple) at 3 wai. Delay or defect of hyphal elongation was observed in the *cerberus* root (D). Bars = 200 μm.

III. Pattern formation by two-layer system

Many multicellular organisms have a layered structure. The interaction between these layers plays an essential role in many developmental processes, and key molecules involved in these processes are often expressed in a layer-specific manner. On the other hand, pattern formation of organisms has been frequently discussed in connection with the Turing system. However, the Turing system has so far been studied mainly in single-layered space. We thus investigate a two-layer Turing system with complementary synthesis, in which two interacting molecules are exclusively synthesized in different layers (Figure 4A).

From a linear stability analysis, we determine the Turing condition of the complementary system, and show that this condition requires stronger regulatory interactions of the molecules than that of the system with usual ubiquitous synthesis. We then confirm that this complementary system affects pattern types in fixed and expanding two-dimensional spaces in a similar way to the system with ubiquitous synthesis. In addition, the two-layer system includes two types of diffusion, lateral and transversal, and these have distinct effects on pattern formation with lateral diffusion mainly determining the periodicity of patterns generated and transversal diffusion affecting pattern type. Finally, we apply this complementary system to explain pattern formation of the shoot apical meristem of plants (Figure 4B-E). These findings provide an understanding of pattern formation caused by the interaction between cell layers in multicellular organisms.

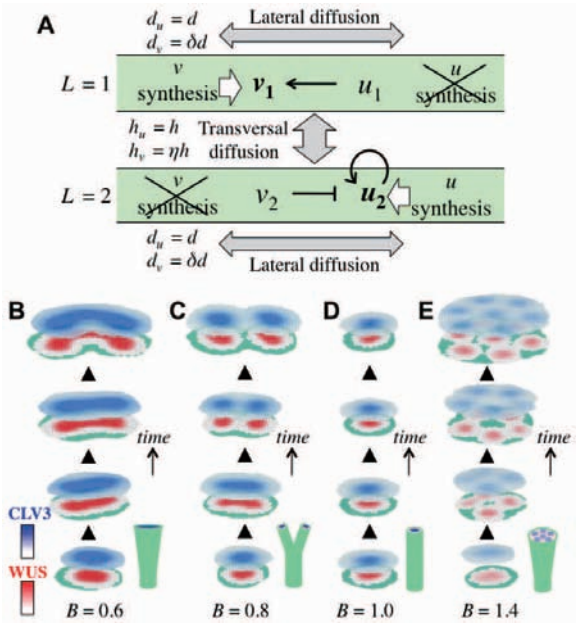

Figure 4. Two-layer system with complementary synthesis. (A) Schematic representation of two-layer activator-inhibitor system with complementary synthesis of *u* (activator) and *v* (inhibitor). (B-E) Examples of time evolution of the SAM in (B) fasciation pattern, (C) dichotomous pattern, (D) homeostasis pattern, and (E) fluctuation pattern.

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- Suzaki, T., Kim, C.S., Takeda, N., Szczyglowski, K., and Kawaguchi, M. *TRICOT* encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in *Lotus japonicus*. Development 2012 Dec 18.

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 as the main target of morphological studies.

The laboratory was moved from Myodaiji-area to Yamate-area in July 2012.

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.

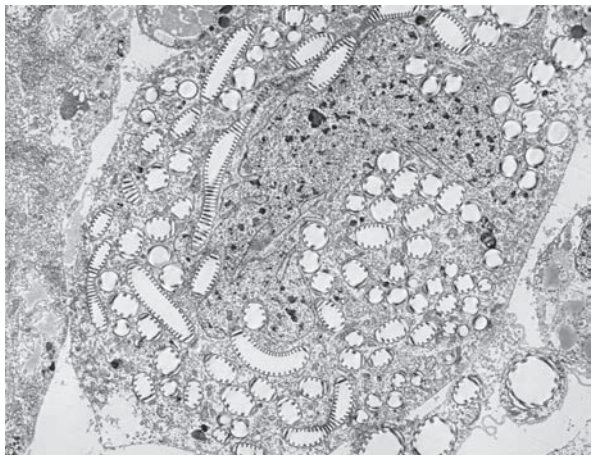

Figure 1. Transmission electron micrograph of the tracheole cell. Many cross-sections of the tracheoles can be observed within the cytoplasm of the tracheole cell. As the cell migrates, the tracheole is laid behind.

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 in collaboration with other laboratories of NIBB. Training in specimen preparation and instrument operation for such observations is also given.

LABORATORY OF BIORESOURCES

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NIBB Research Fellow:	*TAKEHANA, Yusuke**
Postdoctoral Fellow:	*SASADO, Takao*
	SHIBATA, Yasushi
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	NAKAMOTO, Masatoshi
	OKUYAMA, Teruhiro
	KIMURA, Tetsuaki
	NOZU, Ryo
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	TORII, Naoko
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	TESHIMA, Yuuko
	HARA, Ikuyo
	ISHIKAWA, Hiroe
	SHIBATA, Emiko
	TAKAKI, Chikako
	HAMATANI, Ayako
Secretary:	*SUZUKI, Tokiko*

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

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

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

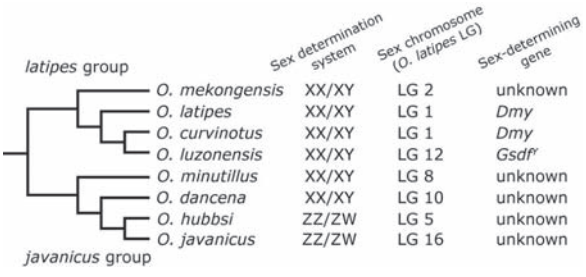

Figure 1. Phylogenetic relationships and sex determination mechanisms in *Oryzias* fishes.

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

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

III. The function of estrogen in the medaka ovary

Estrogens have been generally considered to play a critical role in the ovarian differentiation of teleost fish by Yamamoto's model. In medaka, estrogen treatment has induced functional male-to-female sex reversal. To clarify the function of estrogen during ovarian development, we examined the role of ovarian aromatase, which is responsible for catalyzing the conversion of testosterone to estrogen. We isolated two tilling mutant strains of ovarian aromatase. In these tiling mutants, one amino acid in ovarian aromatase ORF altered the STOP codon. Mutant females seemed to develop normal ovaries but yolk accumulation was not observed in the ovarian follicles and most of the ovarian follicles undergo atresia in the adult ovary. Even more surprisingly, spermatogenesis was observed within the mutant ovary. These results suggest that the hypothesis that endogenous estrogens drive ovarian differentiation needs to be modified to the hypothesis that estrogens are essential for the maintenance of ovarian differentiation.

IV. Positional cloning of pigment cell mutants in medaka

All kinds of pigment cells are derived from neural crest cells. How each type of pigment cell differentiates and what differences are producing which of the various pigment cell types is a very interesting question. Medaka have four types of pigment cell (melanophore, leucophore, xanthophore and iridocyte). The leucophores are unique because only some species have them. To elucidate how leucophores differentiate from neural crest cells and why they exist only in some fishes, we have successfully identified the causal gene of leucophore mutants (leucophore free (*lf*) and leucophre free 2 (*lf-2*)). We identified *slc2a15b* as the causal

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

gene of the *lf* mutant. The *slc2a15b* expression exists but does not persist in the *lf* mutant. *slc2a15b* also exists in species without leucophores, but these use carotenoid as a pigment. Thus, *slc2a15b* may have an important role in use of carotenoid as pigments. The *lf-2* phenotype was rescued by *pax7a*. *pax7a* is needed for differentiation of leucophore and xanthophore in medaka.

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

In 2007, NIBB was selected as the core facility of NBRP Medaka. Our laboratory is taking an active part in this project. With the goal of facilitating and enhancing the use of medaka as a model organism, we provide, maintain and collect living resources such as standard strains, inbred strains, and mutants in addition to frozen resources such as EST/cDNA and BAC/ Fosmid clones and hatching enzymes, as well as integrated information on medaka (Figure 2). We have been providing the TILLING screening system to NBRP Medaka users for promoting the reverse genetic approach since 2011,. NBRP Medaka aims to establish a first rate biological resource with the highest possible levels of accessibility and ease of use.

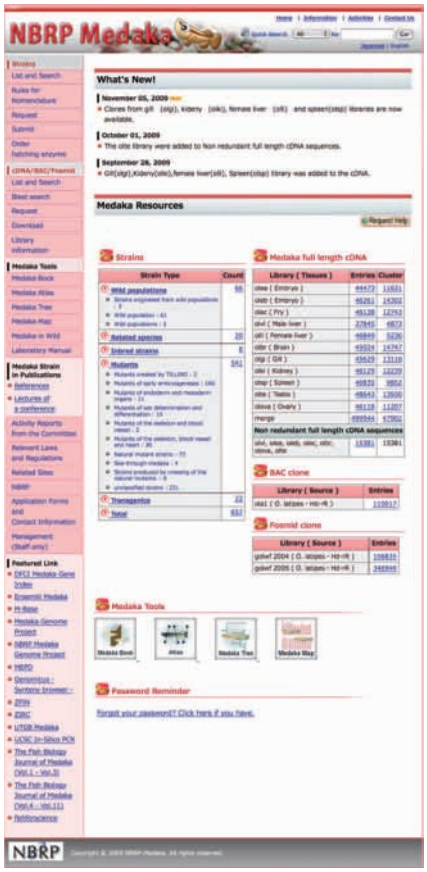

Figure 2. NBRP Medaka website

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LABORATORY OF BIOLOGICAL DIVERSITY

KAMADA Group

Assistant Professor: *KAMADA,Yoshiaki*

Nutrients are indispensable for life. Thus, perception of the nutrient environment is also essential for cells. Eukaryotic cells employ Tor (target of rapamycin) protein kinase to recognize cellular nutrient conditions. Tor forms two distinct protein complexes, TORC (Tor complex) 1 and TORC2. TORC1 regulates rapamycin-sensitive branches of the TOR pathway, such as protein synthesis, cell cycle and autophagy. TORC1 is thought to act as a nutrient sensor, because rapamycin, a TORC1 inhibitor, mimics a starved condition. On the other hand, TORC2, whose function is insensitive to rapamycin, is responsible for actin organization and cell integrity. So far, it is not clear whether TORC2 also perceives nutrient signals.

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

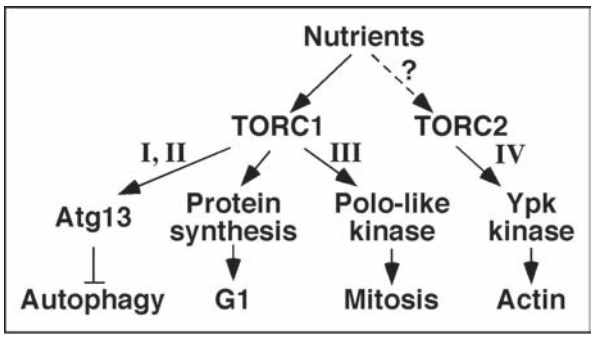

Figure 1. TOR signaling pathway of budding yeast. Our group has found three branches of the TOR pathway.

I. TORC1 phosphorylates Atg13, the molecular switch of autophagy.

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

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

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

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

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

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

We generated a temperature-sensitive allele of *KOG1 (kog1-105)*, which encodes an essential component of TORC1. We found that this mutant, as well as yeast cells treated with rapamycin, exhibit mitotic delay with prolonged G2. We further demonstrated that this G2-arrest phenotype is due to mislocalization and resultant inactivation of Cdc5, the yeast polo-kinase. These results suggest that TORC1 mediates G2/M transition via regulating polo-kinase.

IV. Ypk2 kinase acts at the downstream of TORC2 to control actin organization.

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

Publication List

〔Review article〕

● Klionsky, D.J., Abdalla, F.C., Abeliovich, H., Abraham, R.T., Acevedo-Arozena, A., Adeli, K., Agholme, L., Agnello, M., Agostinis, P., Aguirre-Ghiso, J.A. *et al.* (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy *8*, 445-544.

LABORATORY OF BIOLOGICAL DIVERSITY

OHNO Group

Assistant Professor: *OHNO, Kaoru*

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

I. Gonadotropins in the starfish, *Asterina pectinifera*

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

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

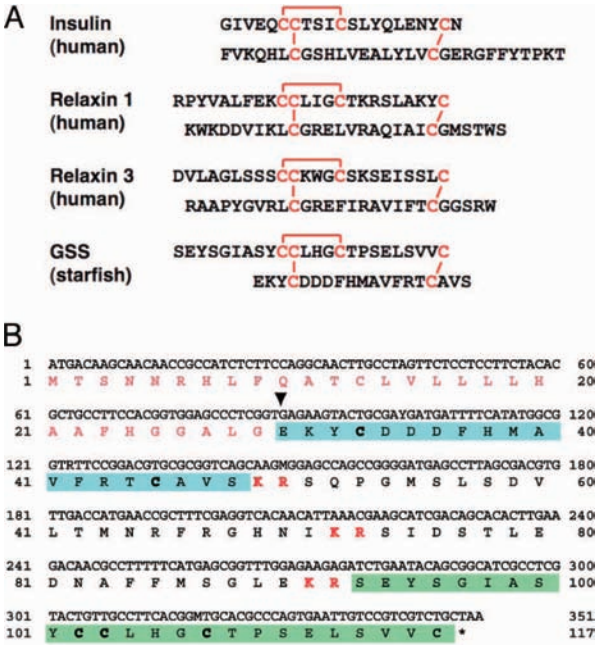

Figure 1. Amino acid sequence of starfish GSS. (A) Comparison of the heterodimeric structure of starfish GSS with those of various representative members of the insulin superfamily. The cysteine bridges are shown in red. (B) Coding DNA sequence and predicted amino acid sequences of GSS. Sequences of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

II. Search for reproductive hormones in invertebrates

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

LABORATORY OF BIOLOGICAL DIVERSITY

HOSHINO Group

Assistant Professor: *HOSHINO, Atsushi*
Technical Assistant: *WATANABE, Seiko*
NAKAMURA, Ryoko
TAKEUCHI, Tomoyo

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

I. Flower pigmentation patterns of the morning glories

Morning glories belong to the genus *Ipomoea* that is the largest group in the family *Convolvulaceae*. Of these, *I. nil* (Japanese morning glory), *I. purpurea* (the common morning glory), and *I. tricolor* have been domesticated well as floricultural plants, and their various spontaneous mutations have been isolated. The wild type morning glories produce flowers with uniformly pigmented corolla, whereas a number of mutants displaying particular pigmentation patterns have been collected (Figure 1). Because flower pigmentation patterns are easily observed, the molecular mechanisms underlying these phenomena provide fine model systems for investigating genome variability.

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

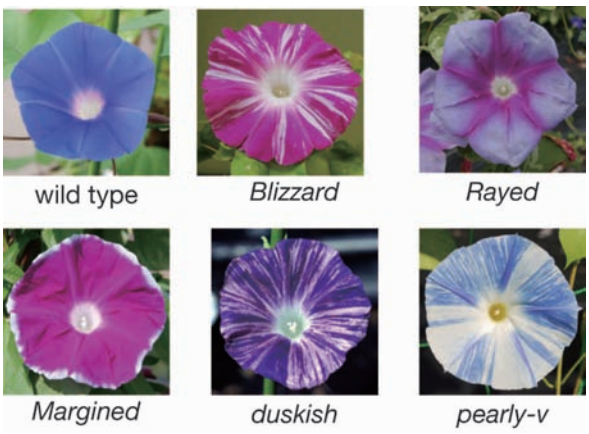

Figure 1. Flower phenotypes of the morning glories.

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

Although morning glories are studied worldwide, especially in plant physiology and genetics, no whole nuclear genome sequences of any *Ipomoea* species are available. To facilitate the studies of our group as well as all morning glory researchers, we are conducting *de novo* genome sequencing of *I. nil*, having a genome of about 800 Mbp. We chose the Tokyo-kokei standard line for genome sequencing, and employed not only shotgun sequencing using high-throughput DNA sequencers but also BAC end sequencing. We are collaborating with several laboratories in Japan.

III. BioResource of morning glories

NIBB is the sub-center for National BioResource Project (NBRP) for morning glory. In this project, we are collecting, maintaining and distributing standard lines, mutant lines for flower pigmentation, and DNA clones from EST and BAC libraries of *I. nil* and its related species. *I. nil* has been one of the most popular floricultural plants since the late Edo era in Japan. It has extensive history of genetic studies and also has many advantages as a model plant; simple genome, large number of mutant lines, and efficient self-pollination. Our collection includes 220 lines and 117,000 DNA clones.

Figure 2. Novel spontaneous mutants isolated in NBRP. From left to right: delayed leaf senescence, morphological alteration of flowers and leaves, flower closure failing, and bushy.

Publication List

〔Original papers〕

- Park, K.I., and Hoshino, A. (2012). A WD40-repeat protein controls proanthocyanidin and phytomelanin pigmentation in the seed coats of the Japanese morning glory. J. Plant Physiol. *169*, 523-528.
- Tong, L., Fukuoka, H., Otaka, A., Hoshino, A., Iida, S., Nitasaka, E., Watanabe, N., and Kumoyama, T. (2012). Development of EST-SRR markers of *Ipomoea nil*. Breed. Sci. *62*, 99-104.

LABORATORY OF BIOLOGICAL DIVERSITY

TSUGANE Group

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

An active nonautonomous DNA transposon, *nDart1-0*, belonging to the *hAT* superfamily, was identified. The transpositions of *nDart1-0* were promoted by an active autonomous element, *aDart1-27*, on chromosome 6. By using the endogenous *nDart1*/*aDart1-27* system in rice, a large-scale *nDart*-inserted mutant population could be easily generated under normal field conditions, and the resulting tagging lines were free of somaclonal variation. The *nDart1* transposons tend to insert into the promoter, 5' UTR region, or exon of a gene, which suggests that the *nDart1*/*aDart1-27* system is a powerful tool for rice functional genomics. Furthermore, we are developing several *indica* lines bearing the active *nDart1*/*aDart1-27* system. These lines would effectively contribute to gene functional analysis and breeding for the *indica* rice varieties.

Activation and Epigenetic Regulation of DNA Transposon *nDart1* in Rice

A large part of the rice genome is composed of transposons. Since active excision/reintegration of these mobile elements may result in harmful genetic changes, many transposons are maintained in a genetically or epigenetically inactivated state. However, some non-autonomous DNA transposons of the *nDart1-3* subgroup, including *nDart1-0*, actively transpose in specific rice lines, such as *pyl-v* which carries an active autonomous element, *aDart1-27*, on chromosome 6. Although *nDart1-3* subgroup elements show considerable sequence identity, they display different excision frequencies. The most active element, *nDart1-0*, had a low cytosine methylation status (Figure1). The *aDart1-27* sequence showed conservation between *pyl-stb* (*pyl-v* derivative line) and Nipponbare, which both lack autonomous activity for transposition of *nDart1-3* subgroup elements. In *pyl-v* plants, the promoter region of the *aDart1-27* transposase gene was more hypomethylated than in other rice lines. Treatment with the methylation inhibitor 5-azacytidine (5-azaC) induced transposition of *nDart1-3* subgroup elements in both *pyl-stb* and Nipponbare plants; the new insertion sites were frequently located in genic regions. 5-azaC treatment principally induced expression of *Dart1-34* transposase rather than the other 38 *aDart1*-related elements in both *pyl-stb* and Nipponbare treatment groups. Our observations show that transposition of *nDart1-3* subgroup elements in the *nDart1*/*aDart1*-tagging system is correlated with the level of DNA methylation. Our system does not cause somaclonal variation due to an absence of transformed plants, offers the possibility of large-scale screening in the field, and can identify dominant mutants. We, therefore, propose that this tagging system provides a valuable addition to the tools available for rice functional genomics.

Publication List

〔Original paper〕

● Eun C.-H., Takagi, K., Park, K.I., Maekawa, M., Iida, S., and Tsugane, K. (2012). Activation and epigenetic regulation of DNA transposon *nDart1* in rice. Plant Cell Physiol. *53*, 857-868.

〔Review article〕

● Saze, H., Tsugane, K., Kanno, T., and Nishimura, T. (2012). DNA methylation in plants: Relationship with small RNAs and histone modifications, and functions in transposon inactivation. Plant Cell Physiol. *53*, 766-784.

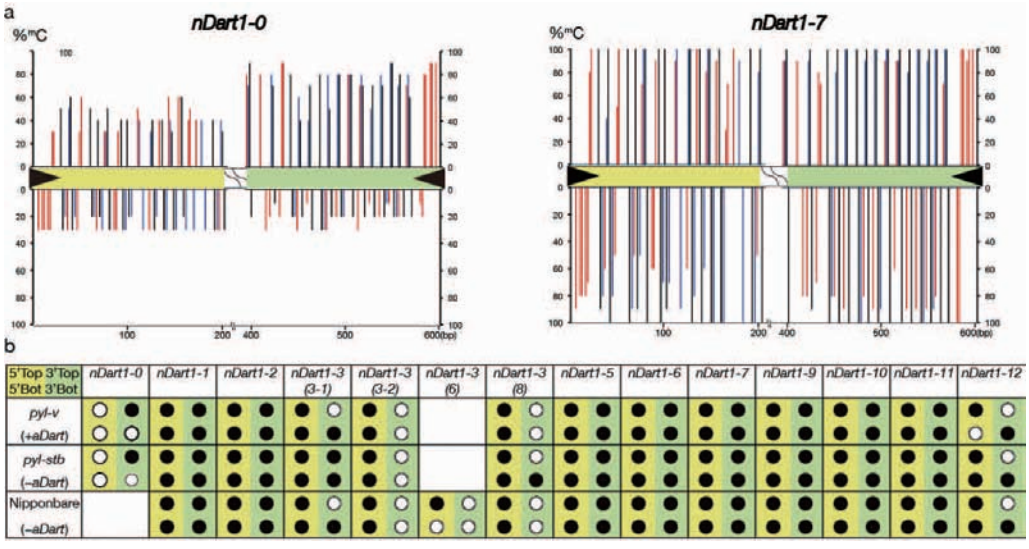

Figure 1. Bisulfite sequencing of *nDart1-3* subgroup elements in rice. (a) Methylation patterns of active *nDart1-0* and inactive *nDart1-7* elements in *pyl-stb* plants. Red, black and blue vertical lines indicate CG, CHG, and CHH, respectively. The top (upper regions) and bottom (lower regions) strands are indicated in the *nDart1-0* and *nDart1-7* boxes. (b) Summary of the bisulfite sequencing analysis of *nDart1-3* subgroup elements in rice. Open and closed circles define the hypomethylation or hypermethylation state of 10 sequenced clones, respectively.

LABORATORY OF BIOLOGICAL DIVERSITY

JOHZUKA Group

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

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

Our research interest is to understand the mechanisms and regulation of chromosome condensation. We have been studying the role of condensin in the budding yeast *Saccharomyces cerevisiae*. Microscopic observation indicated the nucleolar localization of condensin. Consistent with this, the ribosomal RNA gene (rDNA) repeat is the most condensed region in the genome during mitosis. We have found that condensin specifically binds to the RFB site located within the rDNA repeat. By genetic screening, we further discovered the multiple proteins interaction network recruits condensin to the RFB site.

I. Dynamic relocalization of condensin during meiosis

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

II. Condensin-dependent chromatin folding

The RFB site, which consists of a ~150bp DNA sequence, is functioning as a cis-element recruitment of condensin onto chromatin in the yeast genome. If the RFB site is inserted into an ectopic chromosomal locus, condensin can associate with the ectopic RFB site. To explore the role of condensin in chromosome organization, we have constructed a strain in which two RFB sites are inserted on an ectopic chromosome arm at an interval of 15kb in the cell with complete deletion of chromosomal rDNA repeat. Using this strain, condensin-dependent chromatin interaction between two RFBs was examined by chromosome conformation capture (3C) assay. We found condensin-dependent chromatin interaction between the two RFB sites on the chromosome arm. This chromatin interaction is already observed in interphase cells, and its frequency increased in metaphase arrested cells. These results indicate that condensin plays a role in chromatin interaction between condensin binding sites and this interaction lead to creation of a chromatin loop between those sites (Figure 2). It is thought that condensin-dependent chromatin folding is one of basic molecular processes of chromosome condensation. In addition, condensin-dependent chromatin folding observed during interphase cells suggest that other unknown reaction(s) are necessary for mitotic chromosome condensation, in addition to simple chromatin folding.

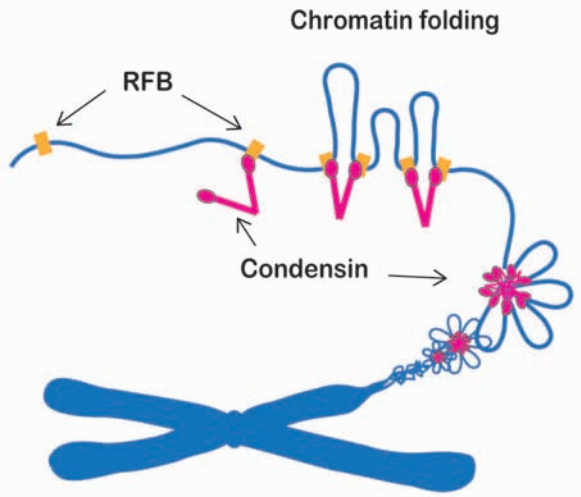

Figure 1. A Schematic model of chromosome condensation. Condensin makes chromatin interactions between adjacent binding sites (RFB, for example). This lead to a folding of chromatin fiber between the sites, as a basic process of chromosome condensation.

LABORATORY OF BIOLOGICAL DIVERSITY

WATANABE Group †

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

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

I. Model systems for studying mechanisms for gene amplification

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

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

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

II. Intensive rearrangement in amplified region

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

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

III. A structural platform for gene amplification

Based on our results, we now focus on a type of genomic structure consisting of two sets of inverted repeats (IR), designated double IR. This structure is found in the human genome and can be observed in the early stages of gene amplification. The double IR constructed in yeast can induce gene amplification as seen in our DRCR systems, suggesting that double IR serves as a source or key intermediate of DRCR amplification in nature. To investigate the molecular mechanisms underlying double IR-based amplification, we have disrupted several genes involved in genome instability in a yeast strain carrying double IR, and constructed double IR in CHO cells.

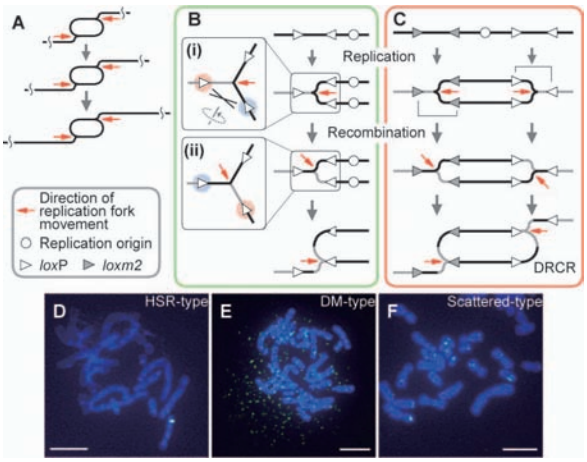

Figure 1. DRCR process, recombinational process coupled with replication, and amplification products in CHO cells.

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

Division of Molecular Environmental Endocrinology

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	TOYOTA, Kenji
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	TOHYAMA, Saki
Technical Assistant:	*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. The immune and nervous systems, bone, muscle, and the liver were also affected. Although many of these chemicals can bind to ERs in wildlife and humans, the molecular basis for the action of environmental estrogens remains poorly understood. Thus, understanding the molecular mechanisms through which environmental estrogens and sex hormones act during critical developmental windows is essential.

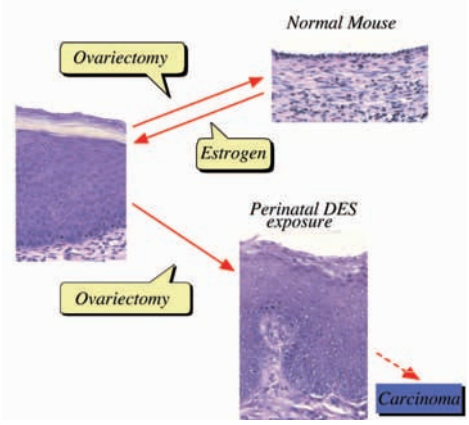

Figure 1. Scheme of estrogen-dependent and -independent vaginal epithelial cell proliferation in mice induced by perinatal estrogen exposure.

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

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

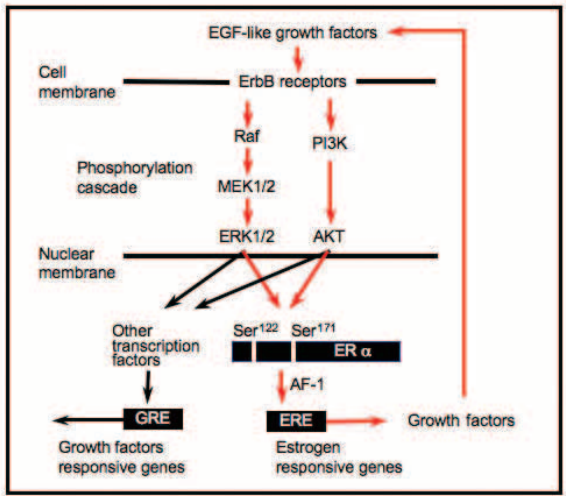

Figure 2. A hypothetical model for the estrogen-independent ER activation pathway in mouse vaginae.

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

Steroid and xenobiotic receptors (SXR) have been cloned from various animal species (fish, amphibians, reptiles, birds and mammals) by our group and we have demonstrated species-specific differences in their responses to various environmental and endogenous chemicals (receptor gene zoo). Thus, simple predictions of chemical effects based on data from a few established model species are not sufficient to develop real world risk assessments. ER and ER-like genes have been cloned from various animal species including rockshell, *Amphioxus*, lamprey, lungfish, sturgeon, gar, roach, stickleback, mosquitofish, mangrove *Rivulus*,

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2012. The former title is indicated by an asterisk (*).

catshark, whale shark, Japanese giant salamander, Tokyo salamander, newt, axolotl, toad, *Silurana tropicalis*, American alligator, Nile crocodile, freshwater turtle, Japanese rat snake, Okinawa habu, and vultures. Functional studies showed that the *Amphioxus* ER sequence does not bind estrogen but *Amphioxus* steroid receptor and lamprey ER exhibited ligand-dependent transactivation, proving that invertebrate and primitive vertebrates, such as the Agnatha, have a functional ER. We found that medaka ER subtypes have their specific functions, and medaka, zebrafish and stickleback ERs are more sensitive to estrogen/estrogen-like chemical exposures than other fishes by reporter gene assay. Thus, these approaches are efficient to evaluate the relationship between species and their sensitivities to chemicals.

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

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

IV. Gene zoo and receptor zoo

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

V. Sex differentiation mechanism in Daphnids

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

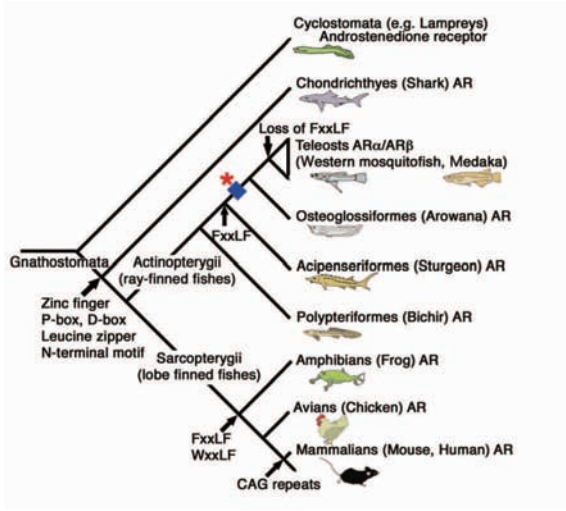

Figure 3. Evolutionary relationships of androgen receptor sequences.

database and developed an oligonucleotide-based DNA microarray with high reproducibility and demonstrated the usefulness of the array for the classification of toxic chemicals as well as for the molecular understanding of chemical toxicity in a common freshwater organism. *D. magna* reproduce asexually (parthenogenesis) when they are in an optimal environment for food, photoperiod and population density. Once environmental conditions become sub-optimal, they alter their reproductive strategy from asexual to sexual reproduction. Chemicals are able to affect the sex determination of *D. magna* and we found that juvenile hormone (JH) agonists (insect growth regulators), for example, induce the production of male offspring. The molecular basis of environmental sex determination is largely unknown in *D. magna*. To understand the molecular mechanisms of this phenomenon we isolated sex determination-related genes. Also, we have developed a method to inject genes into *D. magna* embryos which will allow us to study gain- and loss-of function analyses in more detail in this species. Using these techniques, we demonstrated that DSX1 (double sex 1), one of the DM-domain genes, is essential for male differentiation in *D. magna*. To further explore the signaling cascade of sexual differentiation in *D. magna*, a gene expression profile of

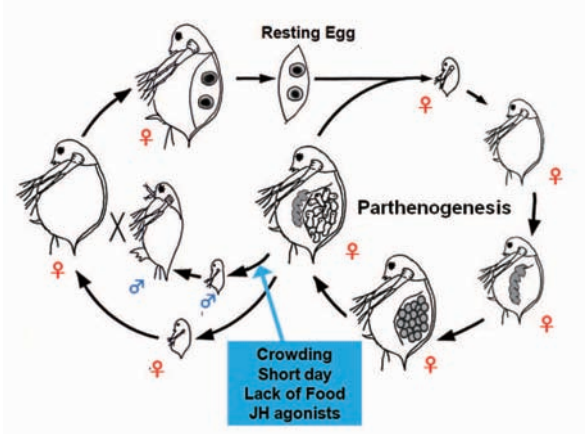

Figure 4. Life cycle of *Daphnia*.

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 identifying JH-responsive genes in the ovary. We are also identifying JH receptor in daphnids.

Publication List

〔Original papers〕

- Brockmeier, E.K., Ogino, Y., Iguchi, T., Barber, D.S., and Denslow, N.D. (2012). Effects of 17β-trenbolone on Eastern and Western mosquitofish (*Gambusia holbrooki* and *G. affinis*) and anal fin growth and gene expression patterns. Aquat. Toxicol. *128-129C*, 163-170.
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DIVISION OF ENVIRONMENTAL PHOTOBIOLOGY

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

I. Macro-organization of photosynthetic machinery

Photosystem (PS) II is a multiprotein complex that splits water and initiates electron transfer in photosynthesis. The central part of PSII, the PSII core, is surrounded by light-harvesting complex II proteins (LHCIIs). In higher plants, two or three LHCII trimers are seen on each side of the PSII core whereas only one is seen in the corresponding positions in a unicellular green alga *Chlamydomonas reinhardtii*. This year, we re-examined the supramolecular organization of the *C. reinhardtii* supercomplex by determining the effect of different solubilizing detergents. When we solubilized the thylakoid membranes with n-dodecyl-β-D-maltoside or n-dodecyl-α-D-maltoside and subjected them to the thylakoid membranes with n-dodecyl-β-D-maltoside gel-filtration, we observed a clear difference in molecular mass. The n-dodecyl-α-D-maltoside-solubilized PSII-LHCII supercomplex bound twice as much LHCII than the n-dodecyl-β-D-maltoside-solubilized supercomplex and retained higher oxygen-evolving activity. Single-particle image analysis from electron micrographs of the n-dodecyl-α-D-maltoside-solubilized and negatively stained supercomplex revealed that the PSII-LHCII supercomplex had a novel supramolecular organization, with three LHCII trimers attached to each side of the core.

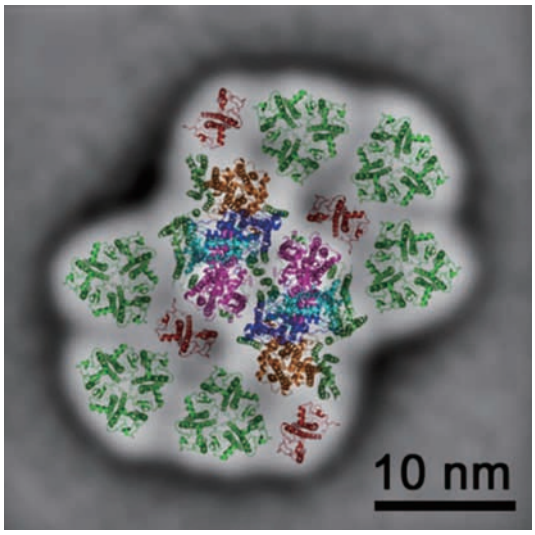

Figure 1. A top view of the PSII-LHCII supercomplex in *C. reinhardtii* as revealed by single particle analysis of electron micrographs.

II. Acclimation of photosynthesis

Using *C. reinhardtii*, we investigated the molecular mechanisms underlying the acclimation processes of the photosynthetic complexes by means of biochemistry, molecular genetics, absorption and fluorescence spectroscopy, and bio-imaging.

II-1 State-transitions

The two photosystems—PSI and PSII—in the thylakoid membranes function as charge-separation devices. Each has a distinct pigment system with distinct absorption characteristics 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. 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. 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.

1I-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.

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.

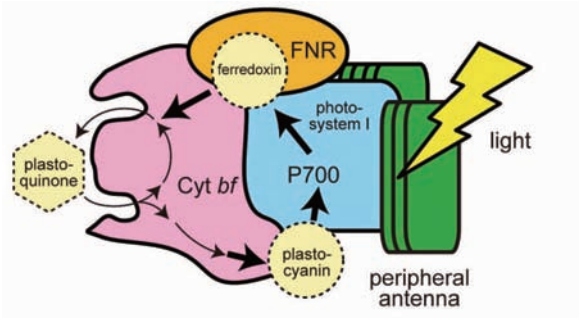

Figure 2. Cyclic electron flow by way of the CEF supercomplex.

III. Ecophysiology of marine phytoplankton

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

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

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

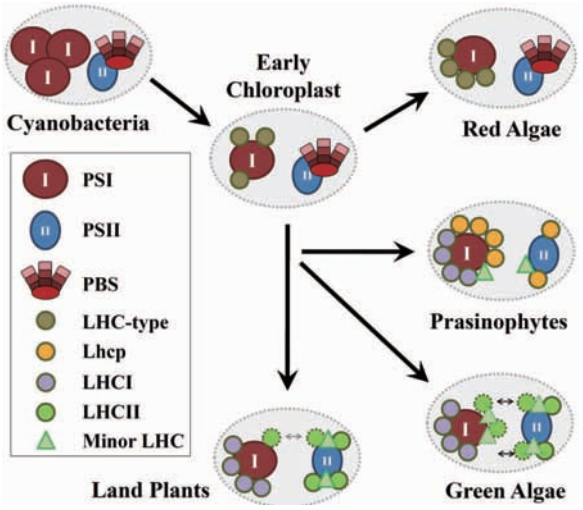

Figure 3. Evolutionary model of LHC affinity in photosynthetic eukaryotes as revealed by biochemical study of the LHC systems in *O. tauri*.

Publication List

〔Original paper〕

● Tokutsu, R., Kato, N., Bui, K. H., Ishikawa, T., and Minagawa, J. (2012). Revisiting the supramolecular organization of photosystem II in *Chlamydomonas reinhardtii*. J. Biol. Chem. *287*, 31574-31581.

LABORATORY OF GENOME INFORMATICS

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

I. Microbial genome database for comparative analysis

The microbial genome database for comparative analysis (MBGD; URL http://mbgd.genome.ad.jp/) is a comprehensive platform for microbial comparative genomics. The central function of MBGD is to create orthologous groups among multiple genomes from precomputed all-against-all similarity relationships using the DomClust algorithm (see Section II below). By means of this algorithm, MBGD not only provides comprehensive orthologous groups among the latest genomic data available, but also allows users to create their own ortholog groups on the fly using a specified set of organisms. In addition, MBGD also provides MyMBGD mode, which allows users to add their own genomes to MBGD.

The database now contains more than 2000 published genomes including 34 eukaryotic microbes and 4 multicellular organisms. This year, we substantially enhanced the database functionality to explore the diversity of microbial genomes. The enhancement includes: 1) To efficiently explore the diversity of the microbial genomic data, MBGD now provides summary pages for pre-calculated ortholog tables among various taxonomic groups; 2) For some closely related taxa, MBGD also provides the conserved synteny information (core genome alignment) pre-calculated using the CoreAligner program (see Section III below); 3) An efficient incremental updating procedure was implemented to create extended ortholog tables by adding additional genomes to the default ortholog table generated from the representative set of genomes (see Section II below).

II. Improvement of the methods for constructing orthologous groups among multiple genomes

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

Although DomClust can rapidly construct orthologous groups, its classification quality has room for improvement since it uses UPGMA like clustering based on pairwise sequence alignment. We are now developing a procedure to refine the DomClust classification based on multiple sequence alignments and phylogenetic trees. Since DomClust constructs orthologous groups at the domain level, the procedure refines not only sequence grouping but also domain splitting. We developed a simple scoring system to identify the most plausible domain boundary or to merge multiple domains into one group by maximizing the sum of the alignment scores of domains under this scoring system (Figure 1). We tested the scoring system using the COG database as a reference and confirmed that changes that increase the score generally improve the agreement with the COG clusters. After determining domain boundaries, our procedure also checks whether the resulting group should be split into subgroups by considering species overlaps between subclusters in the phylogenetic tree (Figure 1).

We are also developing a method to update the clustering result incrementally, by which we can add new genomes to a reference set of ortholog groups that is constructed from a representative set of published genomes available in the MBGD server. This approach allows us to conduct further large-scale comparative genomics based on orthologous groups among thousands of genomic sequences. We are also trying to extend the algorithm for handling metagenomic data. To infer the taxonomic position of the source organism

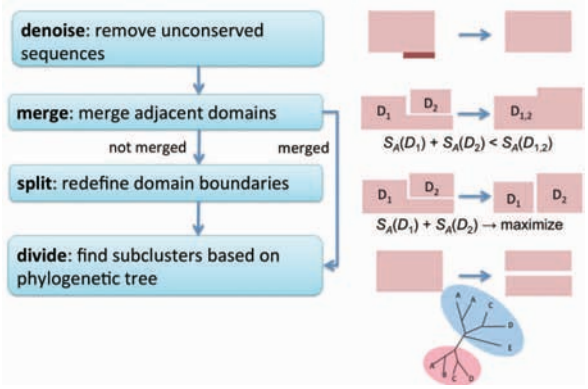

Figure 1. Pipeline for ortholog group refinement. The pipeline consists of several modules including *denoise*, *merge*, *split* and *divide*. Merge and split aim at improving the domain boundary based on the sequence alignment whereas divide aims at improving the subgrouping based on the phylogenetic tree.

of each metagenomic sequence, we have developed a method to map each tree node of the hierarchical clustering tree generated by the DomClust algorithm onto a taxonomic tree node.

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

Horizontal gene transfers (HGT) have played a significant role in prokaryotic genome evolution, and the genes constituting a prokaryotic genome appear to be divided into two classes: a "core gene pool" that comprises intrinsic genes encoding the proteins of basic cellular functions, and a "flexible gene pool" that comprises HGT-acquired genes encoding proteins which function under particular conditions. Thus identification of the set of intrinsically conserved genes, or the genomic core, among a taxonomic group is crucial for understanding prokaryotic diversity and evolution.

Typically "core genome" is defined as a set of genes that are conserved among all the genomes belonging to the given species (called the "universal core"). However, this definition can be too strict since it allows no exceptions. 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 (called "syntenic core"). To find such core structures, we developed a method named CoreAligner, which finds the order of orthologous groups that maximally retains the conserved gene orders.

We systematically applied our method to bacterial taxa (family, genus and species) that contain a sufficient number of completed genomes stored in MBGD. As a result, we can generally obtain more core genes as syntenic core rather than universal core genes, except for some taxonomic groups that have poor syntenic conservation. Moreover, typically the number of syntenic core is more stable than universal core when the number of genomes in the given taxa increases.

The core genome data calculated for various prokaryotic taxa is now available as part of the MBGD database (Figure 2).

Figure 2. Core genome alignment viewer in MBGD showing the core alignment of the family *Bacillaceae*.

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. The ortholog table viewer is a spreadsheet like viewer that can display the entire ortholog table, containing more than a thousand genomes. Using the zoom in/out function, it can display the entire table or a section of the main table with more detailed information. In RECOG, several basic operations on the ortholog table are defined, which are classified into categories such as filtering, sorting, and coloring, and various comparative analyses can be done by combining these basic operations, such as "Neighborhood gene clustering" and "Phylogenetic pattern clustering." In addition, RECOG allows the user to input arbitrary gene properties such as sequence length, nucleotide/amino acid contents and functional classes, and compared these properties among orthologs in various genomes.

Publication List

〔Original papers〕

- Takami, H., Noguchi, H., Takaki, Y., Uchiyama, I., ,Toyoda, A., Nishi, S., Chee, G-J., Arai, W., Nunoura, T., Itoh, T., Hattori, M., and Takai, K. (2012). A deeply branching thermophilic bacterium with an ancient acetyl-CoA pathway dominates a subsurface ecosystem. PLoS ONE *7*, e30559.
- Yahara, K., Kawai, M., Furuta, Y., Takahashi, N., Handa, N., Tsuru, T., Oshima, K., Yoshida, M., Azuma, T., Hattori, M., Uchiyama, I., and Kobayashi, I. (2012). Genome-wide survey of mutual homologous recombination in highly sexual bacterial species. Genome Biol. Evol. *4*, 628-640.

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

- Uchiyama, I., Mihara, M., Nishide, H., and Chiba, H. MBGD update 2013: the microbial genome database for exploring the diversity of microbial world. Nucleic Acids Res. 2012 Oct 30.

LABORATORY FOR SPATIOTEMPORAL REGULATIONS

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ISHIBASHI, Tomoko

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 biological analyses.

I. Initial step for left-right asymmetry

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

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

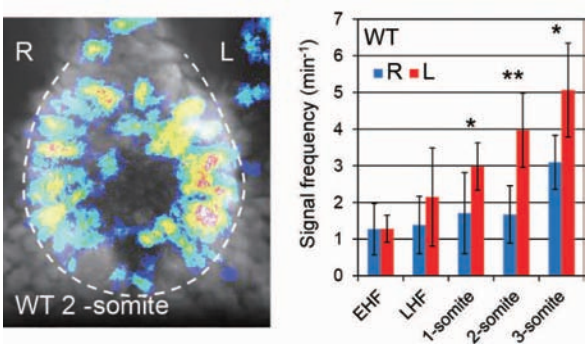


Figure 1. Left: Distribution of Ca^{2+} elevation in a 2-somite wild-type node. Right: Time course of Ca^{2+} elevation frequency at the left and the right sides.

II. Development of light-sheet microscopy

A remarkable characteristic of light-sheet microscopy is high efficiency of light detection against illumination power. This feature results in several advantages in live imaging: detection of weak signals, low phototxicity and bleaching, and fast image acquisition.

We took advantage of light-sheet microscopy by applying long-term observation of intact mouse embryos at gastrulating stages for tracking cell movements, raman imaging of living medaka, and ultra-fast 4D data acquisition of freely moving *Amoeba proteus*.

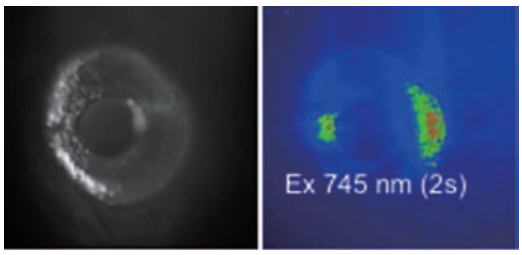


Figure 2. Raman signal of medaka Left: Dark-field image of a living Quintet medaka eye taken by oblique illumination. Right: Raman image of the same area.

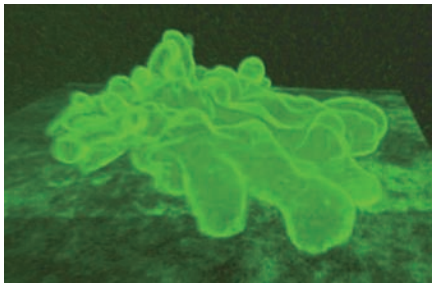


Figure 3. 3D image of Amoeba Proteus reconstructed from 100 optical sections taken in 0.5 sec.

Publication List

〔Original papers〕

- Morita, H., Kajiura-Kobayashi, H., Takagi, C., Yamamoto, T.S., Nonaka, S., and Ueno, N. (2012). Cell movements of the deep layer of non-neural ectoderm underlie complete neural tube closure in Xenopus. Development *139*, 1417-1426.
- Oshima, Y., Sato, H., Kajiura-Kobayashi, H., Kimura, T., Naruse, K., and Nonaka, S. (2012). Light sheet-excited spontaneous Raman imaging of a living fish by optical sectioning in a wide field Raman microscope. Optics Express *20*, 16195-16204.
- Takao, D., Taniguchi, A., Takeda, T., Sonobe, S., and Nonaka, S. (2012). High-speed imaging of amoeboid movements using light-sheet microscopy. PLoS ONE *7*, e50846.
- Yoshiba, S., Shiratori, H., Kuo, I.Y., Kawasumi, A., Shinohara, K., Nonaka, S., Asai, Y., Sasaki, G., Belo, J.A., Sasaki, H., et al. (2012). Cilia at the node of mouse embryos sense fluid flow for left-right determination via Pkd2. Science *338*, 226-231.

NIBB Core Research Facilities

Head
KOBAYASHI, Satoru

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

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

Functional Genomics Facility

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

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

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

Representative Instruments

Genomics

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

During 2012 we carried out 47 NGS projects in collaboration with NIBB laboratories as well as the researchers of other academic institutions. These projects cover a wide range of species (bacteria, animals, plants, and humans) including both model and non-model organisms, and various applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

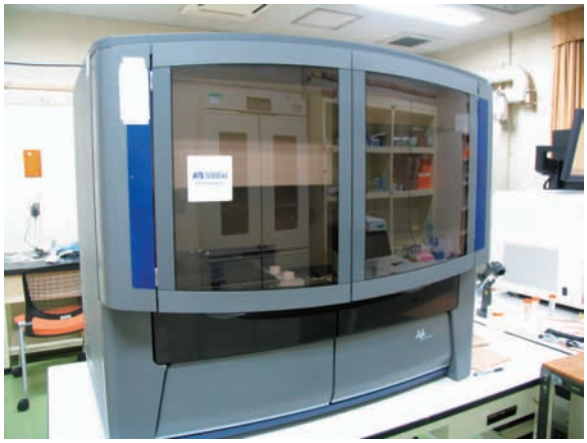


Figure 1. Next-generation sequencer SOLiD5500xl

Proteomics

Three different types of mass spectrometer and two protein sequencers, as listed below, are used for proteome studies in our facility. In 2012, we analyzed approximately 248 samples with mass spectrometers and 37 samples with protein sequencers.

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

Other analytical instruments

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

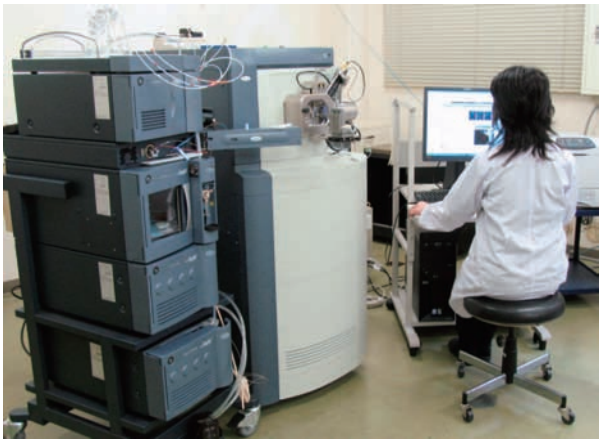


Figure 2. LC-Q-TOF-MS

Genome Informatics Training Course

We organize NIBB Genome Informatics Training Courses every year. In 2012, we provided two two-day training courses on next-generation sequence data analyses and transcriptome analysis. These courses are designed to introduce the basic knowledge and skills of bioinformatics analysis to biologists who are not familiar with bioinformatics.

Figure 3. NIBB Genome Informatics Training Course

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

Associate Professor (Specially appointed)
SHIGENOBU, Shuji

NIBB Research Fellow:	*MAEDA, Taro*
Technical Assistant:	*SUZUKI, Miyuzu*
Visiting Scientist:	*OGAWA, Kota*

Symbiosis Genomics

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

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

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

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

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

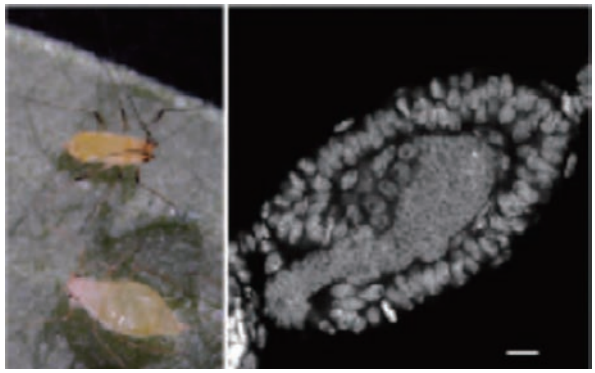


Figure 1. Pea aphids and the bacterial symbiont, *Buchnera*. Adult aphids (Left). A developing viviparous embryo which symbionts are infecting (Right). Scale bar = 20um.

Publication List

〔Original papers〕

- Hojo, M., Maekawa, K., Saitoh, S., Shigenobu, S., Miura, T., Hayashi, Y., Tokuda, G., and Maekawa, H. (2012). Exploration and characterization of genes involved in the synthesis of diterpene defence secretion in nasute termite soldiers. Insect Mol. Biol. *21*, 545-557
- Gallot, A., Shigenobu, S., Hashiyama, T., Jaubert-Possamai, S., and Tagu, D. (2012). Sexual and asexual oogenesis require the expression of unique and shared sets of genes in the insect *Acyrthosiphon pisum*. BMC Genomics *13*, 76

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

● Shigenobu, S., and Stern, D. Aphids evolved novel secreted proteins for symbiosis with bacterial endosymbiont. Proc. Royal Soc. B: Biol. Sci. 2012 Nov 21.

Spectrography and Bioimaging Facility

Associate Professor (Specially appointed)
KAMEI, Yasuhiro

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

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

Representative Instruments:

Okazaki Large Spectrograph (OLS)

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

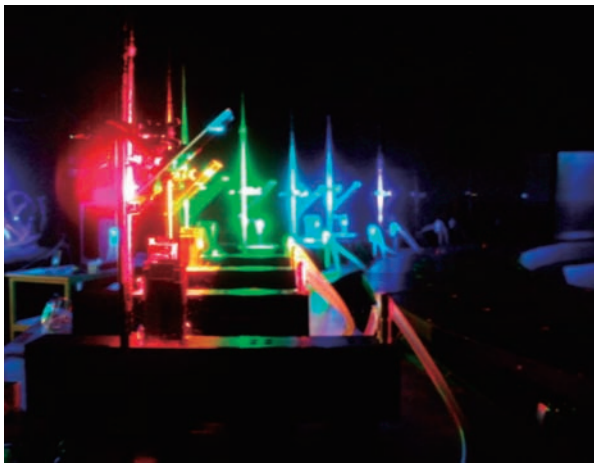


Figure 1. An example of experiments using the Large Spectrograph. Various color rays (monochromatic light from right side and reflected by mirrors) were irradiated simultaneously to samples in cooling chambers.

The 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, Nikon A1R, Nikon A1Rsi, Carl Zeiss Duo 5 and Yokogawa CSU-X1) and other advanced custom-made laser microscopes for special aims (Digital Scanned Light-sheet Microscope: DSLM and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began Collaborative Research Programs using these machines since 2010.

The DSLM was developed by Dr. Ernst Stelzer's group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissue by illuminating from the side of a specimen with a light sheet (more information is described in Dr. Nonaka's section: Lab. for Spatiotemporal Regulations). Dr. Nonaka conducted and supported about 7 projects of the Collaborative Research Program for the Use of the DSLM. On the other, 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 about 10 Individual Collaborative Research projects, including applications for animals and higher plant.

Workshop and Symposium

In 2012, we held workshops (training course) on IR-LEGO for plants (*Arabidopsis*) and fish (medaka) in Japan and Singapore (as a joint workshop by NIBB, the National University of Singapore, and Temasek Lifesciences Laboratory) respectively. We also have been holding a "Bioimaging Forum" every year which discusses Bioimaging from various directions such as microscopy, new photo-technology, and computer science. In 2012, we held the 6th and 7th forums which focused on all imaging sciences, from astronomy to biology, and optogenetics and adaptive optics, respectively.

Publication List on Cooperation

〔Original papers〕

- Moritoh, S., Eun, C-H., Ono, A., Asao, H., Okano, Y., Yamaguchi, K., Shimatani, Z., Koizumi, A., and Terada, R. (2012). Targeted disruption of an orthologue of DOMAINS REARRANGED METHYLASE 2, OsDRM2, impairs the growth of rice plants by abnormal DNA methylation. Plant J. *71*, 85-98.
- Satoh, C., Kimura, Y., and Higashijima, S. (2012). Generation of multiple classes of V0 neurons in zebrafish spinal cord: Progenitor heterogeneity and temporal control of neuronal diversity. J. Neurosci. *32*, 1771-1783.
- Suzaki, T., Yano, K., Ito, M, Umehara, Y., Suganuma, N., and Kawaguchi, M. (2012). Positive and negative regulation of cortical cell division during root nodule development in Lotus japonicus is accompanied by auxin response. Development *139*, 3997-4006.
- Takeda, N., Maekawa, T., and Hayashi, M. (2012). Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates Rhizobial and Mycorrhizai responses in Lotus japonicus. Plant Cell *24*, 810-822.
- Watakabe, A., Kato, S., Kobayashi, K., Takaji, M., Nakagami, Y., Sadakane, O., Ohtsuka, M., Hioki, H., Kaneko, T., Okuno, H., Kawashima, T., Bito, H., Kitamura, Y., and Yamamori, T. (2012). Visualization of Cortical Projection Neurons with Retrograde TET-Off Lentiviral Vector. PLoS ONE *7*, e46157.

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

- Suzaki, T., Kim, C.S., Takeda, N., Szczyglowski, K., and Kawaguchi, M. TRICOT encodes an AMP1-related carboxypeptidase that regulates root nodule development and shoot apical meristem maintenance in Lotus japonicus. Development 2012 Dec. 18.

● *Research activity by Y. Kamei*

Associate Professor (Specially appointed)
KAMEI, Yasuhiro
Technical Assistant: *KANIE, Yuta*

To investigate a gene function in each cell we have to express the gene in the cell *in vivo*, and 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.

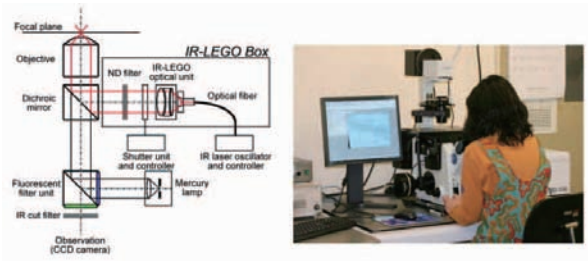


Figure 1. An infrared laser-evoked gene operator (IR-LEGO) microscope system in NIBB.

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 speed of temperature rise and 3-dimensional distribution of temperature during IR irradiation. In a model tissue which contained GFP expressing bacteria in polyacrylamide gel, temperature rose rapidly with IR irradiation and kept a constant level dependant on IR laser power (Figure 2 left). On the other hand, the heated area was limited to a small volume about as large as a typical cell (Figure 2 right).

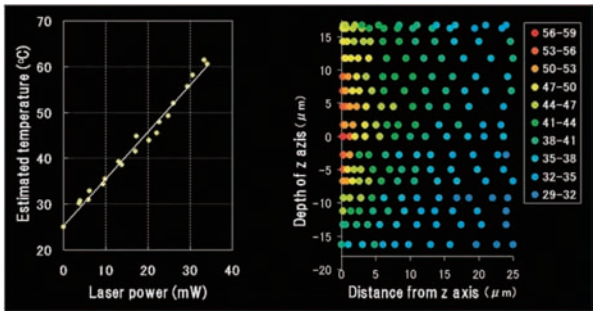


Figure 2. Heating profiles (laser power dependency of focus temperature and 3-D temperature map) of IR irradiation.

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

Studies of cell fates, cell-cell interaction, or analysis of non-cell autonomous phenomena require a fine control system of gene expression in experiments. IR-LEGO will be a powerful

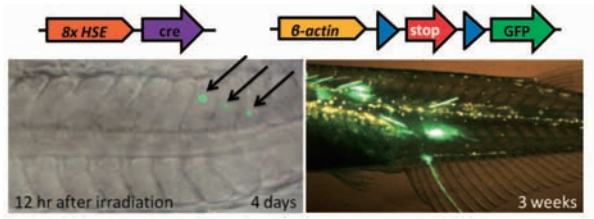


Figure 3. Cre-loxP mediated long-term GFP marking in a living medaka individual for linage tracing.

tool for these studies in combination with molecular biological techniques, such as the cre-loxP system. By Applying IR-LEGO to a mutant and its rescue transgenic strain; using hsp-cre with a rescue gene which is sandwiched by loxP sequences, we will achieve single-cell knockout experiments in living organisms, and reveal fine interaction between the cells. We are now testing this system using medaka. We have already constructed a medaka TILLING library and a screening system for reverse genetic mutant screening, furthermore we have confirmed a system operation of a cre-loxP system in medaka using IR-LEGO (Figure 3).

Publication List

〔Original papers〕

- Ansai, S., Ochiai, H., Kanie, Y., Kamei, Y., Gou, Y., Kitano, T., Yamamoto, T., and Kinoshita, M. (2012). Targeted disruption of exogenous EGFP gene in medaka using zinc-finger nucleases. Dev. Growth Differ. *54*, 546-556.
- Kitano, T., Hayashi, Y., Shiraishi, E., and Kamei, Y. (2012). Estrogen rescues masculinization of genetically female medaka by exposure to cortisol or high temperature. Mol. Reprod. Dev. *79*, 719-726.
- Masuyama, H., Yamada, M. Kamei, Y., Fujiwara-Ishikawa, T., Todo, T., Nagahama, Y., and Matsuda, M. (2012). Dmrt1 mutation causes a male-to-female sex reversal after the sex determination by Dmy in the medaka. Chromosome Res. *20*, 163-176.
- Yasuda, T., Oda, S., Li, Z., Kimori, Y., Kamei, Y., Ishikawa, T., Todo, T., and Mitani, H. (2012). Gamma-ray irradiation promotes premature meiosis of spontaneously differentiating testis-ova in the testis of p53-deficient medaka (Oryzias latipes). Cell Death Dis. *3*, e395

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

- Kobayashi, K., Kamei, Y., Kinoshita, M., Czerny, T., and Tanaka, M. A heat-inducible cre/loxP gene induction system in medaka. Genesis 2012 Nov 3.

Data Integration and Analysis Facility

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

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

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a shared memory parallel computer (DELL PowerEdge R905; 4 nodes/16 cores, 256GB memory), a high-performance cluster system (DELL PowerEdge M1000e+M610; 32 nodes/256 cores, 768GB memory) and a large-capacity storage system (DELL Equallogic; 35TB SAS, 26TB SATA, 750GB SSD). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be

Figure 1. Biological Information Analysis System

processed efficiently. Some personal computers and color/monochrome printers are also available. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for institute members. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

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

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. 65).

NIBB BioResource Center

Head
IGUCHI, Taisen

Vice head: *FUJIMORI, Toshihiko*

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

Model Animal Research Facility

Associate Professor:	*WATANABE, Eiji*
	TANAKA, Minoru
	NARUSE, Kiyoshi
Technical Staff:	*HAYASHI, Kohji*
	NOGUCHI, Yuji
Technical Assistant:	*INADA, Junichi*
	INADA, Yosuke
	MATSUMURA, Kunihiro
	FUJIMOTO, Daiji
	ICHIKAWA, Yoko
	TAKAGI, Yukari
	SUGINAGA, Tomomi
	SUZUKI, Kohta

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.

Figure 1. Mouse (strain B6C3F1)

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

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

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

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

I. Research support activities (mouse)

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

In 2012 (from January 1 to December 31), 2,802 fertilized eggs (*in vitro* fertilization; 2,328 eggs of 8 lines in which 1,825 eggs of 8 lines were frozen for long-term storage, frozen eggs: 474 of 11 lines) and 5,121 mice were brought into the facility in the Yamate area, and 49,541 mice (including pups bred in the facility) and 25 fertilized eggs were taken out.

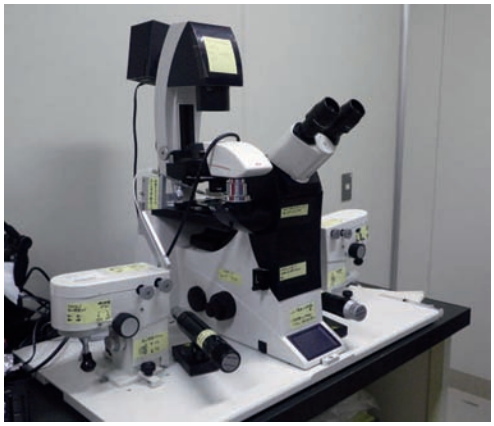


Figure 2. Equipment for manipulating mice eggs.

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 2012 (from January 1 to December 31) 9 mice were brought into the facility in the Myodaiji area, and 318 mice (including pups bred in the facility) were taken out.

Figure 3. Large sized autoclave in the Myodaiji area.

II. Research support activities (small fish, birds, and insects)

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

In 2012 (from January 1 to December 31), 4,419 medaka (1,338 eggs, 10 embryos and 3,071 adults) were brought to the facility and 40,142 medaka (37,785 fertilized eggs and 2,357 adults, including animals bred in the facility) were taken out. In the laboratory for chick embryos there were no fertilized eggs or chicken embryos brought in or taken out this year. These animals were used for research activities in neurobiology and developmental biology.

In 2007 NIBB was approved as a core facility of the National BioResource Project (NBRP) for Medaka by the Japanese Government. We have supported the activities of NBRP Medaka by providing standard strains, mutants, transgenic lines and organizing international practical courses for medaka. In 2010 we began providing the TILLING library screening system to promote the reverse genetic approach. In 2012 we shipped 175 independent medaka strains, 386 cDNA/BAC/Fosmid clones, and 285 samples of hatching enzyme to the scientific community worldwide.

Figure 4. Medaka fish.

III. Research activities

The associate professors of this center - E. Watanabe, T. Naruse and M. Tanaka - are the principal investigators of the Laboratory of Neurophysiology, the Laboratory of Bioresources and the Laboratory of Molecular Genetics for Reproduction, respectively. The Laboratory of Neurophysiology is studying mechanisms of the visual system using a psychophysical approach. The Laboratory of Bioresources has conducted a genetic and genomic analysis of quantitative traits and Mendelian phenotype variations as well as evolution of sex determination systems in medaka related species. The Laboratory of Molecular Genetics for Reproduction is studying the molecular mechanisms of reproductive organ development and sex differentiation using mutagenized or transgenic medaka. For details, please refer to the pages of each laboratory (p. 44, 52, and 32).

Model Plant Research Facility

● *Plant Culture Laboratory*

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

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

The Plant Culture Laboratory equips and manages 48 culture boxes or growth chambers, 6 phytotrons, and 12 rooms with the P1P physical containment level for established and emerging model plants including the thale cress *Arabidopsis thaliana*, several carnivorous plants, the rice *Oryza sativa*, rushes of the *Juncus* sp., the moss *Physcomitrella patens*, and several other flowering plants. An emerging model insect, the tiny moth *Acrocercops transecta* is also reared in this laboratory. Most culture space is fully used the whole year by more than 60 researchers from both outside and inside groups.

As well as regular culture conditions, extreme environmental conditions for light and temperature are available for various types of experiments. Three light environmental simulators are available. Autotrophic and heterotrophic culture devices are also available for researchers using cyanobacteria, algae, and cultured flowering plant cells. Aseptic experiments can be performed in an aseptic room with clean benches.

Next to the institute building of the Myodaiji area, a 386-m^2 experimental farm is maintained for Japanese morning glory and related *Ipomoea* species, several carnivorous plants and other flowering plants necessary to be cultivated outside. Three green houses (44, 44, and 45 m^2) with heating are used for the carnivorous plants, and wild-type strains of medaka fish *Oryzias* sp. Seven green houses (4, 6, 6, 6, 6, 9, and 38 m^2) with air-conditioning are provided for the cultivation of a rice *Oryza* sp., *Lotus japonica* and related legume species, as well as mutant lines of the Japanese morning glory. One green house (18 m^2) with air-conditioning meets the P1P physical containment level and is available for experiments using transgenic plants. The Plant Culture Laboratory also maintains a 46-m^2 building with storage and workspace. Part of the building is used for rearing of the orchid mantis.

In 2012, the green houses with heating were renovated and installed with weather sensors, and three growth chambers were replaced. The chambers (3.4 m^2 each) can control CO_2 and humidity in addition to temperature and light (max 70,000 lux) conditions.

Figure 5. The growth chambers after replacement.

● *Morning Glory BioResource Laboratory*

Assistant Professor: *HOSHINO, Atsushi*

The Japanese morning glory (*Ipomoea nil*) is a traditional floricultural plant in Japan, and is studied worldwide, especially in plant physiology and genetics. NIBB collects, develops and distributes DNA clones, mutant lines for flower pigmentation, and transgenic lines as a sub-center of the National BioResource Project (NBRP) Morning glory, and collaborates with the core organization center, Kyushu University. We collected several mutant lines and DNA clones, and provided 14 DNA clones and 3 BAC clone screening systems to local biologists this year.

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

Cell Biology Research Facility

Assistant Professor: *HAMADA, Yoshio*

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

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

Figure 6. Equipment for tissue and cell culture

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

Head
NISHIMURA, Mikio

Associate Professor
NARUSE, Kiyoshi

Postdoctoral Fellow: *KIMURA, Tetsuaki*
Technical Assistant: *SAKAMOTO, Yuki*

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

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

The IBBP Center includes earthquake proof structures capable of withstanding even very large scale quakes (equipped with emergency backup power generators), cryopreservation facilities equipped with automatic liquid nitrogen feeding systems, deep freezers, and refrigerated storage (mainly for seed stocks), as well as all manner of automated laboratory equipment, cell culture tools, and the latest equipment necessary to back up the genetic resources in a collaborative manner. The specific methods of preservation are freezing of the sperm and eggs of animals, cultured plant and animal cells, and gene libraries. Plant seeds are frozen or refrigerated. The capacity for the backup storage facility is approximately 1.4 million samples.

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

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

Figure 1. Cryogenic storage system. Liquid nitrogen tanks are monitored for 24 hours and are refilled automatically.

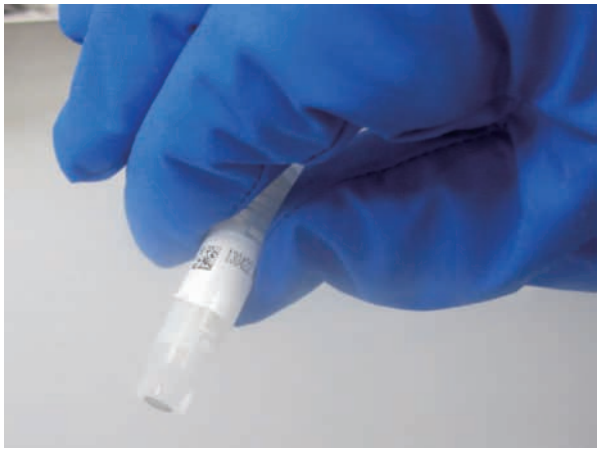


Figure 2. Cryo tube with 2D barcode. Each sample is printed with unique barcode and is managed using database.

CENTER FOR RADIOISOTOPE FACILITIES

Head
HASEBE, Mitsuyasu

Associate Professor
OGAWA, Kazuo
(Radiation Protection Supervisor)

Associate Professor
KODAMA, Ryuji

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

Technical Assistant:
ITO, Takayo
KAMIYA, Kiyomi

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

Ms. Matsuda, Ms. Iinuma, Ms. Ito, and Ms. Kamiya maintained the Myodaiji-Area. Dr. Ogawa and Ms. Sawada worked in the Yamate-Area. Dr. Ogawa, the associate professor of the radioisotope facilities, retired in March 2012 and Dr. Kodama arrived at the post in April, working in both areas.

The number of registrants and the number of users from January 2012 to December 2012 are shown in Table 1.

Users counted by the access control system of the controlled areas numbered 2,491 during this period. The numbers for each area are shown in Table 2. The annual changes of registrants and the number of totals per fiscal year are shown in Figure 1. The balance of radioisotopes received and used at the CRF is shown in Table 3. The training courses on radioisotope handling were given as in Table 4.

The followings are CRF's notable activities in 2012.

1. A lecture was given on the relationship of Radon concentration in the air and earthquakes by an invited speaker (Dr. Yumi Yasuoka, Lecturer, Kobe Pharmaceutical University) as the 2012 special lecture for radiation workers.
2. At the Myodaiji-area, the exhaust fan of line 2 expired and was exchanged. (See Figure 2, A, B.)
3. At the Yamate-area, the access control system of the controlled areas was updated. The new system uses IC cards meeting the FeliCa standard and will be compatible in the future with the access control system of all Yamate-area buildings.

	Myodaiji-Area	Yamate-Area
registrants	97	70
users	42	34

Table 1. Numbers of registrants and users at Myodaiji-Area and Yamate-Area in 2012

	Myodaiji-Area	Yamate-Area	total
users	1737	754	2491
visitors	214	175	389
total	1951	929	2880

Table 2. Users and visitors who entered each controlled area in 2012

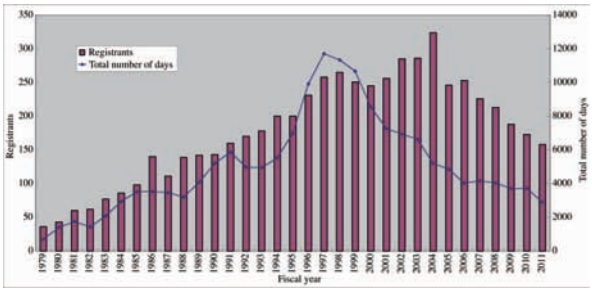


Figure 1. Annual changes of registrants and number of totals per fiscal year

	Myodaiji-Area	Yamate-Area	total
^{125}I Received	370000	966	370966
^{125}I Used	328100	766	328866
^{35}S Received	0	94000	94000
^{35}S Used	0	52600	52600
^{32}P Received	214000	78250	292250
^{32}P Used	142300	71850	214150
^{14}C Received	55500	0	55500
^{14}C Used	52633	0	52633
^{3}H Received	305250	6475000	6780250
^{3}H Used	80357	6937500	7017857

Table 3. Balance of radioisotopes received and used (kBq) at each controlled area in 2012

training course	place	numbers of participant
beginners training course for beginners*	Myodaiji	5
beginners training course for beginners*	Yamate	8
beginners training course for exparts	Myodaiji	7
beginners training course for exparts	Yamate	4
Users traning course*	Myodaiji	72
Users traning course	Yamate	44

*included English course

Table 4. Training courses for radiation workers in 2012

Figure 2. The CRF's notable activities in 2012
A, B: The exhaust fan was exchanged. A shows the old fan and B shows the new fan.
C, D: The access control system of the controlled areas was updated. C shows the card reader of the radioisotope storage room entrance. D shows a card reader for the FeliCa standard card.

STRATEGIC PLANNING DEPARTMENT

Chair
NISHIMURA, Mikio

Associate Professor: *KODAMA, Ryuji*

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

The main activities of the Department

1) Management of external evaluation processes

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

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

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

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

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

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

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

OFFICE OF PUBLIC RELATIONS

Chair
FUJIMORI, Toshihiko

Assistant Professor (Specially appointed): *KURATA, Tomoko*
Technical Assistant: *OTA, Kyoko*
KAWAGUCHI, Colin
BAN, Misato
OKUBO, Masayo

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

The main activities of the office in 2012

1) Press releases

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

2) Updating and maintenance of the NIBB web page

3) Editing of publications, production of posters and leaflets

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

4) Producing Videos

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

5) Organization of scientific outreach programs

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

OFFICE OF INTERNATIONAL COOPERATION

Chair
YOSHIDA, Shosei

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

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

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

The main activities of the office in 2012

1) Supporting coordination of international conferences

The Office of International Cooperation coordinated the following international conferences hosted by NIBB:

The 59th NIBB Conference "Neocortical Organization" Mar. 10-13, 2012

8th Okazaki Biology Conference "Speciation and Adaptation II - Environment and Epigenetics -" Mar. 18-23, 2012

The 58th/60th NIBB Conference "Germline -Specification, Sex, and Stem Cells-" Jul. 17-21, 2012

9th Okazaki Biology Conference "Marine Biology II" Oct. 14-19, 2012

The 4th NIBB-MPIPZ-TLL Symposium "Arabidopsis and Emerging Model Systems" Nov. 19-21, 2012

2) Supporting dispatching of researchers and students to international conferences

The Office of International Cooperation coordinated sending NIBB researchers, and those from the Japanese scientific community outside NIBB, to the following course:

The NUS/TLL/NIBB joint practical workshop on "Genetics, Genomics and Imaging in Medaka & Zebrafish" Jul. 22-31, 2012 (held in Singapore).

3) Management of education related programs

The Office of International Cooperation co-organized the following international training course:

The NUS/TLL/NIBB joint practical workshop on "Genetics, Genomics and Imaging in Medaka & Zebrafish" Jul. 22-31, 2012 (held in Singapore).

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
TANIGUCHI-SAIDA, Misako
Technical Assistant: *ICHIKAWA, Chiaki*
NISHIMURA, Noriko
YAMAMOTO, Kumi
OKA, Naomi
Secretary: *ICHIKAWA, Mariko*
ISHIKAWA, Azusa

● ***NIBB Bioresource Center***

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

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

Unit Chief: *MATSUDA, Yoshimi*

● ***Center for Radioisotope Facilities***

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

Research Support Group

Chief: *KAJIURA-KOBAYASHI, Hiroko*

● ***Cell Biology***

Unit Chief: *KONDO, Maki*

● ***Developmental Biology***

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

● ***Neurobiology***

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

● ***Evolutionary Biology and Biodiversity***

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

● ***Environmental Biology***

Unit Chief: *MIZUTANI, Takeshi*

Reception

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

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

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

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

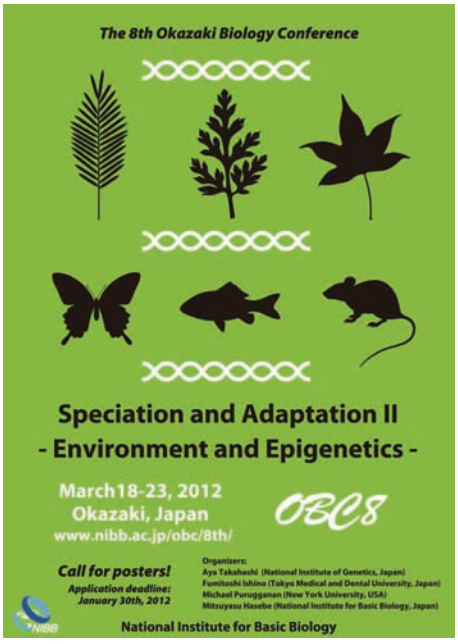
The Technical Division hosts an annual meeting for technical engineers who work in various fields of biology at universities and research institutes throughout Japan. At this meeting, the participants present their own activities and discuss technical problems. The proceedings are published after each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

The Eighth Okazaki Biology Conference "Speciation and Adaptation II - Environment and Epigenetics"

Organizers: Aya Takahashi (National Inst. Genetics), Michael Purugganan (New York Univ.), Fumitoshi Ishino (Tokyo Medical and Dental Univ.), Mitsuyasu Hasebe (NIBB)

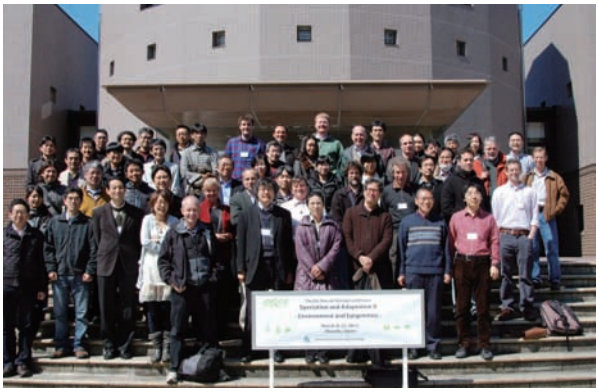
March 18 (Sun)-23 (Fri), 2012

Building on 2007's 5th OBC's topic of "Speciation and Adaptation - Ecological Genetics of Model Organisms and Beyond," the 8th OBC was held with the theme of "Speciation and Adaptation - Environment and Epigenetics." At OBC 8, the foundations were laid for frontier expanding, mold-breaking research investigating how environmental responses relate to evolution, and the relationship epigenetic factors may have on them. Though the event was originally planned for March, 2011, the unfortunate events of the 2011 Tōhoku earthquake and Tsunami forced its postponement until March of the following year. This dark cloud did have a small silver lining for the participating researchers however, as they were granted an additional year within which to gather and analyze data, resulting in more and finer results than may have been presented otherwise.



The topics of discussion included: "major and minor QTL's role in relation to adaptation and wild mutation," "the necessity of analyzing wild populations," "research using fruit flies on the genes controlling speciation and polymorphism in wild populations," "multi-generational polymorphism maintenance mechanisms and their adaptive significance," and "the connection between macro-evolution and epigenetic change," taking place through 7 sessions over 5 days. Through combining the fields of evolutionary, developmental, and ecological science in a single event, researchers joining OBC 8 were able to transcend the boundaries of their own research areas to gain a new understanding of unfamiliar fields, make new ties with colleagues, and create fertile ground for the planning and execution of new research ventures. We at NIBB could not be more pleased.

(Mitsuyasu Hasebe)



Speakers

Chong, Suyinn (Queenlsand Inst. of Medical Research), Colot, Vincent (IBENS), Comai, Luca (UC Davis), Feder, Martin (Univ. of Chicago), Gibson, Greg (Georgia Inst. of Tech.), Graves, Jennifer (La Trobe Inst. of Molecular Sciences), Gresham, David (New York Univ.), Guerrero-Bosagna, Carlos (WSU), Hanzawa, Yoshie (UIUC), Leakey, Andrew (UIUC), Muotri, Alysson (UCSD), Newfeld, Stuart (ASU), Olsen, Kenneth (WUSTL), Presgraves, Daven (Univ. of Rochester), Purugganan, Michael (New York Univ.), Renfree, Marilyn (The Univ. of Melbourne), Schott, Daniel (Harvard Univ.), Shimizu, Kentaro (Univ. of Zurich), Stephan, Wolfgang (LMU Munich), Zhang, Cuicui (HZAU)

Akashi, Hiroshi (NIG), Araki, Kiwako (Kyoto Univ.), Innan, Hideki (SOKENDAI), Ishii, Shunsuke (RIKEN Tsukuba Inst.), Ishino, Fumitoshi (Tokyo Medical and Dental Univ.), Ito, Hidetaka (Hokkaido Univ.), Kakutani, Tetsuji (NIG), Kanaoka, Masahiro (Nagoya Univ.), Kawamura, Shoji (The Univ. of Tokyo), Kinoshita, Tetsu (NAIST), Kitano, Jun (NIG), Kohda, Takashi (Tokyo Medical and Dental Univ.), Kudoh, Hiroshi (Kyoto Univ.), Kuwabara, Tomoko (AIST), Matsui, Takeshi (iCeMS, Kyoto Univ.), Okada, Norihiro (Tokyo Inst. Tech.), Osada, Naoki (NIG), Sawamura, Kyoichi (Univ. of Tsukuba), Takahashi, Aya (NIG), Takahashi, Kazuo (Okayama Univ.), Tamada, Yosuke (NIBB), Tanaka, Kenta (Univ. of Tsukuba)

The Ninth Okazaki Biology Conference "Marine Biology II"

Organizers: Noriyuki Satoh (Okinawa Inst. Sci. Tech.), Thomas C.G. Bosch (Univ. Kiel), Jun Minagawa (NIBB)

October 14 (Sun)-19 (Fri), 2012

The Okazaki Biology Conferences (OBC) are unique retreat style international conferences that focus on discovering new themes in the field of basic biology and forming new communities of researchers.

The 9th OBC, "Marine Biology II", was a continuation of OBC 6 (Marine Biology), focusing on cnidarians, especially corals, and their symbionts, with the first half of the conference taking part in Okazaki from October 14th to the 16th, and the second half in Okinawa from the 17th through the 19th. 44 researchers (24 from overseas) invited from the forefront of 7 fields showing remarkable advancement (Ecophysiology, Genomics, Circadian Rhythms, Photosynthesis, Symbiotics, Evo&Devo), came together to discuss, give presentations, and exchange information on the current challenges and future development of their fields. The conference was organized by Dr. Jun Minagawa of NIBB, Dr. Noriyuki Satoh of the Okinawa Institute of Science and Technology, and Dr. Thomas C. G. Bosch of the University of Kiel. With few opportunities for cutting edge researchers working with cnidarians and their symbionts to gather and share their work, the 9th OBC was an ideal chance for the participants to rethink the striking new developments and new possibilities opening in their respective fields. With a conclusion that Eco-Devo should be advocated as an important direction in the post genomic era, this conference was a perfect example of the OBCs' goal of developing new fields of biology research.

(Jun Minagawa)

Speakers

Allemand, Denis (CSM), Ball, Eldon (ANU), Bosch, Thomas (CAU), Foret, Sylvain (ANU), Fraune, Sebastian (CAU), Gates, Ruth (Univ. of Hawaii, Manoa), Holstein, Thomas (Univ. of Heidelberg), Houliston, Evelyn (UPMC/CNRS), Khalturin, Konstantin (CAU), Lallier, François (UPMC), Larkum, Anthony (Univ. of Sydney), Levy, Oren (BIU), Manuel, François (UPMC), Miller, David (JCU), Pringle, John (Stanford Univ.), Ralph, Peter (UTS), Rosenberg, Eugene (TAU), Smith, Joel (MBL), Takahashi, Shunichi (ANU), Tarrant, Ann (WHOI), Technau, Ulrich (Univ. of Vienna), Vize, Peter (Univ. of Calgary), Weis, Virginia (OSU)
Fujisawa, Toshitaka (SOKENDAI), Hamada, Shun (FWU), Hatta, Masayuki (Ochanomizu Univ.), Hidaka, Michio (Univ. of the Ryukyus), Kawaguchi, Masayoshi (NIBB), Kondo, Takao (Nagoya Univ.), Kurihara, Haruko (Univ. of the Ryukyus), Maruyama, Tadashi (JAMSTEC), Minagawa, Jun (NIBB), Sakai, Kazuhiko (Univ. of the Ryukyus), Satoh, Nori (OIST), Shinzato, Chuya (OIST), Shoguchi, Eiichi (OIST)

The 59th NIBB Conference "Neocortical Organization"

Organizers: Tetsuo Yamamori (NIBB), Yukiko Gotoh (The Univ. of Tokyo), Takuya Shimazaki (Keio Univ.), Ikue Mori (Nagoya Univ.)

March 10 (Sat)-13 (Tue), 2012

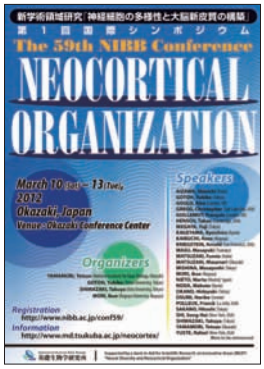
From March 10th to the 13th 2012 the 59th NIBB Conference, in partnership with the Grant-in-Aid for Scientific Research on Inovative Areas "Neural Diversity and Neocortical Organization" was held at the Okazaki Conference Center, with 137 members (31 speakers) gathered together from all around the world.

This conference was held with a special focus on the cerebral neocortex, with the objective of shedding light on the current state of research and to spur on more activity in the research field. This was probably the first international symposium on Neocortical Organization to be held over a period of 4 days in Japan, with top researchers from around the world gathering to discuss the newest research in the field.

The poster session, focusing primarily on younger researchers, was also quite fruitful, with 48 researchers making presentations. Their discussions extended on into the evening dinner parties, some of which went on to become the basis of actual research collaborations.

Though much of the current advances in this field of research pertain to the processes by which diverse controls in neural stem cells give rise to the different types of cells in each layer of the neocortex, as research moves forward we expect that elucidation of these control mechanisms will contribute to our understanding of thalamo – cortical projections and cortico– cortical connections in the brain, and the formation of different areas within the neocortex.

(Tetsuo Yamamori)



Speakers

Guillemot, Francois (NIMR), Hensch, Takao (Harvard Univ.), Kriegstein, Arnold (UCSF), Nieto, Marta (CSIC), Polleux, Franck (TSRI), Shi, Song-Hai (MSKCC), Yuste, Rafael (Columbia Univ.)
Aizawa, Shinichi (RIKEN CDB), Ikegaya, Yuji (The Univ. of Tokyo), Okano, Hideyuki (Keio Univ.), Osumi, Noriko (Tohoku Univ.), Kaibuchi, Kozo (Nagoya Univ.), Kageyama, Ryoichiro (Kyoto Univ.), Kano, Masanobu (The Univ. of Tokyo), Kawaguchi, Yasuo (NIPS), Gotoh, Yukiko (The Univ. of Tokyo), Sakano, Hitoshi (The Univ. of Tokyo), Shimazaki, Takuya (Keio Univ.), Tagawa, Yoshiaki (Kyoto Univ.), Nabekura, Junichi (NIPS), Noda, Makoto (Kyoto Univ.), Hanashima, Carina (RIKEN CDB), Masu, Masayuki (Univ. of Tsukuba), Matsuzaki, Masanori (NIBB), Matsuzaki, Fumio (RIKEN CDB), Mishina, Masayoshi (The Univ. of Tokyo), Murakami, Fujio (Osaka Univ.), Mori, Ikue (Nagoya Univ.), Yamamori, Tetsuo (NIBB)

The 58th/60th NIBB Conference "Germline -Specification, Sex, and Stem Cells-"

Organizers: Robert E. Braun (The Jackson Laboratory), Mark Van Doren (The Jackson Laboratory), Satoru Kobayashi (NIBB), Shosei Yoshida (NIBB)

July 17 (Tue) - 21 (Sat), 2012

Originally the 58th NIBB conference on "Gamete Stem Cells" was to be held in July, 2011, however, due to the March 11th earthquake and tsunami the meeting was postponed. Fortunately, the 60th NIBB conference on "Germline Development" was planned for this year and we were able to hold this unprecedented "58th and 60th 'joint' NIBB conference".

At the symposium we focused on three subjects central to germline research, the formation of the germline, the sexual characteristics of the germline, and gamete stem cells, and thoroughly discussed the universal and distinctive aspects of a wide range of species, including mammals (primates, mice), birds, fish, ascidians, hydra, nematodes, planarians, and fruit flies. Over 130 participants from around the world took part, with four scientists who are pushing the bounds of present research giving special lectures based on data from the cutting edge of this field, which lead to impassioned debate from the beginning to the end of the conference.

Following the special lectures, lectures on the mechanisms regulating specification and sexual development of the germline continued. The topics included identification of the genes that induce feminization or masculinization of primordial germ cells, somatic control of germline sex, and the regulatory mechanisms of gene expression in germline cells. Research using birds, planarians, ascidians, and hydra was also presented, allowing for participants to share the invaluable viewpoint of evolutional conservation and divergence of the regulatory mechanism underlying germline development, and to feel the true breadth of the germline research field.

On the following days, researchers investigating the stem cells of gametogenesis in a variety of animals, including mice, primates, fish and fruit flies, were able to come together and discuss their research in a single venue, making this a historic opportunity. Many debates were presented based on similarities in the gamete stem cell systems in mice, fruit flies, and other animals that display substantial differences in gonadal morphology, as well as computer simulations and quantitative statistical analyses of stem cell dynamics. A variety of revolutionary germ cell manipulating techniques motivated by medical science, animal husbandry, and fisheries science was another important theme of the conference, with much lively debate on the subject to be had.

Though the schedule for the whole event was rather tight, we believe the conference provided ample ground for participants to meet between talk and poster sessions, and at meals and other get-togethers, and to strengthen and broaden their personal bonds. It is our hope that these new connections might become the seeds for new breakthrough research in the field.

Finally, we would like to give our thanks to all the participants for this year's conference for attending, the Office of International Cooperation and the members of Kobayashi and Yoshida Laboratories of NIBB for managing the conference, Grant-in-Aid for Scientific Research on Innovative Areas, "Regulatory Mechanisms of Gamete Stem Cells" from the Ministry of Education, Culture, Sports, Science, and Technology for co-sponsoring the event, and to the Inoue Foundation for Science and the Daiko Foundation for their support.

(Shosei Yoshida)

Speakers

Braun, Robert (The Jackson Laboratory), de Rooij, Dirk (Univ. of Amsterdam), Han, Jae Yong (SNU), Koopman, Peter (Univ. of Queensland), Lehmann, Ruth (NYU), Matunis, Erika (Johns Hopkins Univ.), Newmark, Phillip (UIUC), Orwig, Kyle (Univ. of Pittsburgh), Page, David (Whitehead Institute), Seydoux, Geraldine (Johns Hopkins Univ.), Simons, Benjamin (Univ. of Cambridge), Spradling, Allan (CIW), Van Doren, Mark (Johns Hopkins Univ.), Yamashita, Yukiko (Univ. of Michigan Ann Arbor)
Kobayashi, Kazuya (Hirosaki Univ.), Kobayashi, Satoru (NIBB), Kumano, Gaku (Osaka Univ.), Matsui, Yasuhisa (Tohoku Univ.), Nakamura, Akira (RIKEN CDB), Niki, Yuzo (Ibaraki Univ.), Ogawa, Takehiko (YCU), Saga, Yumiko (NIG), Saitou, Mitinori (Kyoto Univ.), Shinohara, Takashi (Kyoto Univ.), Tanaka, Minoru (NIBB), Yoshida, Shosei (NIBB), Yoshizaki, Goro (TUMSAT),

The 4th NIBB-MPIPZ-TLL Symposium "Arabidopsis and Emerging Model Systems"

Organizers: Masayoshi Kawaguchi(NIBB), Yoshikatsu Matsubayashi (NIBB), Kiyoshi Tatematsu (NIBB), Mitsuyasu Hasebe (NIBB), Mikio Nishimura (NIBB)

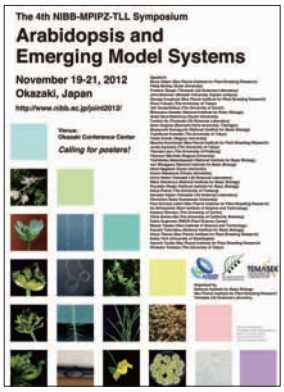
November 19 (Mon) - 21 (Wed), 2012

The National Institute for Basic Biology (NIBB), with the goal of advancing research and providing a venue for academic exchange, held the 4th NIBB-MPIPZ-TLL joint symposium from November 19th ~ November 21st 2012, at the Okazaki Conference Center in Okazaki City, Aichi Prefecture, Japan. This series of symposia began in Cologne in 2009, as a joint venture between NIBB and The Max Planck Institute for Plant Breeding Research (MPIPZ), with which NIBB has formed an academic exchange agreement, the Temasek Life Science Laboratory, which also entered into an academic exchange agreement with NIBB, later joined. The symposia are now being held in rotation between all three institutes. The theme for this year's conference was "Arabidopsis and Emerging Model Systems". Arabidopsis is well known as an excellent model organism, playing a central role in the elucidation of the molecular mechanisms involved in development, physiology, environmental response, and other biological processes. For this year's symposium, we invited many researchers on the forefront of the field to present their research results and engage in lively discourse regarding their work. Notably, Okazaki is known as one of the founding centers of the Arabidopsis community in Japan. In 1990 and 1991, Dr. Kiyotaka Okada, (at the time, an assistant professor at the Dr. Yoshiro Shimura's Lab of NIBB) planned and held the first and second workshops promoting Arabidopsis as a model organism in NIBB, after which its use within Japan rapidly expanded.

The world of plants, however, is quite diverse just like the world of animals or microbes. Many biological phenomena remain which cannot be understood or elucidated through Arabidopsis, and even whether the insights into plant biology provided by Arabidopsis are universal remains unknown. In addition, with recent years bringing revolutions in next generation sequencer technology and genetic analysis techniques, new model lines continue to spring forth at a rapid pace. Thus, at this symposium, we also asked for the participation of researchers engaged in dynamic research utilizing new model plants such as Marchantia Polymorpha, seaweed, algae, or other experimental lines in order to further mutual understanding of research new and old.

This year marks the final year of General Director Okada's tenure at NIBB, and a quarter century of his work in the field of plant biology. The symposium was a lively one, thanks not only to the researchers engaged in pushing forward the state of plant research while expanding the Arabidopsis community, but also thanks to the researchers connected to NIBB, and internationally active women researchers, who were kind enough to take part in this symposium. There were a total of 186 participants, with 67 poster presentations of new research, primarily featuring the work of younger researchers, on exhibit as well. With question and answer sessions running on into lengthy, animated discussions, the 4th NIBB-MPIPZ-TLL joint symposium was a wonderful experience for all.

(Masayoshi Kawaguchi)



Speakers

Albani, Maria (MPIPZ), Benfey, Philip (Duke Univ.), Berger, Frederic (TLL), Bowman, John (Monash Univ.), Coupland, George (MPIPZ), Grossniklaus, Ueli (Univ. of Zurich), Ito, Toshiro (TLL), Juergens, Gerd (Univ. of Tuebingen), Koornneef, Maarten (MPIPZ), Laux, Thomas (Univ. of Freiburg), Nishii, Ichiro (TLL), Palme, Klaus (Univ. of Freiburg), Sarojam, Rajani (TLL), Schulze-Lefert, Paul (MPIPZ), Shimizu, Kentaro (Univ. of Zurich), Somerville, Chris (Univ. of California Berkeley), Theres, Klaus (MPIPZ), Torii, Keiko (Univ. of Washington), Tsuda, Kenichi (MPIPZ)

Fukuda, Hiroo (The Univ. of Tokyo), Hara-Nishimura, Ikuko (Kyoto Univ.), Hasebe, Mitsuyasu (NIBB), Kawaguchi, Masayoshi (NIBB), Komeda, Yoshibumi (The Univ. of Tokyo), Kondo, Takao (Nagoya Univ.), Kyozuka, Junko (The Univ. of Tokyo), Machida, Yasunori (Nagoya Univ.), Matsubayashi, Yoshikatsu (NIBB), Minagawa, Jun (NIBB), Nagatani, Akira (Kyoto Univ.), Nakamura, Kenzo (Chubu Univ.), Nishimura, Mikio (NIBB), Okada, Kiyotaka (NIBB), Sawa, Shinichiro (Kumamoto Univ.), Shimamoto, Ko (NAIST), Sugimoto, Keiko (RIKEN), Tasaka, Masao (NAIST), Tatematsu, Kiyoshi (NIBB), Tsukaya, Hirokazu (The Univ. of Tokyo)

The NUS/TLL/NIBB Joint Practical Workshop "Genetics, Genomics and Imaging in Medaka and Zebrafish"

- Period: July 22(Sun) - July 31(Tue), 2012
- Participants: 16 (3 from Germany, 2 from China, 2 from India, 2 from Italy, one each from Australia, Austria, Canada, France, Japan, Norway, and USA)
- Venue: Natl. Univ. Singapore (NUS) and TLL, Singapore
- Lecturers:
  Dr. Kiyoshi Naruse (NIBB)
  Dr. Atsuko Shimada (Univ. Tokyo)
  Dr. Suresh Jesuthasan (Duke-NUS NRP, Singapore)
  Dr. Zhiyuan Gong (NUS)
  Dr. Thorsten Wohland (NUS)
  Dr. Dipanjan Bhattacharya (NUS)
  Dr. Paul Matsudaira (NUS)
  Dr. Tom Carney (Inst. Mol. Cell Biol., Singapore)
  Dr. Vladimir Korzh (Inst. Mol. Cell Biol., Singapore)
  Dr. Atsuko Sehara-Fujisawa (Kyoto University)
  Dr. Yasuhiro Kamei (NIBB)
  Dr. Hiroyuki Takeda (Univ. Tokyo)
  Dr. Tetsuaki Kimura (NIBB)
  Dr. Sinnakarupan Mathavan (Genome Inst. Singapore)
  Dr. Sudipto Roy (Inst. Mol. Cell Biol., Singapore)

- Course Staff:
  Dr. Mireia Perez Camps, Dr. Takuya Kaneko, Dr. Toru Kawanishi, Dr. Karuna Sampath, Dr. Anand Singh, Dr. Sahar Tavakoli, Dr. Yan Tong, Dr. Yin Wang, Dr. Christoph Winkler, Dr. Chang Woei

- Contents of the course:
  Somite transplantation, Cell transplantation, Cryopreservation of Medaka sperm: In-vitro fertilization, TALENs: Targeted gene modification in zebrafish, Establishment of BAC transgenic medaka, IR-LEGO, SPIM

The NIBB Genome Informatics Training Course

NIBB organizes a series of training courses on up-to-date research techniques for researchers from mainly Japanese universities and institutions. In 2012 we held two training courses on Genome Informatics. The two-day programs offer lectures and hands-on tutorials to introduce basic knowledge and skills to deal with genomic scale large data such as next-generation sequences (NGS). The programs are specially designed for biologists who are not familiar with bioinformatics.

"Introduction to Transcriptome Data Analysis"

March 22 (Thu) -23 (Fri), 2012

- Organizer: Dr. Shuji Shigenobu(NIBB Core Research Facilities)
- Lecturers: Dr. Ikuo Uchiyama, Dr. Masanao Sato, Dr. Katsushi, Yamaguchi, Dr. Shuji Shigenobu
- Participants: 19 (including 6 from NIBB)
- Program:
  1. Overview: Transcriptome data analysis
  2. Introduction to statistics
  3. Introduction to "R"
  4. RNA-seq analysis pipeline
  5. Basic format of mapping data and basic tools
  6. Expression data analysis I: Normalization, differential expression analysis
  7. Expression data analysis II: Multivariate analysis
  8. Excercise

"Introduction to Next-generation DNA Sequence Data Analysis"

September 6 (Thu) -7 (Fri), 2012

- Organizer: Dr. Shuji Shigenobu(NIBB Core Research Facilities)
- Lecturers: Dr. Ikuo Uchiyama, Mr. Tomoki Miwa, Dr. Katsushi, Yamaguchi, Dr. Shuji Shigenobu
- Participants: 16 (including 1 from NIBB)
- Program:
  1. Overview: NGS data analysis
  2. UNIX for beginners
  3. NGS basic data formats
  4. NGS basic tools I: Mapping
  5. NGS basic tools II: Visualization tools
  6. NGS basic tools III: Samtools
  7. Methods for text data processing
  8. Excercise

The NIBB Internship Program

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

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

In FY 2012 there were 26 applicants, of which seven interns were selected. These interns were from universities located in 3 countries (India, Germany and China) and spent periods ranging from 1 week to two months experiencing life as a member of a research team.

Report from a participant
Ji Zhongzhong
Capital Normal University, China

From Sep. 1st to 30th, I joined Prof. Hasebe's laboratory as an intern and focused on roles of a single transcription factor, STEMIN, in the reprogramming of differentiated cells to stem cells. To address how STEMIN induces reprogramming, I investigated spatiotemporal expression patterns of STEMIN in excised leaf cells using the GUS knock-in line and changes in expression of genes involved in reprogramming after the STEMIN induction with advanced techniques.

I successfully completed the work described above and obtained some experimental data. On the last day of my internship program, I gave a short talk about these results and discussed them with Prof. Hasebe and his lab members. It helped me to understand plant stem cell formation.

During my stay at NIBB, everything went well; everyone here was friendly, enthusiastic and accommodating, I felt very lucky for joining Prof. Hasebe's lab. NIBB offered many seminars for students and staff, it is a good platform and helpful for enhancing communication and opening the mind to research here.

The 7th Bio-Imaging Forum "Innovation of the Microscope"

Organizers: Yasuhiro Kamei, Shigenori Nonaka, Masayuki Hattori (NAO), Yosuke Tamada, Takeshi Hiyama

Supervisors: Naoto Ueno, Toshihiko Fujimori

November 26 (Mon)-27 (Tue), 2012

The 7th Bio-Imaging Forum was held on the theme of "Innovation of the Microscope," as a collaboration with the NINS Program for Cross-disciplinary Study by Young Researchers. This NINS Program includes two projects from NIBB: "The establishment of observation techniques for plant cells, utilizing adaptive optics developed for astronomical observation," (lead by Dr. Tamada) focusing on the application of adaptive optics to biological microscopic imaging, and the "Development of 3D optogenetics utilizing new femtosecond ultrashort pulse lasers" project (lead by Dr. Hiyama), focusing on the development and application of new methodologies within the rapidly expanding optogenetics field. For this year's Bio-Imaging Forum, we provided a shared venue for presenting the results of these research projects, and also invited 16 researchers to give lectures on the effects of direct, visual observation in microscopy that adaptive optics provides, the new paradigm shift from "observation" to "manipulation" and the related new potentialities for microscopy made possible by optogenetics, as well as expected future research and developments that may come from these two fields of study.

The forum had 60 participants, with 39 coming from outside of NIBB, including 10 participants from optics-related industries (Olympus, Nikon, Hamamatsu Photonics, Sigma-Koki), and 3 participants from the field of astronomy (National Astronomical Observatory of Japan (NAO), Hawaii Observatory, Subaru Telescope). As a cross-disciplinary gathering of researchers, the forum was host to lively academic exchange, with topics ranging from basic questions about unfamiliar fields, through to rigorous, in depth discussions focusing on specialty research. This exchange continued on into the evening's get-together dinner, with industry affiliated participants providing technical advice and tips, and scientists giving ideas and requests for possible future industrial research. At the end of the forum Professor Ueno also introduced the Center for Novel Science Initiative's (CNSI) Department of Imaging Science stressing the importance of furthering imaging research in the future. Through this year's forum, the necessity of further uniting the field of imaging, and its research, was made clear. It is our hope that the Bio-Imaging Forum will continue to play an active role in bringing the field of imaging research closer together, as well as promoting new front-line imaging research.

(Yasuhiro Kamei)

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Access

From Tokyo

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

From Osaka

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

From Central Japan International Airport

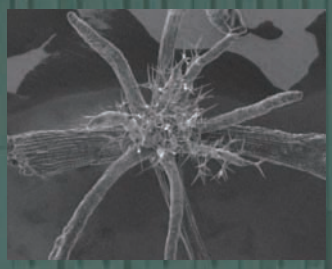
Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes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

From Higashi Okazaki Station to Each Area

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

By car

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



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Issued in June 2013
Edited by the Strategic Planning Department, NIBB